

**Analysis of the June 29, 1998 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 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 29, 1998. Testing results were reported by 166 (91.2%) of the 182 laboratories that received sample panels. Four laboratories returned results more than 2 weeks after the cut-off date and their results are not included in this aggregate report.

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 three p24 antigen test kits, two of which are 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 the CDC test results and interpretations obtained with HIV-1 p24 antigen qualitative 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), 432 (91.3%) of the Qualitative test results indicated the presence of HIV-1 p24 antigen, while 41 (8.7%) of the results did not indicate detection of HIV-1 p24 antigen in these HIV-1 infected donors. For the samples from the two donors not infected with HIV-1 (Donors 3 and 4), laboratories reported 314 (99.7%) results not detecting HIV-1 p24 antigen, and only 1 (0.3%) result detecting HIV-1 p24 antigen.

Neutralization test. For samples from donors that were infected with HIV-1 (Donor 1 and Donor 2), 216 (97.3%) of 222 Neutralization test results confirmed the presence of HIV-1 p24 antigen, while 5 (2.3%) results were reported as indeterminate and one laboratory reported a negative neutralization test result. It is not clear why some laboratories attempted to perform p24 antigen neutralization tests on samples that were negative for p24 antigen in the p24 Qualitative tests. Some laboratories did not perform neutralization tests on negative donor samples, but reported negative neutralization test interpretations for these samples. Data is not included from the few laboratories that did attempt to calculate percent neutralization for p24 antigen-negative samples (Donors 3 and 4).

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. In this survey, 79 (48.8%) laboratories reported only Qualitative test results while an additional 76 (47%) reported both Qualitative and Neutralization test results.

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 (Abbott and Coulter) were used by more than 90% of the laboratories reporting Qualitative results.

HIV-1 p24 Antigen Qualitative Test Results by Kit Manufacturer

Among the 315 interpretations reported for the p24 antigen-negative samples (Donors 3 and 4) there was one false-reactive interpretation reported for Donor 3 by a laboratory using the Organon Teknika test kit (Figure 5).

Of the 473 interpretations reported for the p24 antigen-positive samples (Donors 1 and 2) there were 41 nonreactive interpretations reported by a total of 37 laboratories. Thirty-eight (92.7%) of the 41 false-negative interpretations were reported for Donor 2 while only 3 (7.3%) were reported for Donor 1. Thirty-four (82.9%) of the 41 nonreactive interpretations were reported by 30 laboratories who indicated they used the Abbott HIVAG-1 Monoclonal p24 antigen test kit. Based on our knowledge of kit lot number assignment, provided by Abbott Diagnostics, Inc., 4 of these 34 false-negative results were reported by 4 laboratories who were actually using the Abbott HIVAG-1 (polyclonal) test kit and incorrectly coded their report. In addition, two of the three false-negative results reported by laboratories correctly indicating "Other" test kit were reported by laboratories using the Abbott HIVAG-1 (polyclonal) test kit. Among the 26 laboratories truly using the HIVAG-1 Monoclonal kits, false-negative results were reported using 11 different kit lots; however, only two unique negative control lots were included in all these 11 master kit lots. Since the mean absorbance of the negative control is used to determine the cut-off value for the test, it is possible that these two negative control lots may have impacted testing results reported by these 26 laboratories.

HIV-1 p24 Antigen Neutralization Test Results by Manufacturer

It is unclear why laboratories would attempt to report neutralization test results on samples that were nonreactive in p24 antigen Qualitative assays. In Figure 6, data is not included from laboratories that did not provide absorbance values and/or were not able to calculate percent neutralization values for the p24 antigen-negative samples (Donors 3 and 4) but reported negative interpretations for these samples.

Of the 222 Neutralization test interpretations reported for the p24 antigen-positive samples (Donors 1 and 2) there were 5 indeterminate and one negative interpretation reported. The false-negative and all the indeterminate interpretations were reported for Donor 2 by laboratories indicating they used the Abbott HIVAG-1 Monoclonal Blocking Antibody kit.

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.

Regardless of which test kit was used, laboratories consistently confirmed the presence HIV-1 p24 antigen in the samples obtained from Donor 1. The median percent neutralization 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. With the exception of 6 reports from laboratories using the Abbott Blocking Antibody reagents, p24 antigen confirmation was correctly reported for samples from HIV-1 infected Donor 2.

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.

One laboratory, providing only Quantitative test results, reported 57.0 pg/ml and 52 pg/ml, respectively, for their panel samples from both HIV-1 non-infected donors (Donors 3 and 4). Another laboratory

reported a concentration of 6.5 pg/ml p24 antigen in their sample from Donor 4, which they had reported as negative in the Qualitative screening test.

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.

Among the 159 reports of Qualitative test results, there were 156 containing information regarding the laboratory's use of QC samples. Of these 156 reports, 94 (60.3%) indicated the use of QC samples in addition to kit control samples. Among these 94 reports, the majority indicated that both positive and negative p24 QC samples were used with each plate or set of plates in a run.

Of the 80 reports from laboratories providing Neutralization test results, 78 provided information on the use of external QC materials. Only 20 (25.6%) indicated that external QC samples were used and the majority of these indicated the use of commercially obtained p24 antigen-positive samples with each plate.

Of the 16 laboratories reporting p24 antigen Quantitative test results, only 6 (37.5%) indicated using external QC samples. The majority obtained a p24 antigen-positive QC sample from a commercial source and used it in each plate or in each set or run of plates.

Conclusion

The results of this performance evaluation shipment for HIV-1 p24 antigen determinations showed that most laboratories correctly detected HIV-1 p24 antigen in those samples from HIV-1 infected donors. Most of the false-negative results were reported by laboratories using specific lots of reagents from one manufacturer. With one exception, no laboratories detected HIV-1 p24 antigen in the samples from donors not infected with HIV-1. For the p24 antigen-positive samples from donors infected with HIV-1, the overall analytic sensitivity of the Qualitative test for the results reported was 91.3%. For the samples from donors not infected with HIV-1, the overall analytic specificity was 99.7%.

For these same samples, the overall analytic sensitivity of the Neutralization test was 97.3% and the analytic specificity was 100%.