Analysis of the February 8, 1999 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 8, 1999. Testing results were reported by 178 (89%) of the 199 laboratories who were sent sample panels.

Samples used in the MPEP HIV-1 RNA determinations performance evaluation survey are undiluted, unpooled plasma obtained from individual donors who are HIV-1 infected or uninfected. Before shipment, the CDC tested each donor with at least three test kits which included the 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 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, Donor 3, and Donor 3 duplicate) that were infected with HIV-1, 526 (99.6%) of 528 results reported by the participant laboratories indicated HIV-1 RNA was detected. Two (0.4%) results, false negatives, indicated RNA was not detected. Of the 374 results reported for the samples obtained from a donor not infected with HIV-1 (Donor 4 and Donor 4 duplicate), laboratories reported 359 (96%) results that indicated HIV-1 RNA was not detected, while 15 (4%) results, false positives, indicated detecting HIV-1 RNA.

Types of Laboratories Performing HIV-1 RNA Determinations

The types of laboratories reporting results are shown in Figure 2. Each laboratory type is listed by decreasing frequency. Consistent with previous panel surveys, more than 50% of the laboratories that reported results are hospital 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. The Roche Amplicor HIV-1 Monitor™ test kit, approved by the FDA, was used by more than 70% of the laboratories reporting results.

Aggregate Testing Results Reported by Donor

Aggregate participant laboratories testing results, for each donor sample, by test kit, are shown in Table 2. 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 2 shows the laboratory test results reported for CDC Donor 1 and Donor 2, both HIV-1 infected donors. The second page shows the results reported for Donor 3 and Donor 3 duplicate, an HIV-1 infected donor. The third page shows the results reported for Donor 4 and Donor 4 duplicate, an uninfected donor. For this performance survey shipment, Donor 3 and Donor 4 were samples that were duplicated in each panel, providing participant laboratories an opportunity to review their intra-shipment reproducibility for those donor samples.

Please note that in Table 2, 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.

In general, laboratories performed very well in testing these performance evaluation samples. With the exception of the 2 false negative results, most laboratories detected HIV-1 RNA in those samples obtained from donors infected with HIV-1 and in which CDC detected HIV-1 RNA. Although the data is not shown, some laboratories used either a method to increase the sensitivity of their test kit, e.g., Roche Ultrasensitive Method, or a different version of their manufacturer's test kit. For example, while most laboratories using the Roche Molecular Systems Amplicor HIV-1 Monitor™ indicated a lower limit sensitivity of 400 RNA copies/milliliter, 5 indicated a lower limit sensitivity of 200 copies/ml, 9 indicated a lower limit sensitivity of 50 copies/ml, one indicated a lower limit sensitivity of 20 copies/ml, and one laboratory indicated a lower limit sensitivity of 1,500 copies/ml. In comparison, 6 laboratories using the Chiron Quantiplex™ HIV-RNA assay indicated a lower limit sensitivity of 500 copies/ml, while 34 laboratories reported a lower limit sensitivity of 50 copies/ml. Most laboratories using the Organon Teknika Nuclisens HIV-1 QT (NASBA)™ reported 400 copies/ml as their lower limit sensitivity, 2 laboratories reported 160 copies/ml, and one laboratory reported 80 copies/ml.

Twenty-two (16.5%) of 133 laboratories using the Roche Molecular Systems Amplicor HIV-1 Monitor™ reported inhibition with donor samples in their panels for either the HIV-1 infected or uninfected samples. Since all donor samples are treated in the same manner, the reason for inhibition in only some of the panel samples for only some laboratories using this test kit manufacturer is not known. Some laboratories reported inhibition with a donor sample but did

not report any inhibition with the identical duplicate of that sample contained in their panel.

Most laboratories did not detect viral RNA in the samples obtained from a single donor not infected with HIV-1 (Donor 4 and Donor 4 duplicate). There were 15 false positive determinations reported for these donor samples by laboratories using either a Roche Amplicor HIV-1 Monitor™ or a Chiron HIV-1 Quantiplex™ test kit.

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 used to validate a test run and to quantitate HIV-1 RNA copies/ml, and 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 178 laboratories that reported results, 178 (100%) laboratories provided information on whether they use QC samples other than the controls contained in the test kit. Of these, 70 (39%) indicated they used QC samples other than those contained in the test kit and 69 of these laboratories provided information on their source of QC material. Among these 42 (60.8%) laboratories indicated they obtained their QC material from an in-house source, 25 (36.2%) obtained their QC material from a commercial source, and 2 (3%) laboratories obtained their QC material from both a commercial and in-house source.

Conclusion

The results of this fourth performance evaluation shipment for HIV-1 RNA determinations showed that most laboratories correctly detected HIV-1 RNA in those samples from donors infected with HIV-1. Similarly, most laboratories did not detect HIV-1 RNA in the samples from donors not infected with HIV-1. While there is variability of results within a kit manufacturer and between kit manufacturers, a comparison of the results reported for the duplicate donors for this performance survey shipment showed good reproducibility between testing results. For the samples from donors infected with HIV-1, the analytic sensitivity for the results reported was 99.6%. For the samples from donors not infected with HIV-1, the analytic specificity was 95.9%. The overall analytic performance for this performance survey was 98.1%.