

**Analysis of the March 2, 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 March 2, 1998. Testing results were reported by 168 (91.8%) of the 183 laboratories that received sample panels; however, 2 laboratories returned their results too late to be included in this analysis.

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 neither diluted nor 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.

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 three HIV-1 p24 antigen test kits can be found in Table 1. In preshipment testing by CDC, 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 the donor 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 this donor sample 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 the donor not infected with HIV-1 (Negative). In each panel, duplicated samples from an HIV-1 infected (Donor 2) and an HIV-1 uninfected (Donor 3) individual provide participant laboratories the opportunity to evaluate their intra-shipment test reproducibility.

**Qualitative Test.** Of the 324 results reported for the samples provided by the HIV-1 uninfected donor (Donor 3), laboratories reported only 1 (0.3%) result indicating that HIV-1 p24 antigen was detected.

For the samples from donors that were infected with HIV-1 (Donor 1 and Donor 2), 456 (94.4%) of the Qualitative results indicated the presence of HIV-1 p24 antigen, while 27 (5.6%) of the results did not indicate detection of HIV-1 p24 antigen.

**Neutralization Test.** Four indeterminate p24 antigen Neutralization test interpretations were reported for samples from the HIV-1 uninfected donor (Donor 3); however, no percent neutralization value was reported and these interpretations were not included in this report. For the samples from donors infected with HIV-1 (Donors 1 and 2), 233 (98.3%) of 237 Neutralization test results confirmed the presence of HIV-1 p24 antigen, while 3 (1.3%) results were reported as indeterminate since the percent neutralization calculated was less than the minimum percent neutralization acceptable for the test kit used. One laboratory reported neutralization of 105.3% for a sample from Donor 2, but incorrectly reported a negative neutralization test

interpretation.

### **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 detect and confirm the presence of p24 antigen are shown in Figure 3. Of the 166 laboratories reporting p24 antigen testing results, 47.6% reported only Qualitative test results while 39.7% performed both Qualitative and Neutralization 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 Food and Drug Administration (i.e., Abbott and Coulter) were used by 91.4% of the laboratories reporting Qualitative results.

### **HIV-1 p24 Antigen Qualitative Test Results by Manufacturer**

Among the 324 Qualitative test interpretations reported for the p24 antigen-negative samples (Donor 3) there was one false-reactive interpretation reported by a laboratory using the Abbott HIVAG-1 Monoclonal test kit (Figure 5). This laboratory reported an initial nonreactive Qualitative test on this sample; however, the repeat test and final interpretation were reported as reactive for p24 antigen.

Of the 483 interpretations reported for the p24 antigen-positive samples (Donors 1 and 2) there were 27 nonreactive interpretations. False-negative interpretations were all reported for Donor 2 by laboratories using kits manufactured by Abbott, 20 (74%) of 27; Coulter, 5 (18.5%) of 27; and DuPont, 2 (7.4%) of 27 interpretations. Of the 20 false-negative interpretations reported by laboratories using kits manufactured by Abbott, 14 (70%) of these were reported by seven laboratories using the Abbott HIVAG-1 Monoclonal test kit, lot # 34332M301. **For p24 9806 report indicate this lot number, in spite of coding provided by laboratories, was a POLYCLONAL kit per communication with Abbott “HIVAG Specialty Division”**

### **HIV-1 p24 Antigen Neutralization Test Results by Manufacturer**

There were four indeterminate interpretations among the Neutralization test results reported for the p24 antigen-negative samples (Donor 3) by two laboratories using the Coulter p24 antigen neutralization procedure. However, these laboratories did not provide percent neutralization values and these data are not included in Figure 1 or Figure 6. It is unclear why laboratories performed neutralization tests on samples that were nonreactive in the Qualitative (screening) assay.

Of the 237 Neutralization test interpretations reported for the p24 antigen-positive samples (Donors 1 and 2) there were 3 indeterminate and one false-negative interpretation reported by laboratories using the Abbott HIVAG-1 Monoclonal Blocking Antibody kit. One indeterminate interpretation was reported for Donor 1 while a negative interpretation and two indeterminate interpretations were reported for Donor 2. Interestingly, the laboratory reporting the false-negative interpretation for one of the Donor 2 samples in their panel reported neutralization of 105.26% for the sample. This same laboratory reported neutralization of 104.72% and a positive interpretation for the duplicate Donor 2 sample in their panel. The laboratory reporting the indeterminate interpretation for their Donor 1 sample incorrectly calculated and reported 44.8% neutralization. The correct calculation would have been greater than 65% neutralization and, therefore, interpreted as positive.

### **Aggregate Percent Neutralization Results Reported by Donor**

Aggregate percent neutralization results for samples from HIV-1 infected individuals (Donors 1 and 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. 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.

The median percent neutralization values determined from the results reported for these donors is similar among all the manufacturers. The median percent neutralization values determined from the results reported for the duplicate samples of Donor 2 in each panel reflect a high degree of reproducibility in determining percent neutralization with each manufactured kit.

### **Aggregate p24 Antigen Quantitation Results Reported by Donor**

Aggregate p24 antigen quantitation results 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. 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 manufacturers' reagents were used. The median p24 antigen concentration for duplicate Donor 2 samples in each panel, as calculated from participating laboratory results, generally reflect good reproducibility in quantitation of p24 antigen using reagents from any of the individual manufacturers.

One laboratory, using a proprietary in-house p24 quantitation procedure, reported detection of only 0.1 pg/ml HIV-1 p24 antigen in each of the duplicate Donor 2 samples in their panel. However, there may have been an error in recording results since this same laboratory reported p24 antigen concentration of 38.7 and 32.9 pg/ml

for the p24 antigen-negative samples (Donor 3) duplicated in their panel.

It is unclear why 10 laboratories performed p24 antigen quantitation tests on samples that were nonreactive in the p24 Qualitative (screening) test. None of these laboratories reported significant amounts of p24 antigen in the Donor 3 samples in their panels. One exception was a laboratory that did not perform a p24 Qualitative test and reported 15 and 490 pg/ml p24 antigen, respectively, for the duplicate Donor 3 samples in their panel.

### **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 controls, test standards, and/or test calibrators are provided as internal kit controls. Such internal kit controls are used to validate a test run, to determine percent neutralization, or to quantitate HIV-1 p24 antigen. However, these internal kit controls 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 160 laboratories that reported Qualitative test results, 92 (57.5%) indicated they used QC samples other than those contained in the test kit. Among these 92 laboratories, 64 (69.6%) indicated they obtained QC material only from a commercial source, 20 (21.7%) only used QC material from an in-house source, and 8 (8.7%) used both commercial and in-house QC samples. Although various combinations of QC materials were used, 50 (54.3%) laboratories indicated they used both a positive and negative p24 antigen control and 27 (29.3%) indicated using only a p24 antigen-positive control. Of the 92 laboratories using QC material in addition to test controls contained in their test kit, 83 (90.2%) used their QC material with each plate or set of plates in a run.

Only 23 (28.4%) of the 81 laboratories reporting neutralization test results indicated using external QC samples, and 20 (86.9%) of these used commercially obtained p24 antigen-positive or both antigen-positive and p24 antigen-negative samples with each plate or set of plates in a run.

Of the 20 laboratories reporting p24 antigen quantitative test results, only 9 (45%) indicated using external QC samples. Six of these 9 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 second MPEP performance evaluation survey 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. Of the 160 laboratories reporting p24 Qualitative results, 14 laboratories (8.75%) reported false-negative results for one or more of the p24 antigen-positive samples in their panel. With one exception, no laboratories detected HIV-1 p24 antigen in the samples from the donor not infected with HIV-1.

For the results reported for samples from donors infected with HIV-1, the overall analytic sensitivity of the qualitative test was 94.4%. For the results reported for samples from the donor not infected with HIV-1, the

overall analytic specificity of the qualitative test was 99.7%. The overall analytic sensitivity of the neutralization test was 98.3% and the analytic specificity was 100%.

A single donor provided the Donor 1 samples used in this survey as well as the Donor 2 samples for the first p24 antigen survey mailed in June 1997. By comparing the report of the June 1997 survey with this current survey, laboratories can determine the intershipment reproducibility of qualitative, neutralization, and quantitative test results for samples from this donor.