Analysis of the July 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 in July 1999. Testing results were reported by 804 (89.8%) of 895 laboratories that received sample panels.

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 treated with an organic solvent-detergent mixture known to inactivate blood borne viruses including HIV-1, human T-lymphotropic virus, and hepatitis B and C. Before shipment, each donor sample was tested with three 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. Donor samples were not tested prior to shipment with any HIV-1 indirect immunofluorescence (IIF) test.

In pre-shipment testing, the strong-positive HIV-1 donor sample (Donor 1) was 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. The negative donor sample (Donor 2) was EIA repeatedly non-reactive and demonstrated no bands with any FDA-licensed HIV-1 WB kit. Donor samples 3 and 4, 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 preshipment testing. Testing information for sequential serum samples from Donors 3 and 4 demonstrated factors consistent with seroconversion such as a positive p24 antigen test, positive test for HIV-1 ribonucleic acid (RNA), rising HIV-1 antibody titers in all 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 762 EIA interpretations reported for the HIV-1-negative sample, 4 (0.52%) were incorrectly reported as reactive. There were 106 (2.76%) false-negative EIA interpretations among the 3,837 interpretations reported for the HIV-1-positive samples. Of 134 WB interpretations reported for the HIV-1-negative samples, no reactive or indeterminate WB interpretations were reported. Among the 1,322 WB interpretations reported for the HIV-1-positive samples, there were 12 (0.91%) false-negative and 248 (18.76%) indeterminate interpretations reported. Among the 24 IIF interpretations reported for HIV-1-negative samples, there were no false-positive or indeterminate interpretations reported. Of the 164 IIF interpretations reported for HIV-1-positive samples, there were 12 (7.30%) indeterminate and 46 (28.05%) 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 (61.1%), while some laboratories performed both EIA and supplemental

tests (37%), and others (1.9%) performed only supplemental tests. There were 158 laboratories that performed other tests in addition to EIA, WB and IIF, and 74 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 manufacturer code provided in the survey report form and these responses have been grouped as "Other" manufacturer kits. Some “Other” kits reported as being used for EIA include Organon Teknika Vironostika HIV Uniform II Plus O (20 laboratories), Abbott HIV-1/HIV-2 3rd Generation PLUS (18 laboratories), Ortho Diagnostics HIV-1/HIV-2 Ab Capture EIA (8 laboratories), and Innogenetics Innotest HIV-1/HIV-2 (4 laboratories). 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, the four false-positive EIA interpretations were reported for Donor 2 by laboratories using EIA kits from four different manufacturers.

Of the 106 nonreactive EIA interpretations reported for the HIV-1-positive samples (Donors 1, 3, and 4), 83 (78.3%) were reported for Donor 3 by laboratories using at least 8 different EIA kits. Laboratories using the Genetic Systems (Sanofi) HIV-1 rLAV kit reported 66 (62.3%) of the 106 false-negative interpretations and, 63 (95.5%) of the 66 were reported for Donor 3 samples. The following table shows the mean determined for the cut-off values and mean determined for sample absorbances (Abs) reported by laboratories using the Genetic Systems (Sanofi) HIV-1 rLAV kit and reporting either nonreactive or reactive EIA interpretations for samples from Donor 3.

Analysis of Data Reported by Laboratories using the Genetic Systems (Sanofi) rLAV EIA kit to Test Donor 3 Samples:

Initial Test Interpretation	Number of Samples	Cut-off Mean	Cut-off Range	Mean Sample Absorbance	Sample Abs Range
Non-Reactive	63	0.284	0.248 - 0.336	0.248	0.062 - 0.730
Reactive	21	0.297	0.275 - 0.358	0.441	0.250 - 1.029

Eight different lots of the Genetic Systems (Sanofi) rLAV kit were used by laboratories in this survey and false-negative results were not associated with any specific lot number. Donor 4 samples accounted for 19 (17.9%) of the false-negative interpretations, and laboratories using the Organon Teknika Vironostika HIV-1 kit reported 10 (52.6%) of the 19 false-negative interpretations for Donor 4 samples. Interestingly, there were 4 (3.8%)

false-negative EIA interpretations reported for the HIV-1 strong-positive samples from Donor 1, and 2 (50%) of these were reported by laboratories using EIA kits manufactured by Abbott Diagnostics. No false-negative EIA interpretations were reported by laboratories using 4 different manufactured kits not marketed in the United States.

WB Results

Of the 804 laboratories reporting test results in this survey, only 268 (33%) performed WB testing. Since laboratories are asked to test these performance samples as they would patient or donor samples, it is unclear why 128 laboratories would perform WB testing on donor samples that they reported as nonreactive in EIA tests. None of the laboratories reporting WB results for the HIV-1-uninfected donor samples indicated the detection of any HIV-1 viral bands.

All of the 248 indeterminate WB results were reported for samples from the 2 HIV-1-infected seroconverting donors (Donors 3 and 4). Indeterminate WB interpretations for the seroconversion samples were reported by laboratories using WB kits provided by seven different manufacturers (Figure 6). Laboratories using WB kits manufactured by BioRad accounted for 11 (91.7%) of the 12 false-negative WB interpretations reported for samples from HIV-1-infected donors in this survey, including a false-negative report for one of the HIV-1 strongly-positive samples from Donor 1 that was duplicated in each panel.

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

Of the 268 laboratories reporting WB test results, 246 indicated which WB criteria were used to interpret their WB tests. The ASTPHLD/CDC WB interpretive criteria were used by 214 (87%) of these 246 laboratories. The WB interpretive guidelines published by all the FDA-licensed WB kit manufacturers are identical to the ASTPHLD/CDC HIV-1 WB interpretive criteria. Please recall that the Association of State and Territorial Public Health Laboratory Directors (ASTPHLD) is now called the Association of Public Health Laboratories (APHL). Eight laboratories indicated they were using interpretive criteria different from that recommended by the kit manufacturer as licensed 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 more than 100 WB results were reported for the samples from an HIV-uninfected donor (Donor 2) 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 (Donor 1), laboratories had no difficulty in detecting antibodies to gag, pol, and env antigens regardless of the HIV-1 WB kit used. The donor samples obtained from HIV-1 infected individuals during seroconversion appeared to cause more difficulty. Indeterminate interpretations

reported for these samples most often resulted from failure to detect antibody to envelope (env) antigens (gp41, gp120, gp160) or to detect env-antibody reactivity resulting in bands with less than the required intensity. Indeterminate WB interpretations reported for the seroconversion samples from Donor 3 resulted from inability to detect antibody to viral envelope (env) antigen and/or group-associated gene (gag) antigen in these donor samples. Indeterminate WB interpretations reported for the seroconversion samples from Donor 4 resulted primarily from failure to detect antibody to viral envelope (env) antigen at sufficient intensity to be determined reactive. 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 164 IIF interpretations reported for the HIV-1-positive samples, 46 (28%) false-negative and 12 (7.3%) indeterminate interpretations were reported. These false-negative and indeterminate interpretations were reported only for the samples from seroconverting Donors 3 and 4.

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 the sample from HIV-1-uninfected Donor 2. Also note that 34 (54%) of the 63 IIF reports received for samples from Donor 3 indicated no fluorescence observed and 12 (40%) of the 30 IIF reports received for Donor 4 samples indicated that no fluorescence was observed.

Other Tests Performed

Figure 10 provides information on the test results and interpretations provided by 158 laboratories that perform HIV-1 antibody tests in addition to or other than microtiter-format EIA or 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. Of the 158 laboratories reporting results on the form for “Other” types of tests, 86 (54.4%) are US or US-territory laboratories. Of these 86 laboratories, 83 (96.5%) reported results using the FDA-approved Murex SUDS HIV-1 test. Sixty-six (41.8%) of the 158 laboratories reporting results of “Other” types of tests did not report results using EIA or WB or IIF tests.

The procedures used by 91 (57.6%) of these 158 laboratories can be described as "rapid" micro-filtration EIA procedures (e.g., SUDS HIV-1, Testpack HIV-1/HIV-2, and MultiSpot 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. Fourteen laboratories tested samples using a gelatin particle agglutination test (Fujirebio Serodia HIV) and four laboratories 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 16 laboratories. Note that most laboratories using the Abbott AXSYM or PRISM systems correctly reported their results on the “Other” test type result form since these tests are based on microparticle capture and chemiluminescence measurements and differ from the traditional microtiter-format EIA tests.

Among the 165 final interpretations reported for HIV-1-negative sample (Donor 2) tested by laboratories using these "Other" procedures, 3 false-positive and 3 indeterminate interpretations were reported by a total of 6 laboratories using the Murex/Abbott SUDS HIV-1 test. One false-positive and one indeterminate interpretation were reported by a total of two laboratories using the Fujirebio Serodia HIV particle agglutination test.

Among the 875 interpretations reported for the HIV-1-positive samples tested by procedures other than EIA, WB, or IIF, there were 180 false-negative interpretations and 22 indeterminate interpretations. All of the indeterminate and all but one of the false-negative interpretations were reported for HIV-antibody weakly reactive samples from Donors 3 and 4. Of the 180 false-negative interpretations, 149 (82.8%) were reported for samples from Donor 3 and 135 (90.6%) of these 149 false-negative interpretations for Donor 3 samples were reported by laboratories using the Murex/Abbott SUDS HIV-1 test. Since the samples used for this survey are not fresh serum samples, individuals performing the SUDS HIV-1 test should note the statement in the package insert for this kit that indicates centrifugation at 15,000 rpm for 15 minutes should be carried out prior to testing survey samples.

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 478 (66%) indicated they used quality control samples other than those provided with the manufactured test kit. Of these 478 laboratories, 275 (57.5%) used samples obtained commercially, 174 (36.4%) used QC samples from in-house sources, and 24 (5.0%) 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 268 laboratories reporting WB test results, only 85 (31.7%) laboratories described their external QC samples. Of these 85 laboratories, 54 (63.5%) used samples prepared in-house, 24 (28.2%) used QC samples obtained commercially, and 6 (7.1%) used QC material from both commercial and in-house sources. One laboratory 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 35 laboratories reporting IIF results, 14 (40.0%) used IIF external QC samples. Of these, 9 (64.3%) used samples from in-house sources, 4 (28.6%) used QC samples obtained commercially, and one laboratory did not indicate the source of external QC samples used for IIF. Most of the 14 laboratories included at least a weak-positive external QC sample with each set/run of slides.

Of the 158 laboratories reporting results of tests other than EIA or WB or IIF, only 48 (30.4%) indicated the use of external QC samples. Of these, 24 (50.0%) used samples from in-house sources, 19 (39.6%) used

samples from commercial sources, 4 (8.3%) indicated using QC material obtained from both of these sources, and one laboratory did not indicate the source of external QC samples used. 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. However, some false-negative results were reported for EIA (2.8%), WB (0.91%) and IIF (28%) after laboratories tested the HIV-1-positive samples (Donors 1, 3, and 4). False-positive EIA (0.52%) results were reported infrequently for samples negative for HIV-1 antibody (Donor 2). No false-positive WB or 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 97.2% and 99.5%, respectively. When indeterminate and reactive WB interpretations are combined, the WB analytic sensitivity was 99.1%. The WB analytic specificity for this survey was 100%. When indeterminate and reactive IIF interpretations are combined for the HIV-1-positive samples, the IIF analytic sensitivity was 72%. The IIF analytic specificity was 100% for this survey. The analytic sensitivity and specificity of the "Other" test procedures vary greatly, depending on which test method results are analyzed (Figure 10). When 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 97.6%, 99.2%, and 75.5% respectively.

Please note that we plan to ship the next panels of MPEP HIV-1 antibody samples to participating laboratories on January 10, 2000, to laboratories located outside the United States and on January 24, 2000, to laboratories located within the United States.