Analysis of the October 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 October 9 and October 16, 2001. Of those laboratories receiving specimen panels, 263 (90.4%) of 291 reported testing results. Three laboratories were unable to report results due to equipment malfunction or lack of reagents. Two laboratories were unable to return results due to personnel shortages (out of country, on strike). 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 October 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 one day for one laboratory and five days for another laboratory due to overnight carrier (FedEx) problems. Eleven laboratories reported a one day delay, one laboratory reported a two day delay, and one laboratory reported a five day delay in receiving their specimens due to delivery problems within their institution. Additionally, 44 (16.7%) of 263 laboratories reported they did not process the MPEP TLI specimens on the day they were received (39 laboratories, one day delay; three laboratories, two day delay; one laboratory, three day delay; one laboratory, 5 day delay).

Summary of Results

The types of laboratories participating in the October 2001 TLI shipment are shown in Figure 1. The majority of laboratories participating during this shipment period are classified as Hospital, 166 (63.1%) of 263, or Independent, 51 (19.4%) of 263.

Figure 2 of the report shows the methods used by the laboratories to prepare specimens for TLI. The majority of laboratories, 201 (76.4%) of 263, 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, 30 (11.4%) of 263; Flow Count, 14 (5.3%) of 263; FACSCount, 12 (4.5%) of 263; and one method described as "Other".

Of those laboratories reporting absolute cell counts, 57 of 210 (27.1%) laboratories reported using single-platform methods in the October 2001 shipment compared with 57 of 205 (27.8%) laboratories in the April 2001 shipment, 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, 26 (10.4%) of 251, 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, 109 (42.1%); FACS Calibur, 92 (35.5%); FACScan, 42 (16.2%); FACSCount, 6 (2.3%); and FACSort, 5 (1.9%). Other types of flow cytometers were used, each with a frequency of two 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, or Flow-Count) or in a single procedural assay (e.g., Coulter manual CD4, CD4Trax, or Zymmune). The majority of laboratories, 153 (72.8%) of 210, used only a multi-platform method to derive these absolute cell counts. Some laboratories, 56 (26.7%) of 210, used only a single-platform method. One laboratory (0.5%) of 210 provided absolute cell counts derived from both multi-platform and single-platform methods. Since reagents for the Becton Dickinson Biosciences Imagn2000 system were recalled in late 2000 and have not again been released, laboratories which had used this instrument in previous MPEP TLI surveys were unable to use it to participate in the October 2001 shipment.

Since not all laboratories provided results for absolute cell counts derived by multi-platform methods, only 170 (64.6%) of 263 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 (52.3%); Abbott, 29 (17.1%); Roche/Sysmex, 29 (17.1%); Bayer/Technicon, 21 (12.3%); Baker/Biochem Immunosystems, 1 (0.6%); 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, CD45/CD3/CD4, 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 207 (1.9%) of 10,840 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, 95.2%; CD8, 94.6%; CD14, 96.0%; CD19, 95.6%; CD45, 96.6%; and CD56/16, 95.3%.

The percentages of participating laboratory results within the 95% confidence limits established for the hematology data are: white blood cell count, 92.7%; lymphocyte percentage, 91.4%; and absolute lymphocyte count, 91.1%.

The percentages of participating laboratory results within the 95% confidence limits established for the absolute cell counts are: CD4, 92.8%; and CD8, 92.2%. As can be seen in the table on the following page, 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, B1	1	1 - 24	1 - 80	188 - 355	12 - 1540	35 - 912
A3, B4	2	1219 - 1786	842 - 4636	459 - 694	304 - 1862	287 - 6730
A1, B5	3	1051 - 1358	678 - 3578	601 - 802	316 - 2050	225 - 3420
A2, A4	4	525 - 992	390 - 1603	841 - 1533	591 - 2204	232 - 4250
B2, B3	5	711 - 1018	609 - 2046	1072 - 1502	341 - 2816	1743 - 3577
C2, D4	6	690 - 1026	106 - 1697	380 - 624	61 - 1494	225 - 3822
C3, C4	7	417 - 648	375 - 1265	286 - 454	248 - 866	826 - 3040
C5, D1	8	3 - 127	4 - 88	218 - 578	282 - 776	470 - 1927
D2, D5	9	1171 - 1745	38 - 2336	1178 - 2072	42 - 2652	2035 - 6468
C1, D3	10	1047 - 1466	1024 - 1798	774 - 1034	713 - 1349	2216 - 4087

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

In all cases, but one (Donor 8, CD4 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, 4, and 9). 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 all 5 specimens tested reported lymphocyte count results that were in error by a factor of ten (e.g., the laboratory reported a WBC of 2040 and a lymphocyte percent of 17, which should have yielded a lymphocyte count of 347, and the laboratory reported a lymphocyte count of 35). There were a total of 13 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 13, two laboratories reported inaccurately calculated lymphocyte counts on all 5 specimens tested and one laboratory reported inaccurately calculated lymphocyte counts on three 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 October 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.