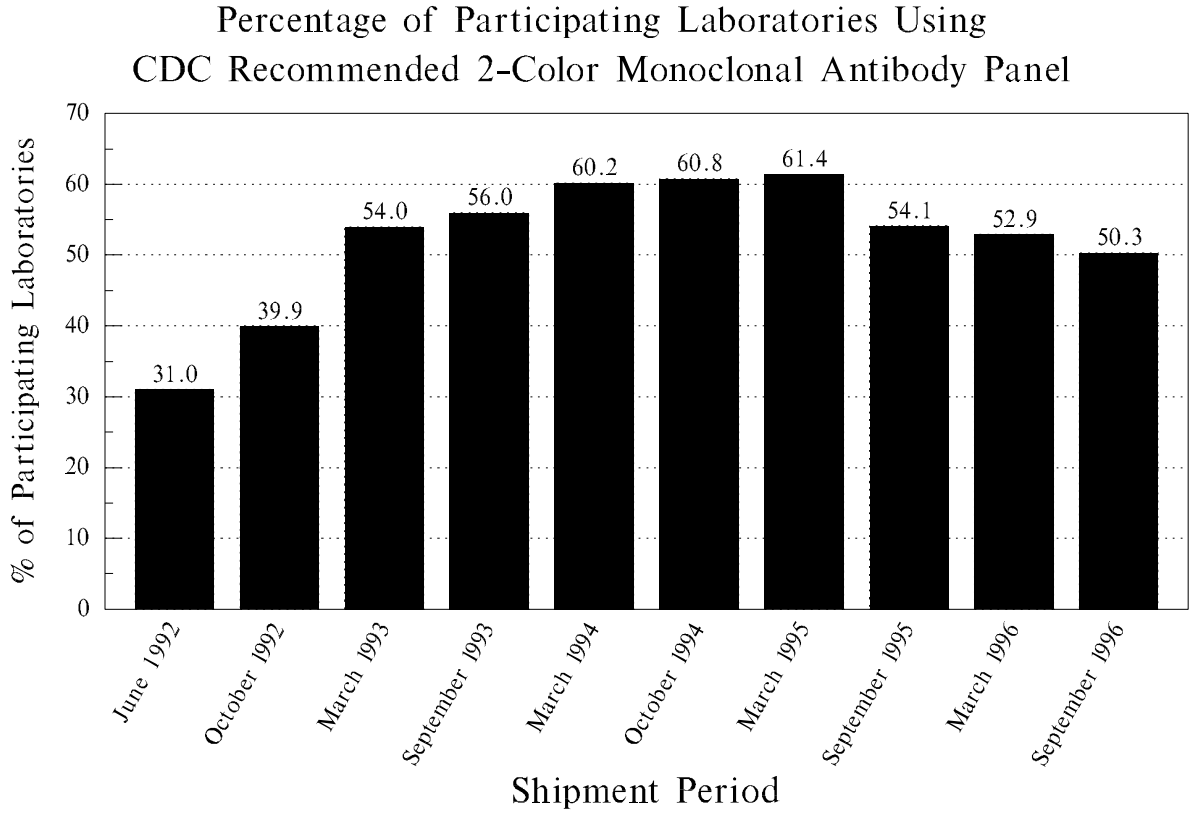
Analysis of the September 1996 Performance Evaluation Testing Results for T-Lymphocyte Immunophenotyping Reported to the Centers for Disease Control and Prevention by Participating Laboratories

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 in September 1996. Of those laboratories receiving specimen panels, 300 (90.9%) of 330 reported testing results. Receipt of panel shipments by some participating laboratories were delayed due to inclement weather conditions (hurricanes) in September. Fresh panels were sent in subsequent weeks of the shipment period to those laboratories requesting reshipment. Two laboratories returned incomplete result reports stating the samples received were too old to test. One laboratory reported only absolute count results (rather than percentages), therefore, their results were not included in the aggregate report.

Each laboratory received a total of six specimens: three HIV-1 antibody-positive and two HIV-1 antibody-negative whole blood specimens, and an instrument performance control sample consisting of fluorescenated beads. 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 three pages immediately following the title page contain the specimen numbers and donor information for each performance evaluation specimen.

The result reporting booklet used for the September 1996 specimen shipment was designed to be consistent with the CDC guidelines for $CD4^+$ cell testing (MMWR, vol. 43, no. RR-3, March 4, 1994). Laboratories have been encouraged by the MPEP to utilize these guidelines in performing TLI on specimens from HIV-infected patients.

Laboratories have been allowed to report results using not only 2-color but also 3-color analyses of the specimens for the last three shipment periods. As can be seen in the graph on the following page, the ability to report 3-color data has resulted in a decreased number of participant laboratories, 151 (50.3%) of 300, that used the 2-color monoclonal antibody panel recommended by the CDC for CD4+ cell testing. Twenty-six (8.7%) of 300 laboratories performed 2-color analyses for some cell marker determinations and 3-color for other cell marker determinations on the same specimen. Forty-two (14.0%) of 300 laboratories performed only 3-color analyses for all the markers on all the specimens they tested.



The types of laboratories participating in the September 1996 TLI shipment are shown in Figure 1. The majority of laboratories participating during this shipment period are classified as Hospital, 195 (65.0%) of 300, or Independent, 49 (16.3%) of 300.

Figure 2 of the report shows the methods used by the laboratories to prepare specimens for TLI. The majority of laboratories, 292 (97.7%) of 299, reported using a method of whole blood lysis to prepare specimens for TLI.

Figure 3 shows the methods used by the laboratories to fix their TLI specimens before flow cytometric analysis. Of laboratories reporting testing results, 20 (6.8%) of 295, reported 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: FACScan, 112 (37.3%); EPICS XL, 95 (31.7%); EPICS Profile II, 36 (12.0%); Ortho CytoronAbsolute, 19 (6.3%); EPICS Elite, 11 (3.7%) and FACSort, 7 (2.3%). Other types of flow cytometers were used, each with a frequency of less than 2%.

Results reported by those laboratories which used the 2-color monoclonal antibody panel recommended in the CDC $CD4^+$ testing guidelines were used to calculate 90% reference ranges for each donor and cell marker involved in two-color analysis using the Statistical Analysis System (SAS) procedure PROC UNIVARIATE. Before calculation, data were analyzed for possible outliers. There were 75 (1.0%) of 7597 results returned by laboratories using the monoclonal antibody panel recommended in the CDC guidelines that were considered to be outliers. Of the 75 outliers detected, 21 (28.0%) were results reported by two laboratories and all data reported by these two laboratories were removed for calculation of reference ranges. No data from any laboratory, however, were removed from the table comparing values against the 90% reference ranges.

Results reported by all laboratories were grouped by donor number and cell marker, and compared to the 90% reference ranges as determined for each donor and marker involved in two-color analysis. Cell markers for each donor for which there were insufficient data for determination of 90% reference ranges are not included in the tables.

The overall frequency of 2-color laboratory results, by cell marker within, above, or below the 90% reference ranges are shown in Table 1. Single-color and 3-color results and results using reagents not included in the CDC $CD4^+$ cell testing guidelines (e.g., CD2 and CD57) are not presented. The percentage of participating laboratory results within the 90% reference ranges for the cell markers specified in Table 1 are: CD3 average, 91.88%; CD4, 94.29%; CD8, 91.35%; CD14, 96.50%; CD19, 96.49%; CD45, 93.50%; and CD56/16, 93.93%.

Due to insufficient data, 90% reference ranges could not be calculated for CD16 alone and CD56 alone. Table 2 shows the entire range of laboratory results (maximum and minimum) reported for these two cell markers.

The percentage of laboratory results, by monoclonal antibody manufacturer, that were within the 90% reference ranges are shown in Table 3. Other manufacturers included Dako, GenTrak, Immunotech, In-house, Ortho, Pharmingen, or Sigma.

The percentage of laboratory results, by flow cytometry instrument manufacturer, that were within the 90% reference ranges are shown in Table 4.

Laboratories indicated they sometimes use one manufacturer's flow cytometer and another manufacturer's monoclonal reagents; e.g., a laboratory using a flow cytometer manufactured by Coulter may be using monoclonal antibody reagents manufactured by Becton Dickinson, or vice versa. Laboratories also indicated that they do not use monoclonal antibodies from one manufacturer exclusively in the battery of tests used to analyze these specimens; e.g., the manufacturer of the CD3/CD4 reagent may be different from the manufacturer of the CD3/CD56+CD16 reagent. Laboratories also indicated that they may use antibodies from different manufacturers within a single tube, e.g., for a CD3/CD4 tube the laboratory might use the CD3 reagent from Coulter and the CD4 reagent from Becton-Dickinson. Analysis of reported cell marker percentages by flow cytometer and by monoclonal antibody manufacturer

(Tables 1, 2, 3, and 4) indicated the cell marker percent positive values differed depending on the flow cytometer or monoclonal antibody manufacturer used by the laboratories. These associations were made by comparing the results with each monoclonal antibody manufacturer to the respective 90% reference ranges as shown in Table 3, and by comparing the results with instrument manufacturer to the respective 90% reference ranges as shown in Table 4. It is unclear whether these differences are related to either the flow cytometer, the monoclonal antibody manufacturer, the combination of the flow cytometer and monoclonal antibody manufacturers, or whether other factors may be involved, e.g., the method used to prepare the specimens for analysis.

The percentages of laboratory results within, above, or below the 90% reference ranges for the fluorescenated bead instrument performance control sample are shown in Table 5. The results from the same laboratories that were used to generate the 90% reference ranges for Table 1 were used to generate the 90% reference ranges for Table 5. Most laboratory results, 972 (88.6%) of 1,097, were within the 90% reference ranges.

The overall frequency of laboratory results using 3-color analyses, by cell marker within, above, or below the 90% reference ranges are shown in Table 6. The ranges used for comparison are the same 2-color derived ranges shown in Table 1. Results for 3-color analyses, for nearly comparable cell markers, were compared against the same 2-color ranges, e.g., CD45+/CD3+/CD4+ and CD3+/CD4+/CD8- were compared against the 2-color range for CD3+/CD4+, CD45+/CD3+CD8+ and CD3+/CD4-/CD8+ were compared against the 2-color range for CD3+CD8+, CD45+/CD3-CD19+ was compared against the 2-color range for CD3-/CD19+, and CD45+/CD3-/CD56&16+ was compared against the 2-color range for CD3-/CD56&16+. The percentage of participating laboratory results within the 90% reference ranges for the cell markers specified in Table 6 are: CD45+/CD3+/CD4+, 96.5%; CD45+/CD3+/CD8+, 89.5%; CD3+/CD4+/CD8-, 90.6%; CD3+/CD8+/CD4-, 84.3%; CD45+/CD3-/CD19+, 88.4%; and CD45+/CD3-/CD56/16+, 86.2%.

In summary, most laboratories performed well on the donor specimens in the September 1996 shipment. Not all laboratories used the 2-color monoclonal antibody combinations recommended in the CDC MMWR $CD4^+$ cell testing guidelines. Of the 2-color results listed in Table 1, 5,259 (94.0%) of 5,596 were within the 90% reference range. Of the 3-color results listed in Table 6, 921 (89.4%) of 1,030 were within the 2-color derived 90% reference range. Differences in laboratory performance of cell marker analysis may be related to the use of the CDC $CD4^+$ cell testing guidelines, the use of different flow cytometer and reagent manufacturer combinations, or to other factors associated with specimen preparation.