Analysis of the February 1998 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 16, 1998. Testing results were reported by 177 (91%) of the 195 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.

Please note that the samples used in this February 1998 shipment were the same samples used in the June 1997 shipment with the exception of Donor 4. Donor 1, obtained from an HIV-1 infected patient, was used in the June 1997 shipment and was duplicated in that shipment. Donor 2, also obtained from an HIV-1 infected patient, was used in the June 1997 shipment and was duplicated in this February 1998 shipment. Donor 3, not infected with HIV-1, was used in the June 1997 shipment and was duplicated in this February 1998 shipment. Donor 4, used in the June 1997 shipment, was not used in this February 1998 shipment.

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) that were infected with HIV-1, 536 (99.6%) of the results indicated HIV-1 RNA was detected, while only 2 (0.4%) of the results indicated that HIV-1 RNA was not detected. Conversely, of the 363 results reported for the samples obtained from a donor not infected with HIV-1 (Donor 3 and Donor 3 duplicate), laboratories reported 341 (93.9%) results that indicated not detecting HIV-1 RNA, yet laboratories reported 22 (6.1%) results that indicated detecting HIV-1 RNA. These 22 results included values that were above and below the lower limit sensitivities of the test kits used by the laboratories.

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. 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 68% of the laboratories reporting results.

Aggregate Testing Results Reported by Donor

Aggregate testing results, for each donor by test kit, reported by participant laboratories, are shown in Table 2. Since the lower limit sensitivities of the reported test kits 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. 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. The second page shows the results reported for Donor 2 and Donor 2 duplicate. The third page shows the results reported for Donor 3 and Donor 3 duplicate. For this shipment, Donor 2 and Donor 3 were samples that were duplicated in each panel providing participant laboratories the opportunity to review their intra-shipment reproducibility for those donor samples. Similarly, since the samples in this shipment were replicates of those used in the June 1997 shipment, laboratories are provided the opportunity to examine their reproducibility between shipments.

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 viral RNA, followed by the minimum, median, and maximum result value listed for each test kit manufacturer.

In general, laboratories performed well in testing these performance evaluation samples. Most laboratories detected HIV-1 RNA in those samples obtained from donors infected with HIV-1 and in which CDC detected viral RNA. Of the two testing results reported by laboratories not detecting viral RNA in these HIV-1 RNA positive samples, one incorrect result was reported for Donor 2; yet, the laboratory reporting this result did not report an incorrect result for Donor 2 duplicate. Similarly, another laboratory that reported an incorrect result for Donor 2 duplicate also reported a correct result for the Donor 2 sample.

Similarly, most laboratories did not detect viral RNA in the duplicated sample obtained from the donor who was not infected with HIV-1. Of the laboratories using a Roche Amplicor HIV-1 Monitor™ kit that detected viral RNA, 5 incorrect determinations were reported for Donor 3 and a corresponding 5 incorrect determinations were reported for Donor 3 duplicate. Reported values ranged from 0 to 24,665 with a median of 299, while for Donor 3 duplicate, the reported values ranged from 0 to 492 with a median of 266. Please note that 2 laboratories using the Roche

Amplicor HIV-1 Monitor™ kit reported zeros for their Donor 3 and Donor 3 duplicate results, instead of checking the box on the result form that would indicate their results were less than the test kit lower limit sensitivities, i.e., no HIV-1 RNA detected. Consequently, the insertion of a zero in the result boxes was concluded to be their calculated result based upon directions in the manufacturer's insert. Similarly, of the laboratories using a Chiron HIV-1 Quantiplex™ kit, 6 incorrect determinations were reported for Donor 3 and a corresponding 6 incorrect determinations were reported for Donor 3 duplicate. Reported values ranged from 50 to 13,390 with a median of 850, while for Donor 3 duplicate, the reported values ranged from 50 to 1,852 with a median of 581.

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 177 laboratories that reported results, 177 (100%) laboratories provided information on their use of QC samples other than the controls contained in the test kit. Of these, 46 (26%) indicated they used QC samples other than those contained in the test kit. Among these 46 laboratories, 34 (74%) indicated they obtained their QC material from an in house source and 12 (26%) obtained their QC material from a commercial source. Although, some laboratories indicated using a single serum/plasma, or multiple serum/plasma, 4 laboratories indicated they used VQA standards obtained through AIDS Clinical Trial Group (ACTG) participation. Although various combinations of QC materials were used, e.g., high RNA copies plus a negative control or low RNA copies plus a negative control, 12 (34%) laboratories indicated they used a high RNA copy control, low RNA copy control, and negative control all in combination. Of the 46 laboratories using QC material in addition to that contained in their test kit, 17 (37%) used their QC material with each set of tests, 14 (30%) used QC material only with each new test kit, 9 (19%) used QC material with each new test, and 6 (13%) indicated an "Other" use frequency.

Conclusion

The results of this second 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. Only a few laboratories did not detect HIV-1 RNA. Similarly, most laboratories did not detect HIV-1 RNA in the samples from donors not infected with HIV-1 RNA, while only a few laboratories did detect HIV-1 RNA in these donor samples. While there is variability of results within a kit manufacturer and between kit manufacturers, a comparison of the results reported for the June 1997 shipment and results for this replicate February 1998 shipment showed that most results were reproducible. For the samples from donors infected with HIV-1, the overall analytic sensitivity for the results reported was 99.6%. For the samples from donors not infected with HIV-1, the overall analytic specificity was 93.9%.