Analysis of the January 25, 1999 Performance Evaluation
HIV-1 Antibody Testing Results
Reported to the Centers for Disease Control and Prevention (CDC)
by Laboratories Participating in the Model Performance Evaluation Program

This report is an analysis of results provided to the Centers for Disease Control and Prevention (CDC) by laboratories participating in the Model Performance Evaluation Program (MPEP) after they tested the human immunodeficiency virus type 1 (HIV-1) performance evaluation samples shipped to them January 25, 1999. Testing results were reported by 804 (90.2%) of 891 laboratories that were sent sample panels. One laboratory returned results but failed to identify the sample codes for the samples they tested; consequently, test results from this laboratory are not included in the analysis. Additionally, 4 laboratories returned results more than 4 weeks after the cutoff date and these results are not included in the analysis.

Samples used in the MPEP surveys are undiluted, defibrinated plasma obtained from individual donors who are HIV-1-infected (positive) or HIV-1-uninfected (negative). The samples from HIV-1-infected donors were heat treated at 60^0C for 60 minutes. Before shipment, the CDC tested each donor sample with four HIV-1 and two HIV-1/HIV-2 enzyme immunoassay (EIA) kits licensed by the Food and Drug Administration (FDA). Supplemental testing was performed with three FDA-licensed HIV-1 Western blot (WB) kits and one HIV-2 WB kit. Donor samples were not tested by CDC with any HIV-1 indirect immunofluorescence (IIF) test. Please note that the samples sent to laboratories on January 25, 1999 are replicates of samples previously sent on August 17, 1998.

In pre-shipment testing performed by CDC, the strong-positive HIV-1 donor samples (Donors 4 - 7, 10 - 14, and 17 - 18) were EIA repeatedly reactive with all of the HIV-1 and HIV-1/HIV-2 EIA kits and WB reactive with all HIV-1 FDA-licensed WB kits used by CDC. The negative donor samples (Donors 15 and 16) were EIA repeatedly non-reactive and demonstrated no bands with any FDA-licensed HIV-1 WB kit. Donor samples 1-3, and 8 - 9, obtained from individual donors recently infected with HIV-1, were weakly positive for HIV-1 antibody and demonstrated variable EIA and WB reactivity with the FDA-licensed EIA and WB kits used for testing. Testing information for sequential serum samples from these donors demonstrated factors consistent with seroconversion such as a positive p24 antigen test, rising HIV-1 antibody titers in both lysate-based and recombinant antigen EIA tests with S/C ratios increasing as much as 10-fold between two bleeds, and WB reactivity changing from nonreactive (no bands) to indeterminate or reactive from one donation to the next.

Figure 1 shows the cumulative frequency of test result interpretations reported by participating laboratories, arranged according to donor reactivity, for the EIA, WB, and IIF methods. Of the 1,452 EIA interpretations reported for HIV-1-negative samples, 4 (0.28%) were incorrectly reported as reactive. There were 5 (0.17%) false-negative EIA interpretations among the 2,919 interpretations reported for the HIV-1-positive samples. Of 258 WB interpretations reported for the HIV-1-negative samples, one (0.39%) positive interpretation and four (1.55%) indeterminate WB interpretations were reported. Among the 1,070 WB interpretations reported for the HIV-1-positive samples, there were two (0.19%) false-negative and 82 (7.66%) indeterminate interpretations reported. Among the 44 IIF interpretations reported for HIV-1-negative samples, there were no false-positive or indeterminate interpretations reported. Of the 143 IIF interpretations reported for HIV-1-positive samples, there were 5 (3.50%) indeterminate and 2 (1.4%) false-negative interpretations.

The types of laboratories that reported results to CDC are shown in Figure 2. Each laboratory type is listed, by decreasing frequency, for each of the test methods.

The combinations of test methods used by the laboratories and the frequency of use are shown in Figure 3. Most

laboratories performed only EIA (61.3%), while some laboratories performed both EIA and supplemental tests (36.4%), and others (2.3%) performed only supplemental tests. There were 88 laboratories that performed other tests in addition to EIA, WB and IIF, and 61 laboratories that performed only tests other than EIA, WB, or IIF. The data for tests performed in addition to or other than EIA, WB, or IIF are presented in Figure 10.

The types of test kits used, by kit manufacturer, for the EIA, WB, and IIF methods are shown, by decreasing frequency, in Figure 4. For each test method, some laboratories indicated using test kits for which there was no unique glossary code provided in the survey report form and these responses have been grouped as "Other" manufacturer. Some "Other" kits reported as being used for EIA include Abbott HIV-1/HIV-2 3rd Generation PLUS, Murex ICE HIV 1.O.2 Detection, Innogenetics Innotest HIV-1/HIV-2, Organon Teknika Vironostika HIV Uniform II Plus O, and Ortho Diagnostics HIV-1/HIV-2 Ab Capture EIA. Some laboratories, located outside the United States, used the Abbott AXSYM system or the Abbott PRISM analyzer and reported results as S/CO (sample/cutoff ratio). Since the S/CO data can not be entered correctly on the MPEP EIA result form, the data from laboratories using either AXSYM or PRISM systems is reported with "Other" tests in Figure 10.

The results reported for the EIA, WB, and IIF methods, listed by kit manufacturer, for the HIV-1-positive and HIV-1-negative samples are shown in Figures 5, 6, and 7. Results reported by the participant laboratories reflect their testing performance using manufactured kits to evaluate MPEP samples and do not necessarily reflect an evaluation of these manufactured kits.

EIA Results

In Figure 5, three of the four false-positive EIA interpretations were reported for Donor 15 and the other for Donor 16 by laboratories using EIA kits from two different manufacturers.

The five nonreactive EIA interpretations were reported for five different HIV-1-positive donor samples by laboratories using two different FDA-licensed EIA kits. Four of the 5 EIA false-negative interpretations were reported once each for HIV-1 strong-positive donor samples (Donor numbers 10,14,17, and 18). Since there were duplicate samples from Donors 10 and 14 in some panels, it is evident that one was reported incorrectly as EIA nonreactive and the other correctly as EIA reactive.

WB Results

Of the 703 laboratories reporting test results in this survey, only 267 (38%) performed WB testing. Since laboratories are asked to test these performance samples as they would patient or donor samples, it is unclear why many laboratories would perform WB testing on HIV-1-negative donor samples that were nonreactive in EIA tests. It is possible that the one reactive WB interpretation (for Donor 16) and the four indeterminate WB interpretations (for Donor 15) were reported by laboratories that also reported false-positive EIA results for these samples. The types and number of WB bands reported for the HIV-1-uninfected donor samples varied from only a gp160 band in one report to the full gamut of HIV-1 WB bands (17, 24, 31, 51, 66, 120, and 160) reported by another laboratory. Although two reports noted the presence of a p24 band for Donor 16, a WB nonreactive interpretation was indicated.

Of the 82 indeterminate WB interpretations reported for samples from the 14 HIV-1 infected donors (Donors 1-14), 81 (98.8%) were reported for samples from the seroconverting donors (Donors 1, 2, 3, 8, and 9). Indeterminate WB interpretations for the seroconversion samples were reported by laboratories using WB kits provided by five different manufacturers (Figure 6). Indeterminate interpretations reported for these samples most often resulted from failure to detect antibody to envelope (env) antigens (gp41, gp120, gp160) or detection of env-

antibody reactivity resulting in bands with less than the required intensity.

The WB bands for these donor samples, as determined in pre-shipment testing by CDC with three FDA-licensed WB test kits, are shown in Table 2. Only bands of greater than or equal to 1+ intensity are listed in Table 2.

Of the 267 laboratories reporting WB test results, 241 indicated which WB criteria were used to interpret their WB tests. The ASTPHLD/CDC WB interpretive criteria was used by 210 (87.1%) of these 241 laboratories. The WB interpretive guidelines published by the FDA-licensed WB kit manufacturers are identical to the ASTPHLD/CDC HIV-1 WB interpretive criteria. Eight laboratories indicated they were using interpretive criteria different from that recommended by the kit manufacturer as approved by the FDA.

WB Band Patterns

The protein band patterns for the major viral proteins, as reported by participant laboratories for each donor sample, are shown in Figure 8. The frequency of a reported band is listed above the column. The number of WB reports received for the donor sample is indicated in the far right column. This figure **does not** include WB bands reported as 'W', indicating intensity less than that of the designated band of the weak positive control provided in the WB kit nor does it include bands of greater than 1+ intensity reported for p15, p17, p51, p55, or p66. Note that 20 or more WB results were reported for the samples from HIV-uninfected donors (Donors 15 and 16) although most laboratories do not normally include the testing of EIA-nonreactive donor samples,in their routine algorithm for HIV antibody testing.

For the HIV-1 antibody strong-positive samples (Donors 4 - 7, 10 - 14, 17 and 18), laboratories had no difficulty in detecting antibodies to gag, pol, and env antigens with any HIV-1 or HIV-1/HIV-2 WB kit used. The donor samples obtained from HIV-1 infected individuals during seroconversion appeared to cause more difficulty. Most of the indeterminate WB interpretations reported for the seroconversion samples (Donors 1, 2, 3, 8, and 9) resulted from inability to detect antibody to viral envelope antigen and, infrequently, to gag antigen in these donor samples. These findings are consistent with the CDC WB test results as shown in Table 2 of the results report accompanying this analysis.

IIF Results

No false-positive or indeterminate IIF interpretations were reported for the HIV-1-negative donor samples (Figure 7). Among the 143 IIF interpretations reported for the HIV-1-positive samples, 2 (1.4%) false-negative and 5 (3.5%) indeterminate interpretations were reported only for the samples from seroconverting Donors 2, 3,and 9.

Fluorescence Intensity Patterns

The IIF intensity patterns for HIV-1 infected cells, as reported by participating laboratories, are shown in Figure 9. The frequency of reports of fluorescence intensity for each donor is listed in the far right column. A scoring of fluorescence intensity is not required for interpretation of seroreactivity with the FDA-licensed Waldheim Fluorognost HIV-1 IFA kit; therefore, some laboratories provided interpretation, but did not score fluorescent intensity. Data from these laboratories were included in Figures 1 and 7, but cannot be included in Figure 9. No fluorescence intensity was reported for either of the samples from HIV-1-uninfected donors (Donors 15 and 16).

Other Tests Performed

Figure 10 provides information on the test results and interpretations provided by laboratories that perform

HIV-1 antibody tests in addition to or other than microtiter-format EIA, WB or IIF. The first graphic of this figure shows manufacturers of the "Other" types of tests and frequency of use. The rest of this figure shows the results reported by laboratories after testing the HIV-1-negative and HIV-1-positive samples in this shipment. The procedures used by 88 (59.1%) of these 149 laboratories can be described as "rapid" micro-filtration EIA procedures (e.g., SUDS HIV-1, Testpack HIV-1/HIV-2, MultiSpot HIV-1/HIV-2, and HIV-Spot HIV 1+2). These tests are generally provided as kits that use micro-particles, such as latex, or nitrocellulose membranes coated with purified lysate, synthetic, or recombinant HIV-1 (and often HIV-2) antigens. Eighteen laboratories tested samples using a gelatin particle agglutination test (Fujirebio Serodia HIV) and one laboratory used a latex agglutination test (Capillus). Results of "Line or Strip Immunoassay" tests such as Liatek (Organon Teknika), INNO-LIA (Innogenetics) and RIBA (Chiron) were appropriately reported on the "Other Test" results form by 10 laboratories. Note that most laboratories using the Abbott AXSYM or PRISM systems correctly reported their results as "Other" test type since these tests are based on microparticle capture and chemiluminescence measurements and differ from the traditional microtiter-format EIA tests.

Of the 149 laboratories reporting results on the form for "Other" types of tests, 84 (56.4%) are US or US-territory laboratories. Of these 84 laboratories, 82 reported results using the FDA-approved Murex SUDS HIV-1 test. Sixty-one (40.9%) of the 149 laboratories reporting results of "Other" types of tests did not report results using EIA, WB or IIF tests.

Among the 304 final interpretations reported for HIV-1-negative samples (Donors 15 and 16) tested by laboratories using these "Other" procedures, 5 false-positive interpretations were reported by 3 laboratories using the Fujirebio Serodia HIV particle agglutination test. Two of these laboratories reported false-positive results for each of the duplicated Donor 16 samples in their panels and one laboratory reported a false-positive result for only one of the duplicated Donor 15 samples in their panel. One false-positive was reported by a laboratory using the Murex SUDS rapid test device for only one of the duplicated Donor 16 samples in their panel.

Among the 628 interpretations reported for the HIV-1-positive samples tested by procedures other than EIA, WB, or IIF, there were no false-negative interpretations and only two indeterminate interpretations. The two indeterminate interpretations were reported for samples from Donor 11 by a single laboratory using the Serodia particle agglutination test.

Quality Control Testing

Information was sought on the use of quality control (QC) samples **other than the controls provided in various test kits**. Positive and negative samples included in manufactured kits are internal kit control material used to validate the test run, calculate test run cut-off values, and may not validate the analytic testing process which may include testing problems such as faulty pipettors, inadequate incubation conditions, or kit lot sensitivity. Most laboratories completing the QC section of the form adhered to the instructions pertaining to this section and described only external QC samples used in their HIV testing procedures.

Of the 724 laboratories that reported EIA test results, only 434 (59.9%) indicated they used quality control samples other than those provided with the manufactured test kit. Of these 434 laboratories, 248 (57.1%) used samples obtained commercially, 156 (35.9%) used QC samples from in-house sources, and 25 (5.8%) used QC material from both commercial and in-house sources. Five laboratories did not indicate the source of their external QC samples. The majority indicated the use of either weak-positive or weak-positive and negative serum/plasma with each set or run of EIA plates.

Of the 267 laboratories reporting WB test results, only 81 (30.3%) laboratories described their external QC samples. Of these 81 laboratories, 49 (60.5%) used samples prepared in-house, 26 (32.1%) used QC samples obtained commercially, and 3 (3.7%) used QC material from both commercial and in-house sources. Three laboratories did not indicate the source of external QC samples used in WB. Most laboratories used at least a weak-positive serum/plasma and included this sample in each set/run of WB strips.

Of the 36 laboratories reporting IIF results, only 7 (19.4%) used IIF external QC samples. Of these, 5 (71.4%) used samples from in-house sources and 2 (28.6%) used QC samples obtained commercially. Most laboratories included at least a weak-positive external QC sample with each set/run of slides.

Of the 149 laboratories reporting results of tests other than EIA, WB or IIF, only 34 (22.8%) indicated the use of external QC samples. Of these, 18 (52.9%) used samples from in-house sources, 13 (38.2%) used samples from commercial sources and 3 (8.8%) indicated using QC material obtained from both of these sources. The majority of these laboratories indicated they used at least a weak-positive QC sample with each run or at least with each new kit lot.

Conclusion

Most participant laboratories performed well in testing the HIV-1 donor samples in this shipment. Only a few laboratories reported false-negative EIA (0.17%), false-negative WB (0.19%) or false-negative IIF (1.4%) results for the HIV-1-positive samples (Donor numbers 1-14 and 17-18). False-positive EIA (0.28%) and false-positive (0.39%) or indeterminate WB (1.6%) results were reported infrequently for samples that CDC tested and found negative for HIV-1 antibody in both EIA and WB tests (Donors 15 and 16). No false-positive IIF results were reported in this survey.

The following information regarding overall analytic performance, analytic sensitivity, and analytic specificity is determined from the results reported by laboratories testing performance evaluation samples and is not intended to reflect the actual sensitivity and specificity of the manufactured test kits. For this survey, the overall EIA analytic sensitivity and specificity was 99.8% and 99.7%, respectively. When indeterminate and reactive WB interpretations are combined, the WB analytic sensitivity was 99.8%. If indeterminate interpretations are considered incorrect for HIV-1 antibody-negative samples, the WB analytic specificity was 98.1%. When indeterminate and reactive IIF interpretations are combined for the HIV-1-positive samples, the IIF analytic sensitivity was 98.6%; the IIF analytic specificity was 100% for this survey. The analytic sensitivity and specificity of the test procedures other than EIA, WB, and IIF vary greatly, depending on which test method results are analyzed (Figure 10). If indeterminate WB and IIF interpretations for the HIV-1 antibody-positive samples are combined with reactive interpretations, the overall analytic performance for laboratories testing these performance evaluation samples by EIA, WB, and IIF procedures was 99.8%, 99.5%, and 98.9% respectively.

Please note that we plan to ship the next panels of MPEP HIV-1 antibody samples to participating laboratories on July 26, 1999.