Analysis of the April 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 April 6 and April 13, 1999. Of those laboratories receiving specimen panels, 289 (89.5%) of 323 reported testing results. Four laboratories reported shipment problems (specimens received late), equipment problems, or specimen mishandling upon receipt (specimens frozen) which prevented participation in the survey. One laboratory returned results too late to be included in the aggregate analysis.

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 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.

The types of laboratories participating in the April 1999 TLI shipment are shown in Figure 1. The majority of laboratories participating during this shipment period are classified as Hospital, 185 (64.0%) of 289, or Independent, 50 (17.3%) of 289.

Figure 2 of the report shows the methods used by the laboratories to prepare specimens for TLI. The majority of laboratories, 256 (88.6%) of 289, 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, 22 (7.61%) of 289; FACSCount, 6 (2.1%) of 289; Imagn2000, 5 (1.7%) of 289; and Flow Count, 1 (0.3%) of 289. Forty-two laboratories reported using single-platform methods in the April 1999 shipment compared with 35 laboratories in the September/October 1998, 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, 24 (8.6%) of 279, 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, 106 (37.6%); FACScan, 82 (29.1%); FACS Calibur, 54 (19.1%); Ortho CytoronAbsolute, 16 (5.7%); and EPICS Profile II, 11 (3.9%). 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, 166 (79.8%) of 208, used only a multi-platform method to derive these absolute cell counts. Some laboratories, 40 (19.2%) of 208, used a single-platform method. Two laboratories (1.0%) of 208, 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 195 (67.5%) of 289 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, 121 (62.1%); Sysmex, 30 (15.4%); Abbott, 22 (11.3%); Bayer/Technicon, 19 (9.7%); and Other, 3 (1.5%).

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 225 (1.8%) of 12,332 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, 94.9%; CD4, 95.5%; CD8, 94.8%; CD14, 93.4%; CD19, 96.1%; CD45, 95.6%; and CD56/16, 96.1%.

The percentage of participating laboratory results within the 95% confidence limits established for the hematology data are: white blood cell count, 91.9%; lymphocyte percentage, 91.8%; and absolute lymphocyte count, 92.1%.

The percentage of participating laboratory results within the 95% confidence limits established for the absolute counts are: CD4, 92.6%; and CD8, 92.9%. 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	499 - 774	45 - 1794	418 - 655	38 - 1379	437 - 3901
2	598 - 857	437 - 1013	240 - 360	179 - 466	1121 - 2279
3	555 - 755	433 - 1260	347 - 451	36 - 820	1031 - 2090
4	444 - 609	408 - 2229	443 - 567	332 - 2074	1008 - 5690
5	63 - 158	9 - 190	673 - 1008	72 - 1420	962 - 2108
6	331 - 1039	278 - 664	344 - 534	278 - 620	331 - 1039
7	220 - 364	184 - 911	276 - 448	250 - 720	500 - 1710
8	19 - 1029	442 - 1267	116 - 601	249 - 757	726 - 2579
9	1 - 1353	448 - 1713	37 - 2184	676 - 1567	1413 - 4520
10	0 - 225	115 - 531	66 - 1598	790 - 3476	894 - 4828

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

The multi-platform ranges were larger than the corresponding single-platform ranges in 16 (80%) 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 (Donor 1, ~ nine fold difference between smallest and largest absolute lymphocyte count determinations).

For this performance survey, some changes were made in the manner in which K_3EDTA was prepared for specimen collection. Thirty (10.4%) of 289 reporting laboratories described problems with the shipped specimens, particularly with regard to hematology analysis. The wide range of absolute lymphocyte counts, and therefore the wide range of absolute CD4 and CD8 T-cell counts using multi-platform techniques, may be related to the condition of the shipped specimens. As a result, a protocol has been implemented to ensure standardized preparation of K_3EDTA.

In summary, most laboratories performed well on the donor specimens in the April 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, or to other factors associated with specimen preparation.