Analysis of the September 13, 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 September 13, 1999. Testing results were reported by 169 (88.9%) of the 190 laboratories that received sample panels. Two laboratories returned results more than 2 weeks after the cut-off date and two laboratories returned result forms with no testing results included. The data from these four laboratories are not included in this aggregate report.

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 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 2. HIV-1 p24 antigen was detected in the plasma from both of the HIV-1 infected donors (Donors 3 and 4) 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 1 and 2) had no HIV-1 p24 antigen detected, as defined by the test kit manufacturer's 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 498 results reported for samples from donors that were infected with HIV-1 (Donor 3 and Donor 4), 114 (22.9%) did not indicate detection of HIV-1 p24 antigen in these HIV-1 infected donors. For the 336 results reported for samples from the donors not infected with HIV-1 (Donor 1 and Donor 2), only 2 (0.6%) results indicated the detection of HIV-1 p24 antigen in these HIV-1 uninfected donors.

Neutralization Test. Of the 384 HIV-infected donor samples in which p24 antigen was detected by qualitative tests, only 188 of these were tested with a supplemental p24 neutralization test. The presence of p24 antigen was confirmed in 182 (96.8%) of these 188 samples; 5 samples

(2.7%) were reported negative, and 1 sample was reported as indeterminate. 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. The laboratories that did perform neutralization tests for the p24 antigen-negative samples in their panel reported four negative, one indeterminate, and one positive neutralization test result.

Data is excluded from 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 169 laboratories reporting results in this survey, 92 (54.4%) 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 the 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 336 interpretations reported for the p24 antigen-negative samples (Donor 1 and Donor 2) there were only two false-reactive interpretations reported, both for samples provided by Donor 1, by laboratories using the Coulter p24 Antigen Assay after performing an immune complex dissociation procedure on these HIV-1 uninfected donor samples (Figure 5).

Of the 498 interpretations reported for the p24 antigen-positive samples (Donors 3 and 4) there were 114 non-reactive interpretations reported by a total of 110 laboratories. All of the 114 non-reactive interpretations were reported by laboratories using 28 different master lots of the Abbott HIVAG-1 Monoclonal p24 antigen test kit. Of the 114 false-negative interpretations, 110 (96.5%) were reported for Donor 3 samples. Five laboratories reported their Donor 3 sample as initially reactive but non-reactive on duplicate repeat testing. There were only 4 (3.5%) false-negative interpretations reported by two laboratories for Donor 4 samples. There were duplicate

samples from Donor 4 in each panel and one laboratory reported both Donor 4 samples initially reactive but non-reactive on the repeat test using the same kit lot. The other laboratory reported one of their Donor 4 samples initially reactive and repeat test non-reactive, but found the duplicate Donor 4 sample non-reactive in both the initial and repeat test, using the same kit lot for both initial and repeat testing.

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 p24 antigen-negative panel samples (Donor 1 and Donor 2) but reported negative neutralization test interpretations for these samples. Four laboratories reported absorbance values and calculated percent neutralization for six HIV-uninfected donor samples. Two laboratories using the Abbott HIVAG-1 Monoclonal Blocking Antibody kit reported negative neutralization results for both the Donor 1 and Donor 2 samples in their panels accompanied with percent neutralization values of 0.00%, 72%, 80% and 128.5%, respectively. It is unclear why a laboratory would report percent neutralization greater than 50% and a negative test result. These same samples had been tested by these two laboratories in the Abbott qualitative p24 antigen assay and found to be negative.

Of the 188 neutralization test interpretations reported for the p24 antigen-positive samples (Donors 3 and 4) there were one indeterminate and five negative interpretations reported by a total of five laboratories. Two laboratories reported indeterminate and negative results, respectively, for one of the Donor 4 samples in their panel and positive results for the duplicate Donor 4 sample in the panel. One laboratory reported negative neutralization test results for both of the Donor 4 samples in their panel but also indicated percent neutralization calculated at 94.4% and 125%, respectively, for these samples.

Aggregate Percent Neutralization Results Reported by Donor

Aggregate percent neutralization results for HIV-1 infected Donor 3 and Donor 4 (duplicate samples), by test kit, are shown in Table 3. Information listed in these tables also includes the identity of panel vials containing plasma from these donors. For this shipment, Donor 4 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 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.

A comparison of the median percent neutralization values determined from the results reported for the duplicated Donor 4 samples in each panel reflects a strong degree of reproducibility in determining percent neutralization by each manufactured kit. With the exception of six reports from laboratories using the Abbott Blocking Antibody reagents, p24 antigen confirmation was correctly reported for samples from HIV-1 infected Donor 3 and 4.

Aggregate p24 Antigen Quantitation Results Reported by Donor

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

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 4 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 2.9 pg/ml for their panel sample from HIV-1 uninfected Donor 3; however, this laboratory reported a negative Abbott HIVAG-1 qualitative test result for this same sample.

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, 114 (69.1%) contained information regarding the use of QC samples. Of these 114 reports, 95 (83.3%) indicated the use of commercially manufactured QC samples in addition to kit control samples. Of these 95 reports, 74% indicated that both positive and negative p24 QC samples were used in each test run.

Of the 75 reports from laboratories providing neutralization test results, only 23 (30.7%) indicated that external QC samples were used and the majority of these indicated the use of a commercially obtained p24 antigen-positive QC sample with each plate.

Among the 18 reports received from laboratories performing p24 antigen quantitative tests, there were 9 (50%) that indicated using external QC samples. The majority described the use of commercially obtained p24 antigen-positive samples that were used in each set or run of test plates.

Conclusion

With two exceptions, no laboratories detected HIV-1 p24 antigen in the samples from donors not infected with HIV-1 resulting in an overall analytic specificity of 99.4% 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 only 77.1%. Most of the false-negative qualitative assay results (110/114) were reported for the samples provided by a single donor (Donor 3) whose plasma contained low levels of p24 antigen. The donor samples for this survey were entirely different from the donor samples used in the March 1999 survey, where the overall analytic sensitivity determined for the qualitative assay was 85.1%. In the March 1999 survey, the false-negative error rate determined from results reported by laboratories using the Coulter assay was 35.7% while in the present survey the false-negative error rate determined from results reported by laboratories using this same Coulter assay is 0%. In contrast, for the March 1999 survey, the false-negative error rate determined from results reported by laboratories using the Abbott HIVAG-1 Monoclonal p24 antigen assay was 7.3% while in the present survey the false-negative error rate determined from results reported by laboratories using this Abbott assay is 34.2%.

The overall analytic sensitivity determined from the results reported for the neutralization test was 96.8% as a result of four laboratories reporting negative neutralization test results for samples from an HIV-1 infected donor.

As calculated from the data obtained in this performance survey, the overall analytic performance of laboratories testing these samples for the presence of HIV p24 antigen was 86.1% for the qualitative screening assay and 95.9% 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 6, 2000.