

**Analysis of the September 18, 2001 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 Centers for Disease Control and Prevention (CDC) by laboratories participating in the Model Performance Evaluation Program (MPEP) after they performed p24 antigen determinations on HIV-1 performance evaluation samples shipped to them September 18, 2001. Testing results were reported by 138 (88.5%) of the 156 laboratories that received sample panels.

Samples used in the MPEP HIV-1 p24 antigen performance evaluation survey are plasma obtained from single donors who are HIV-1 infected or uninfected and are not diluted or pooled with plasma from other individuals. In order to obtain the volumes of plasma required for this performance evaluation survey, plasma units obtained sequentially from an individual donor are combined to obtain the final product. Before shipment, the CDC tested the final plasma product from each donor with two p24 antigen test kits, which are approved by the Food and Drug Administration (FDA).

Table 1 lists the panel and vial designations, the CDC donor numbers, CDC test results, donor HIV status, and a section where laboratorians can insert their test results to compare with the CDC test results.

Table 2 lists the CDC panels for this shipment, the labeled vials contained in each panel, the CDC donor numbers, the CDC test results obtained with each test kit manufacturer, and the CDC interpretation of the results based on the manufacturers' criteria. HIV-1 p24 antigen was detected in the plasma from both of the HIV-1 infected donors (Donors 2 and 3) by each of the test kits used, and the CDC interpretation for these donors was positive for p24 antigen. The plasma from the HIV-1 uninfected donors (Donors 4 and 5) had no HIV-1 p24 antigen detected, as defined by the test kit manufacturers' criteria, and 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 402 results reported for samples from donors that were infected with HIV-1 (Donors 2 and 3), two (0.5%) results did not indicate detection of HIV-1 p24 antigen in these HIV-1 infected donors. For the 268 results reported for samples from the donors not infected with HIV-1 (Donors 4 and 5), one (0.4%) result indicated the detection of HIV-1 p24 antigen in an HIV-1 uninfected donor.

Neutralization Test. The neutralization test is the confirmatory test for the qualitative assay. Although 400 reactive results were reported for the HIV-1 infected donor samples in the qualitative assay, only 170 (42.5%) were confirmed with a supplemental p24 antigen neutralization test. Of the 400, the presence of p24 antigen was confirmed in 168 sample testing results, and two results were reported as indeterminate. Two laboratories reported four negative p24 antigen neutralization results on samples that were negative for p24 antigen in the qualitative tests. One laboratory reported a negative p24 antigen neutralization test on an HIV-1 uninfected donor sample that it had reported reactive for p24 antigen in the qualitative test. One laboratory reported a positive p24 neutralization test on an HIV-1 uninfected donor.

Data is excluded for laboratories that did not provide absorbance values and/or percent neutralization data for negative donor samples, but still reported negative neutralization test interpretations for these samples.

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 is shown in Figure 3. Of the 138 laboratories reporting results in this survey, 79 (57.3%) reported only qualitative test results. Fifty (36.2%) reported both qualitative and neutralization test results. The remaining nine laboratories (6.5%) reported results using various combinations of tests.

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 the FDA (Abbott and Coulter) were used by 96.2% of the laboratories reporting qualitative results.

HIV-1 p24 Antigen Qualitative Test Results by Kit Manufacturer

Among the 268 interpretations reported for the p24 antigen-negative samples (Donors 4 and 5) one laboratory reported one false-reactive interpretation using the Coulter HIV-1 p24 Antigen Assay (Figure 5).

Of the 402 interpretations reported for the p24 antigen-positive samples (Donors 2 and 3), two laboratories reported two non-reactive interpretations using the Abbott HIVAG-1 Monoclonal kit.

HIV-1 p24 Antigen Neutralization Test Results by Manufacturer

Two laboratories reported a total of four neutralization results on samples that were reported non-reactive in p24 antigen qualitative assays (Figure 6). It is unclear why laboratories would attempt to report neutralization test results on these samples. Additionally, one laboratory reported a negative neutralization result for a nonreactive sample which it had reported as reactive for the qualitative test, and another laboratory reported a positive p24 neutralization test on an HIV-1 uninfected donor using the Coulter HIV-1 p24 Neutralization Kit. In Figure 6, data are not included from laboratories that did not provide absorbance values and/or percent neutralization values for the p24 antigen-negative panel samples (Donors 4 and 5) but reported negative neutralization test interpretations for these samples.

Of the 170 neutralization test interpretations reported for the p24 antigen-positive samples (Donors 2 and 3), two were reported indeterminate by two laboratories using the Abbott HIVAG-1 Monoclonal kit.

Aggregate Percent Neutralization Results Reported by Donor

Aggregate percent neutralization results for HIV-1 infected Donor 2 and Donor 3 are shown in Table 3. The columns under each donor sample list, by test kit manufacturer, the test interpretation, number of laboratory results for each interpretation, and the minimum, median, and maximum percent neutralization values determined from the data provided by reporting laboratories for each donor sample. Information listed in this table also includes the identity of panel vials containing plasma from these donors. For this shipment, Donor 2 provided duplicate samples for each panel allowing participant laboratories the opportunity to review their intra-shipment reproducibility for that donor sample.

A comparison of the median percent neutralization values determined from the results reported for the duplicated Donor 2 samples in each panel reflects a strong degree of reproducibility in determining percent neutralization by each manufactured kit.

Aggregate p24 Antigen Quantitation Results Reported by Donor

Aggregate p24 antigen quantitation data for HIV-1 infected Donor 2 and Donor 3, by test kit, are shown in Table 4. 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. Information listed in this table also includes the identity of panel vials containing the plasma from these donors. For this shipment, Donor 2 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.

The range in the quantity of p24 antigen detected, as determined from the results reported for these donors, varied depending on which manufactured reagents were used. The median p24 antigen concentration for duplicate Donor 2 samples in each panel, as determined from participating laboratory results, reflects good reproducibility in the quantitation of p24 antigen using reagents from any of the individual manufacturers.

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 to 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 130 reports of qualitative test results, 103 (79.2%) indicated that external QC was used in addition to manufacturers' test kit controls. Of these 103 reports, 91 (88.4%) indicated the use of commercially manufactured QC samples in addition to kit control samples. Additionally, of these 103 reports, 68 (66.0%) indicated that both positive and negative p24 QC samples were used, and 54 (52.4%) reports indicated the use of QC material with each set/run of plates.

Of the 55 reports from laboratories providing neutralization test results, only 17 (30.9%) indicated that external QC samples were used. The majority of these indicated the use of commercially obtained, multiple-reactivity p24 antigen QC material with each plate.

Among the eight reports received from laboratories performing p24 antigen quantitative tests, four (50.0%) indicated using external QC samples. The majority described the use of p24 antigen-positive samples with each plate or set/run of plates.

Conclusion

The results of this performance evaluation survey for HIV-1 p24 antigen determinations showed that most laboratories correctly detected HIV-1 p24 antigen in those samples from HIV-1 infected donors. The overall analytic specificity for the qualitative assay results reported for this survey was 99.6%. From the results reported for the p24 antigen-positive samples of donors infected with HIV-1, the overall analytic sensitivity of the qualitative test for this survey was 99.5%.

If indeterminate results are considered incorrect for the HIV-1 p24 antigen positive donors, the analytic sensitivity determined from the results reported for the neutralization test was 98.8%.

As calculated from the data obtained in this performance survey, the overall analytic performance of laboratories testing these samples for the presence of HIV-1 p24 antigen was 99.6% for the qualitative screening assay and 98.3% for the supplemental neutralization test.

Please note that the next MPEP HIV-1 p24 antigen performance survey panels will be mailed to participating laboratories on March 5, 2002.