Analysis of the February 12, 2002 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 12, 2002. Testing results were reported by 175 (86.2%) of the 203 laboratories who were sent sample 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 two viral RNA test kits approved by the Food and Drug Administration (FDA), and one test kit 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 1 duplicate, and Donor 3) infected with HIV-1, 534 (98.9%) of 540 results reported by the participant laboratories indicated HIV-1 RNA was detected. There were six (1.1%) results which were false negatives, indicating RNA was not detected. Of the 361 results reported for the samples obtained from donors not infected with HIV-1 (Donor 4 and Donor 5), laboratories reported 356 (98.6%) results that indicated HIV-1 RNA was not detected, while 5 (1.4%) 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 175 laboratories reporting results, each laboratory type is listed by decreasing frequency. Similar to previous performance surveys, Hospital laboratories (86, 49.1%) reported most of the testing results, followed by Independent (39, 22.3%), Health Department (34, 19.4%), Other (14, 8.0%), and Blood Bank (2, 1.1%) 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, (125, 69.4 %), in reporting results. Of other test kits used, 45 (25.0%) laboratories used the Bayer HIV-1 RNA 3.0 Quantitative Assay (bDNA)®, eight (4.4%) laboratories used bioMeriéux NucliSens® HIV-1 QT (formerly, Organon Teknika), which is also approved by the FDA, one (0.6%) laboratory used an In House kit, and one laboratory used an "other" test kit. While not included in this analysis, the participating American Red Cross laboratories used the Gen-Probe developed HIV-1/HCV assay, also approved by the FDA, marketed by Chiron under the name of the Procleix™ HIV-1/HCV Assay. This testing system will be included in future performance evaluations.

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 400 RNA copies/ml, the results are shown for each individual donor by test kit and listed according to the minimum, maximum, and median calculated values reported by the laboratories 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 and the duplicate of Donor 1, an HIV-1 infected donor. The second page shows the results reported for Donor 3, also an infected donor. The third page shows the results reported for Donor 4 and Donor 5, both uninfected donors. For this performance survey shipment, Donor 1 was duplicated in each panel, providing participant laboratories an opportunity to review their intra-shipment reproducibility for these donor samples.

Laboratories performed generally well in the testing of these performance evaluation samples. The reporting of 5 false positive results was a significant decrease from the number of false positives noted in the last performance survey where 12 false positive results were reported. These 5 false positive results were reported for Donor 4 and Donor 5; four results were obtained using the Roche Amplicor HIV-1 Monitor™ test and one result was obtained using Bayer HIV-1

RNA 3.0 Quantitative Assay (bDNA)®.

Six false negative results were reported in this survey. The six false negative results reported for this performance survey were all associated with Donor 1. All six results were obtained using the Roche Amplicor HIV-1 Monitor™ test. The reporting of 6 false negative results was a decrease from the number of false negatives noted in the last performance survey where 8 false negative results were reported.

Of the laboratories that indicated using the Roche Amplicor HIV-1 Monitor™, 67.2% indicated that they used a lower limit sensitivity of 400 copies/ml, while other lower limit sensitivities reported were 450 copies/ml (0.8%), 200 copies/ml (2.4%), 50 copies/ml (23.2%), and 40 reports that did not indicate the lower limit sensitivity (6.4%). Of the laboratories using the Bayer HIV-1 RNA 3.0 Quantitative Assay (bDNA)® test, 93.3% used the version 3 with a lower limit sensitivity of 50 copies/ml, while 6.7% of laboratories did not indicate a lower limit sensitivity. Among laboratories using the bioMeriéux NucliSens® test, 25% reported 400 copies/ml as their lower limit sensitivity, 25% reported 200 copies/ml, 25% reported 160 copies/ml, 12.5% reported 25 copies/ml, and 12.5% of the laboratories failed to report lower limits of sensitivity.

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 175 laboratories that reported results for this performance survey, 165 (94.3%) laboratories provided information on whether they use QC samples other than the controls contained in the test kit. Of these, 87 (52.7%) indicated they used QC samples other than those contained in the test kit. Among these 87 laboratories, 44 (50.6%) laboratories obtained their QC material from an in-house source, while 48 (55.2%) obtained their QC material from a commercial source. Five laboratories reported using QC material from both commercial and in-house sources.

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. 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 98.9%. For the samples from donors not infected with HIV-1, the analytic specificity was 98.6%. The overall analytic accuracy of this performance survey was 98.8%.