

**Analysis of the March 1, 1999 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 March 1, 1999. Testing results were reported by 170 (91.9%) of the 185 laboratories that received sample panels. One laboratory returned results more than 2 weeks after the cut-off date and one laboratory reported results for the p24 antigen panel mailed in June, 1998, rather than the current panel. The results from these two laboratories 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 uninfected and are not 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 (Donors 1 and 2) by each of the test kits used; the CDC interpretation for these donors was positive for p24 antigen. The plasma from the HIV-1 uninfected donor (Donor 3) 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), 416 (85.1%) of the qualitative test results indicated the presence of HIV-1 p24 antigen, while 73 (14.9%) of the results did not indicate detection of HIV-1 p24 antigen in these HIV-1 infected donors. For the samples from the donor not infected with HIV-1 (Donor 3), laboratories reported 327 (99.7%) results not detecting HIV-1 p24 antigen, and only 1 (0.3%) result detecting HIV-1 p24 antigen.

Neutralization test. Of the 416 samples in which p24 antigen was detected by the qualitative test, only 194 of these were tested with a p24 neutralization test. The presence of p24 antigen was confirmed in 189 (97.4%) of these 194 samples; 4 samples (2.1%) were reported indeterminate, and 1 sample was reported as negative. It is not clear why laboratories would attempt to perform p24 antigen neutralization tests on samples that were negative for p24 antigen in the qualitative tests. Data were not included from the two laboratories that did perform neutralization tests and attempted to calculate percent neutralization for the p24 antigen-negative samples in their panel. Also excluded is data from some 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 168 laboratories reporting results in this survey, the majority (52.4%) reported only qualitative 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 (Figure 4).

HIV-1 p24 Antigen Qualitative Test Results by Kit Manufacturer

Among the 328 interpretations reported for the p24 antigen-negative samples (Donor 3) there was only 1 false-reactive interpretation reported by a laboratory using the Abbott HIVAG-1 Monoclonal test kit (Figure 5).

Of the 489 interpretations reported for the p24 antigen-positive samples (Donors 1 and 2) there were 73 non-reactive interpretations reported by a total of 47 laboratories. Forty-six (63%) of the 73 false-negative results were reported by 28 laboratories using 12 different master lots of the Coulter p24 antigen kit and 24 (32.9%) of the 73 non-reactive interpretations were reported by 17 laboratories using 12 different master lots of the Abbott HIVAG-1 Monoclonal p24 antigen test kit. Seventy-one (97.3%) of the 73 false-negative interpretations were reported for Donor 2 samples. This same donor (Donor 2) provided samples (A3, B4, C5, and D1) for the June 1998 survey, and accounted for 38 (92.7%) of the 41 false-negative qualitative assay interpretations reported in that survey. In the present survey, there were duplicate samples from Donor 2 in each panel and 11 laboratories using the Abbott kit as well as 11 laboratories using the Coulter kit reported one of the Donor 2 samples positive and the other negative in the same run.

The data provided by each laboratory for the cut-off value determined for the initial qualitative assay and the absorbance value reported for the Donor 2 samples in each panel were analyzed in an attempt to determine the reason for the high percentage of false-negative qualitative test interpretations for the HIV-infected donor samples. The following table shows, for each manufacturer, the mean cut-off value and mean sample absorbance reported in the initial qualitative test that were associated with the reported negative and positive interpretations:

Data Reported by Participating Laboratories for the Initial p24 Qualitative Assay for Donor 2

Manufacturer	Initial Test Interpretation	Number of Samples	Cut-off Mean	Cut-off Range	Mean Sample Absorbance	Sample Abs Range
Abbott	Non-Reactive	23	0.079	0.060 - 0.115	0.072	0.032 - 0.096
Abbott	Reactive	197	0.074	0.059 - 0.088	0.094	0.062 - 0.230
Coulter	Non-Reactive	45	0.085	0.061 - 0.117	0.076	0.028 - 0.140
Coulter	Reactive	41	0.090	0.061 - 0.131	0.117	0.065 - 0.243

It is evident that the mean sample absorbance and range of absorbance (Abs) values indicated were significantly lower for the samples with false-negative interpretations. Based on the mean values determined for the assay cut-off and sample absorbance in the above table, the mean sample to cut-off (S/C) ratio for the Donor 2 samples reported non-reactive by laboratories using the Abbott assay is 0.911 while the mean S/C ratio for the Donor 2 samples reported reactive by this assay is 1.27. Similarly, the mean sample to cut-off (S/C) ratio for the Donor 2 samples reported non-reactive by laboratories using the Coulter assay is 0.894 while the mean S/C ratio for the Donor 2 samples interpreted as reactive in the Coulter assay is 1.30.

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 non-reactive in p24 antigen qualitative assays. In Figure 6, data are not included from laboratories that did not provide absorbance values and/or percent neutralization values for the duplicated p24 antigen-negative panel samples (Donor 3) but reported negative interpretations for these samples. The laboratory that reported the false-positive qualitative test result for one of the duplicate Donor 3 samples in their panel also performed an Abbott HIVAG-1 Monoclonal Blocking Antibody assay on this sample and reported 566.7% neutralization. Another laboratory did not report qualitative test results; however, using the Coulter p24 antigen neutralization assay, they reported 100% neutralization for one of the duplicate Donor 3 samples in their panel.

Of the 194 neutralization test interpretations reported for the p24 antigen-positive samples (Donors 1 and 2) there were 1 negative and 4 indeterminate interpretations 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 assay. It is unclear why the false-negative result was reported since the laboratory indicated 90.6% neutralization had been achieved for this sample in the blocking antibody assay. One laboratory reported indeterminate neutralization test results for each of the duplicated Donor 2 samples in their panel, but indicated 18.3% and 72.9% neutralization, respectively, for these samples. The two remaining indeterminate interpretations were reported for the initial Donor 2 panel sample by two laboratories indicating 48.9% and 57.6% neutralization, respectively. The duplicated Donor 2 sample in the panels of these two laboratories were reported as positive with 61.7% and 86.1% neutralization, respectively.

Aggregate Percent Neutralization Results Reported by Donor

Aggregate percent neutralization results for HIV-1 infected Donor 1 and Donor 2 (duplicate samples), 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 2 provided duplicate samples for each panel allowing 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 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.

Regardless of which neutralization assay was used, laboratories consistently confirmed the presence of HIV-1 p24 antigen in the samples provided by Donor 1. 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. With the exception of five 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 data for HIV-1 infected Donor 1 and Donor 2 (duplicate samples), 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 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.

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.

The range in the 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 concentration for duplicate Donor 2 samples in each panel, as determined from participating laboratory results, reflects a good reproducibility in the quantitation of p24 antigen using reagents from any of the individual manufacturers.

One laboratory, using Abbott reagents, reported 26.7 pg/ml and 6.7 pg/ml, respectively, for their duplicate panel samples from HIV-1 uninfected Donor 3. Another laboratory, using an immune complex dissociation procedure and Coulter reagents, reported a concentration of 521 pg/ml p24 antigen in one of their duplicate Donor 3 samples, 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 165 reports of qualitative test results, 161 contained information regarding the use of QC samples. Of these 161 reports, 106 (65.8%) indicated the use of QC samples in addition to kit control samples. Among these 106 reports, the majority indicated that both positive and negative p24 QC samples obtained from a commercial source were used in each set or run.

Of the 77 reports from laboratories providing neutralization test results, 76 provided information on the use of external QC materials. Only 25 (32.9%) indicated that external QC samples were used and the majority of these indicated the use of a commercially obtained p24 antigen-positive sample with each plate.

Of the 15 laboratories reporting p24 antigen quantitative test results, 7 (46.7%) 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/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. With one exception, no laboratories detected HIV-1 p24 antigen in the samples from donors not infected with HIV-1 resulting in an overall analytic specificity of 99.7% for the qualitative assay results reported for this survey. From the results reported for the p24 antigen-positive samples from donors infected with HIV-1, the overall analytic sensitivity of the qualitative test for this survey was calculated to be 85.1%. Most of the false-negative qualitative assay results were reported for the samples provided by a single donor (Donor 2). The donor samples for this survey were identical to the donor samples used in the June, 1998 survey and, for comparison, the overall analytic sensitivity determined for the qualitative assay in the June, 1998 survey was 91.3%. In the June, 1998 survey the false-negative error rate determined from results reported by laboratories using the Coulter qualitative p24 antigen assay was 7.3% while in the present survey the false-negative error rate determined from results reported by laboratories using this same Coulter assay is 63%. In contrast, for the June, 1998 survey the false-negative error rate determined from results reported by laboratories using the Abbott HIVAG-1 Monoclonal p24 antigen assay was 73.2% while in the present survey the false-negative error rate determined from results reported by laboratories using this Abbott assay is 32.9%.

The overall analytic sensitivity determined from the results reported for the neutralization test was 97.4% and only two laboratories incorrectly reported the presence of p24 antigen in samples from an HIV-uninfected donor.