Analysis of the June 23, 1997 Performance Evaluation
HIV-1 p24 Antigen Testing Results
Reported to the Centers for Disease Control and Prevention
by Laboratories Participating in the Model Performance Evaluation Program

This is the first report of the Centers for Disease Control and Prevention (CDC) Model Performance Evaluation Program human immunodeficiency virus type 1 (HIV-1) p24 antigen determination survey which was newly implemented in 1997. This report is an analysis of results reported to the CDC by laboratories participating in the MPEP after they performed p24 antigen determinations on HIV-1 performance evaluation samples shipped to them June 23, 1997. Testing results were reported by 178 (92.7%) of the 192 laboratories that received sample panels.

Samples used in the MPEP HIV-1 p24 antigen performance evaluation survey are obtained from donors who are HIV-1 infected or non-infected and are neither diluted or pooled so that each donor sample consists of plasma from an individual donor. Before shipment, the CDC tested plasma from each donor with two p24 antigen test kits approved by the Food and Drug Administration (FDA).

The CDC panels for this shipment, the labeled vials contained in each panel, the CDC donor numbers, and CDC test results and interpretations obtained with FDA-approved test kits can be found in Table 1. HIV-1 p24 antigen was detected in the plasma from both of the HIV-1 infected donors by each of the test kits used; the CDC interpretation for these donors was positive for p24 antigen. The plasma from donors not infected with HIV-1 had no HIV-1 p24 antigen detected, as defined by the test kit manufacturer's criteria; the CDC interpretation for these donors was negative for p24 antigen.

Summary of Results

Figure 1 shows the cumulative frequency of HIV-1 p24 Qualitative and Neutralization test results reported by laboratories for HIV-1 infected donors (Positive) and for those donors not infected with HIV-1 (Negative).

Qualitative Test. For the samples from donors that were infected with HIV-1 (Donor 1 and Donor 2), 508 (98.8%) of the Qualitative results indicated the presence of HIV-1 p24 antigen, while 6 (1.2%) of the results did not indicate detection of HIV-1 p24 antigen. Of the 340 results reported for the two donors not infected with HIV-1 (Donors 3 and 4), laboratories reported 339 (99.7%) results not detecting HIV-1 p24 antigen, and only 1 (0.3%) result detecting HIV-1 p24 antigen.

Neutralization test. For the samples from donors that were infected with HIV-1 (Donor 1 and Donor 2), 199 (98.5%) of 202 Neutralization test results confirmed the presence of HIV-1 p24 antigen, while 3 (1.5%) results were reported as indeterminate since the percent neutralization achieved was less than the minimum neutralization percent acceptable for the test kit used. Data is not included from two laboratories, unable to calculate a percent neutralization for a reactive sample, that incorrectly reported indeterminate neutralization test interpretations. No indeterminate or negative interpretations were reported for samples from donors not infected with HIV-1 (Donors 3 and 4). However, data is not included from two laboratories, unable to calculate percent neutralization for a nonreactive sample, that incorrectly reported indeterminate neutralization test interpretations. Likewise, data is not included from

two laboratories that incorrectly reported negative neutralization test interpretation for a negative donor sample without performing a neutralization test on the sample.

Types of Laboratories Performing HIV-1 p24 Antigen Determinations

The types of laboratories reporting results for the Qualitative, Neutralization, and Quantitative tests are shown in Figure 2. Each laboratory type is listed by decreasing frequency. Blood bank laboratories performed the most Qualitative and Neutralization tests while Independent laboratories reported the most Quantitative test results.

Combination of HIV-1 p24 Antigen Tests Performed

The combination of tests performed by laboratories to determine and confirm the presence of p24 antigen are shown in Figure 3. More than 50% of the laboratories participating in this survey reported only Qualitative test results. Many of these laboratories indicated there was insufficient sample to perform Neutralization tests as they do routinely to confirm the presence of p24 antigen.

Types of Test Kits Used

The types of test kits used by laboratories reporting HIV-1 p24 antigen test results are shown in Figure 4, by test type and manufacturer. Test kits approved by FDA were used by more than 90% of the laboratories reporting Qualitative results.

HIV-1 p24 Antigen Qualitative Test Results by Manufacturer

Among the 340 interpretations reported for the p24 antigen-negative samples (Donors 3 and 4) there was one false-reactive interpretation reported for Donor 4 by a laboratory using the Organon Teknika test kit. This false-reactive interpretation was reported by a laboratory that incorrectly recorded repeat reactive qualitative test results for a positive donor sample (Donor 1) using the wrong sample code. Of the 514 interpretations reported for the p24 antigen-positive samples (Donors 1 and 2) there were 6 nonreactive interpretations reported. Four nonreactive interpretations reported for Donor 1 by laboratories using the DuPont Alliance kit and two nonreactive interpretations were reported for Donor 1 by a laboratory using proprietary reagents being developed by a commercial manufacturer of HIV-1 diagnostic kits.

HIV-1 p24 Antigen Neutralization Test Results by Manufacturer

There were no false-positive or indeterminate results among the 8 Neutralization test results reported for the p24 antigen-negative samples (Donors 3 and 4). It is unclear why laboratories performed neutralization tests on samples that were nonreactive in the Qualitative assay. Of the 202 Neutralization test interpretations reported for the p24 antigen-positive samples (Donors 1 and 2) there were 3 indeterminate interpretations reported. The indeterminate interpretation reported by the laboratory using

the Coulter kit was reported for Donor 1 while two laboratories, using the Organon Teknika reagent kit, reported indeterminate interpretations for Donor 2.

Aggregate Percent Neutralization Results Reported by Donor

Aggregate percent neutralization results for HIV-1 infected Donor 1 (duplicate samples) and Donor 2, by test kit, are shown in Table 2. Information listed in these tables also includes the identity of panel vials containing plasma from these donors. For this shipment, Donor 1 was the only sample that was duplicated in each panel providing participant laboratories the opportunity to review their intra-shipment reproducibility for that donor sample.

Please note that in Table 2 the columns under each donor sample list, by test kit manufacturer, the number of laboratory results confirming HIV-1 p24 antigen or not confirming HIV-1 p24 antigen, followed by the minimum, median, and maximum percent neutralization values determined from the results reported.

Laboratories consistently reported HIV-1 p24 antigen neutralization for the samples obtained from donors infected with HIV-1. The median percent neutralization values determined from the results reported for these donors is remarkably similar among all the manufacturers. The median percent values determined from the results reported for the duplicate samples of Donor 1 in each panel reflect a strong degree of reproducibility in determining percent neutralization by each manufactured kit.

No laboratories reported HIV-1 p24 antigen neutralization for samples obtained from donors who were not infected with HIV-1; therefore, percent neutralization data is not shown for Donors 3 and 4.

Aggregate p24 Antigen Quantitation Results Reported by Donor

Aggregate p24 antigen quantitation results for HIV-1 infected Donor 1 (duplicate samples) and Donor 2, by test kit, are shown in Table 3. Information listed in these tables also includes the identity of panel vials containing the plasma from these donors. For this shipment, Donor 1 was the only sample that was duplicated in each panel providing participant laboratories the opportunity to review their intra-shipment reproducibility for that donor sample.

In Table 3, the columns under each donor sample list, by test kit manufacturer, the number of laboratory results reporting the quantity of HIV-1 p24 antigen detected, followed by the values for minimum, median, and maximum quantity of p24 antigen, as determined from the results reported.

Laboratories were readily able to quantitate HIV-1 p24 antigen in those samples obtained from donors infected with HIV-1. The median quantity of p24 antigen detected, as determined from the results reported for these donors, varied widely depending on which manufactured reagents were used. The median p24 antigen values for duplicate Donor 1 samples in each panel, as determined from participating laboratory results, reflect a good reproducibility in quantitation of p24 antigen using reagents from any of the individual manufacturers.

No laboratories reported detection of any quantity of HIV-1 p24 antigen in samples obtained from donors who were not infected with HIV-1; therefore, quantitation data is not shown for Donors 3 and 4.

Use of Quality Control Testing Material

Information was collected on the use of quality control (QC) samples in addition to the controls contained in the test kits. Depending on the manufactured test kit used, positive and negative test controls, test standards, and/or test calibrators are internal kit control samples used to validate a test run and to determine percent neutralization or quantitate HIV-1 p24 antigen. However, these internal kit control samples may not be sufficient to validate the analytic testing process which may include testing problems related to pipetting, inadequate incubation conditions, inadequate washing, or variability in kit lot sensitivity.

Of the 170 laboratories that reported Qualitative test results, 84 (49.4%) indicated they used QC samples other than those contained in the test kit. Among these 84 laboratories, 58 (69%) indicated they obtained QC material only from a commercial source, 21 (25%) only used QC material from an in house source, 4 (4.8%) used both commercial and inhouse QC samples, and 1 (1.2%) laboratory did not provide a source for their QC samples. Although various combinations of QC materials were used, 39 (46.4%) laboratories indicated they used both a positive and negative p24 antigen control and 30 (35.7%) indicated using only a p24 antigen-positive control. Of the 84 laboratories using QC material in addition to test controls contained in their test kit, 73 (87%) used their QC material with each plate or set of plates in a run.

Only 20 (30%) of the 69 laboratories reporting neutralization test results indicated using external QC samples and the majority of these used commercially obtained p24 antigen-positive samples with each plate.

Of the 24 laboratories reporting p24 antigen quantitative test results, 15 (62.5%) indicated using external QC samples. More than 80% of these laboratories obtained their QC samples from a commercial source and used either a positive or both a positive and negative QC sample on each plate or in each set of plates in a run.

Conclusion

The results of this first performance evaluation shipment for HIV-1 p24 antigen determinations showed that most laboratories correctly detected HIV-1 p24 antigen in those samples from donors infected with HIV-1. Only a few laboratories did not detect HIV-1 p24 antigen. With one exception, no laboratories detected HIV-1 p24 antigen in the samples from donors not infected with HIV-1. For the samples from donors infected with HIV-1, the overall analytic sensitivity of the qualitative test for the results reported was 98.8%. For the samples from donors not infected with HIV-1, the overall analytic specificity was 99.7%. The overall analytic sensitivity of the neutralization test was 98.5% and the analytic specificity was 100%.