Analysis of the February 22, 2000 Performance Evaluation
HIV-1 RNA Determinations (Viral Load) 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 ribonucleic acid (RNA) determinations on human immunodeficiency virus type 1 (HIV-1) performance evaluation samples shipped to them February 22, 2000. Testing results were reported by 192 (91%) of the 210 laboratories who were sent sample panels.

Please note the original performance survey date was February 15, 2000; however, panels sent on this date did not contain enough dry ice and, as a result, many panel samples had thawed upon reaching the participant laboratory. Therefore, panels were resent to participant laboratories on February 22, 2000. If your laboratory tested and reported results for the thawed samples in the panel sent to you on February 15, 2000, we would appreciate any feedback regarding how your results compared to results reported by other laboratories. Similarly, if your laboratory tested both the thawed samples sent to you on February 15, 2000 and the panel samples sent on February 22, 2000, we would appreciate any information about comparisons made between the testing results from the two panels.

Samples used in the MPEP HIV-1 RNA determinations performance evaluation survey are plasma obtained from individual donors (not pooled or diluted with plasma from other donors) who are HIV-1 infected or uninfected. Before shipment, the CDC tested each donor with at least three test kits which included one viral RNA test kit approved by the Food and Drug Administration (FDA), and two test kits not approved by the FDA and designated for research use only.

The second page following the report title page, 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.

The third page following the report title page, Table 2, lists the CDC panels for this shipment, the labeled vials contained in each panel, the CDC donor numbers, the CDC results obtained with each test kit manufacturer, and the CDC interpretation of the results based on the manufacturers' criteria. For all the HIV-1 infected donors, HIV-1 RNA was detected by all the test kits used and the CDC interpretation for these donors was positive for RNA. Conversely, the donors not infected with HIV-1 did not have HIV-1 RNA detected consistent with the criteria contained within the test kit manufacturer's insert. Based upon the lower limits of the test kit sensitivities, these donors were interpreted by CDC as negative for HIV-1 RNA.

Summary of Results

Figure 1 shows the cumulative frequency of test results reported by laboratories for donors who were HIV-1 infected and had detectable HIV-1 RNA, and for donors not infected with HIV-1 and in whose donor plasma HIV-1 RNA was not detectable. For the samples obtained from donors (Donor 1, Donor 2, and Donor 2 duplicate) infected with HIV-1, 597 (99.3%) of 601 results

reported by the participant laboratories indicated HIV-1 RNA was detected. There were four (0.7%) results which were false negatives, indicating RNA was not detected. Of the 403 results reported for the samples obtained from donors not infected with HIV-1 (Donor 3 and Donor 4), laboratories reported 396 (98.3%) results that indicated HIV-1 RNA was not detected, while seven (1.7%) results, false positives, indicated HIV-1 RNA was detected.

Types of Laboratories Performing HIV-1 RNA Determinations

The types of laboratories reporting results are shown in Figure 2. Among the 192 laboratories reporting results, each laboratory type is listed by decreasing frequency. Similar to previous performance surveys, Hospital laboratories (100, 52.1%) reported most of the testing results, followed by Independent (42, 21.9%), Health Department (32, 16.7%), Other (17, 8.9%), and Blood Bank (1, 0.5%) laboratories.

Types of Test Kits Used by Laboratories

The types of test kits used by laboratories performing viral RNA determinations are shown in Figure 3 and are listed by decreasing frequency. Please note that some laboratories used more than one test kit which is why the "N" number for this figure exceeds the number of laboratories reporting results. The Roche Amplicor HIV-1 Monitor™ test kit, approved by the FDA, was used most frequently, (141, 69.8 %), in reporting results. The frequency of other test kits used were Bayer (Chiron) Quantiplex™, (46, 22.8%), and Organon Teknika NucliSens NASBA™ , (10, 5.0%).

Aggregate Testing Results Reported by Donor

Aggregate participant laboratory testing results, for each donor sample, by test kit manufacturer, are shown in Table 3. Please note that in Table 3, the columns under each donor sample provide the number of laboratory results detecting HIV-1 RNA or not detecting HIV-1 RNA, followed by the minimum, median, and maximum result values listed for each test kit manufacturer. Although the lower limit sensitivities of the reported test kits generally ranged from 20 RNA copies/ml to 500 RNA copies/ml, the results are shown for each individual donor by test kit and listed according to the minimum, maximum, and median values that were calculated from the reported results regardless of the kit lower limit sensitivity. Information listed in the results section for each individual donor also includes the HIV-1 infection status of the donor and which panel vials contained the donor material. The first page of Table 3 shows the laboratory test results reported for CDC Donor 1, an HIV-1 infected donor. The second page shows the results reported for Donor 2, and the duplicate of Donor 2, also an HIV-1 infected donor. The third page shows the results reported for Donor 3 and Donor 4, both uninfected donors. For this performance survey shipment, only Donor 2 was duplicated in each panel, providing participant laboratories an opportunity to review their intra-shipment reproducibility for this donor sample.

Laboratories performed well in testing these performance evaluation samples and the reporting of four false negative and seven false positive results was a substantial decrease in the number of false negatives and false positives when compared with the last performance survey. It should be

noted that the samples contained in this survey panel were identical to the samples used in the previous performance survey which was sent to laboratories in August 16, 1999. All four of the false negative results were associated with donor 1. Among the seven false positive results reported, three false positive results were associated with donor 3 and four false positive results were associated with donor 4.

Of the laboratories that indicated using the Roche Amplicor HIV-1 Monitor™, most reports, 104 (74%) of 140, were results determined using a lower limit sensitivity of 400 copies/ml which is comparable to the number of results reported for the previous performance survey using this lower limit sensitivity. Among these same laboratories using the Roche Amplicor HIV-1 Monitor™, other lower limit sensitivities reported were 25 copies/ml (one report), 50 copies/ml (20 reports), 100 copies/ml (one report), 200 copies/ml (six reports), and nine reports that did not indicate lower limit sensitivity. In contrast, of the laboratories using the Bayer (Chiron) Quantiplex™, 45 (98%) of 46 used the version 3 with a lower limit sensitivity of 50 copies/ml. Only one laboratory using the Bayer (Chiron) Quantiplex™ reported a lower limit sensitivity of 272 copies/ml. Among laboratories using the Organon Teknika NucliSens HIV-1 QT (NASBA)™, six laboratories reported 400 copies/ml as their lower limit sensitivity, two laboratories reported 160 copies/ml, one laboratory reported 80 copies/ml, and one laboratory reported 40 copies/ml.

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, or test calibrators are internal kit control samples and are used to validate a test run, and to quantitate HIV-1 RNA copies/ml. These kit controls may not 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 192 laboratories that reported results for this performance survey, 187 (97 %) laboratories provided information on whether they use QC samples other than the controls contained in the test kit. Of these, 85 (45%) indicated they used QC samples other than those contained in the test kit. Among these, 46 (54%) laboratories indicated they obtained their QC material from an in-house source and 39 (46%) obtained their QC material from a commercial source.

Conclusion

The results of this performance evaluation shipment for HIV-1 RNA determinations showed a decrease in the number of false positive and false negative results when compared with the previous performance survey which contained identical samples. While there is continued variability of results within a kit manufacturer and between kit manufacturers across all performance surveys, a comparison of the results reported for the duplicate donor in this performance survey showed good reproducibility within the results reported for each kit manufacturer. For the samples from donors infected with HIV-1, the analytic sensitivity for the results reported was 99.3%. For the samples from donors not infected with HIV-1, the analytic specificity was 98.3%. The overall analytic performance for this performance survey was 98.9%.