Analysis of the September/October 1998 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 September 22 or October 6, 1998. Of those laboratories receiving specimen panels, 286 (92.6%) of 309 reported testing results. Two laboratories reported shipment problems (specimens received late) which prevented participation in the survey.

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 first two pages immediately following the acknowledgment page contain the specimen numbers and donor information for each performance evaluation specimen.

The result reporting booklet used for the September/October 1998 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 September/October 1998 TLI shipment are shown in Figure 1. The majority of laboratories participating during this shipment period are classified as Hospital, 179 (62.6%) of 286, or Independent, 50 (17.5%) of 286.

Figure 2 of the report shows the methods used by the laboratories to prepare specimens for TLI. The majority of laboratories, 258 (90.8%) of 284, 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, 14 (4.9%) of 284; FACSCount, 8 (2.8%) of 284; Imagn2000, 3 (1.1%) of 284; and Flow Count, 1 (0.4%) of 284. Thirty-five laboratories reported using single-platform methods in the September/October 1998 shipment compared with 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, 27 (9.7%) of 278, 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, 104 (36.4%); FACScan, 86 (31.1%); FACS Calibur, 42 (11.9%); EPICS Profile II, 17 (7.3%); and Ortho CytoronAbsolute, 16 (6.6%). 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 STKS, Flow-Count, or Imagn2000) or in a single procedural assay (e.g., Coulter manual CD4, CD4Trax, or Zymmune). The majority of laboratories, 153 (81.4%) of 188, used only a multi-platform method to derive these absolute cell counts. Some laboratories, 33 (17.6%) of 188, used a single-platform method. Two laboratories (1.1%) of 188, 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 184 (64.3%) of 286 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 (64.7%); Sysmex, 27 (14.7%); Abbott, 19 (10.3%); Technicon, 17 (10.1%); and Other, 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+ 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 291 (2.4%) of 12,208 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, 94.0%; CD4, 94.4%; CD8, 95.6%; CD14, 94.2%; CD19, 94.7%; CD45, 96.7%; 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, 94.7%; lymphocyte percentage, 92.9%; and absolute lymphocyte count, 92.6%.

The percentage of participating laboratory results within the 95% confidence limits established for the absolute counts are: CD4, 93.3%; and CD8, 92.4%.

In summary, most laboratories performed well on the donor specimens in the September/October 1998 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.