

**Analysis of the January 2000 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 January 2000. Testing results were reported by 800 (89.7%) of 892 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 enzyme immunoassay (EIA) kits, two HIV-1/HIV-2 enzyme immunoassay (EIA) kits, and one rapid test (RT) kit (SUDS HIV-1) licensed by the Food and Drug Administration (FDA). Supplemental testing was performed with four 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 EIA kits, the HIV-1/HIV-2 EIA kits, and the RT kit, 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, RT, 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, 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 1,508 EIA interpretations reported for the HIV-1-negative samples, 6 (0.4%) were incorrectly reported as reactive. There were 14 (0.5%) false-negative EIA interpretations among the 3,053 interpretations reported for the HIV-1-positive samples. Of 270 WB interpretations reported for the HIV-1-negative samples, 2 (0.7%) reactive and 1 (0.4%) indeterminate WB interpretations were reported. Among the 1,113 WB interpretations reported for the HIV-1-positive samples, there were 3 (0.3%) false-negative and 141 (12.7%) indeterminate interpretations reported. Among the 42 IIF interpretations reported for HIV-1-negative samples, there were no false-positive or indeterminate interpretations reported. Of the 145 IIF interpretations reported for HIV-1-positive samples, there were 15 (10.3%) indeterminate and 23 (15.9%) 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. Of

the 800 laboratories reporting results, 378 (47.3%) performed only EIA, 246 (30.8%) performed EIA and a supplemental test, and 6 (0.8%) performed only a supplemental test. There were 170 (21.3%) laboratories that performed an “Other” test in addition to EIA, WB and IIF. The data for these “Other” tests 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 Ortho Diagnostics HIV-1/HIV-2 Ab Capture EIA (8 laboratories), Behring Enzygnost anti-HIV1/2 PLUS (6 laboratories), BioChem ImmunoSystems Detect HIV (6 laboratories) and Innogenetics Innostest HIV-1/HIV-2 (5 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 6 false-positive EIA interpretations were reported for Donor 2 by laboratories using EIA kits from 3 different manufacturers. For the 4 false positive results reported by laboratories using the Abbott HIV-1/HIV-2 (rDNA) kit, 3 different lot numbers were used.

Of the 14 nonreactive EIA interpretations reported for the HIV-1 weakly-positive samples (Donors 3 and 4), 10 (71.4%) were reported for Donor 4 by laboratories using 4 different EIA kits, and 4 (28.6%) were reported for Donor 3 by laboratories using 4 different EIA kits. No false-negative EIA interpretations were reported for the HIV-1 strong-positive sample from Donor 1.

### **WB Results**

Of the 800 laboratories reporting test results in this survey, 281 (35.1%) performed WB testing. (One laboratory did not report WB results for its EIA repeat-reactive samples, therefore, N=280 for Figure 6). Since laboratories are asked to test these performance samples as they would patient or donor samples, it is unclear why many laboratories performed WB testing on donor samples that they reported as nonreactive in EIA tests. Two reactive WB interpretations and one indeterminate WB interpretation were reported for the HIV-1 uninfected donor sample (Donor 2). One of these reactive reports indicated WB bands 24, 160 and the other indicated bands 24, 66, and 160. The indeterminate report did not indicate the presence of any WB bands, so it is unclear why it was interpreted as indeterminate. Additionally, the presence of viral bands was noted on 2 reports for the HIV-uninfected donor (p55 on one, and 17, 24 on the other) with nonreactive interpretations indicated.

Of the 141 indeterminate WB results reported for samples from the 2 HIV-1-infected seroconverting donors (Donors 3 and 4), 135 (95.7%) were reported for Donor 4. Indeterminate WB interpretations for the

seroconversion samples were reported by laboratories using WB kits provided by 5 different manufacturers (Figure 6). Laboratories using WB kits manufactured by BioRad accounted for 71 (50.4%) of the indeterminate WB results, and 2 of the 3 false-negative interpretations.

The WB bands for the donor samples in this survey, as determined in pre-shipment testing with four 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 281 laboratories reporting WB test results, 251 indicated which WB criteria were used to interpret their WB tests. The ASTPHLD/CDC WB interpretive criteria were used by 216 (86.1%) of these 251 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). Five 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', or "weak", 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 200 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 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 145 IIF interpretations reported for the HIV-1-positive samples, 23 (15.9%) false-negative and 15 (10.3%) indeterminate interpretations were reported. These false-negative and indeterminate interpretations were reported only for the samples from seroconverting Donors 3 and 4. Nineteen (82.6%) of the false-negative interpretations and 10 (66.7%) of the indeterminate interpretations were reported for samples from Donor 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 4 (12.1%) of the 33 IIF reports received for samples from Donor 3 indicated no fluorescence observed and 19 (27.5%) of the 69 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 170 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 170 laboratories reporting results on the form for "Other" types of tests, 100 (58.8%) are US or US-territory laboratories. Of these 100 laboratories, 99 (99%) reported results using the FDA-approved Murex SUDS HIV-1 test. Sixty-three (37.1%) of the 170 laboratories reporting results of "Other" types of tests did not report results using EIA or WB or IIF tests.

The procedures used by 103 (60.6%) of these 170 laboratories can be described as "rapid" micro-filtration EIA procedures (e.g., Murex/Abbott SUDS HIV-1, Abbott Testpack HIV-1/HIV-2, and Genelabs Diagnostics HIV-Spot). 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. Seventeen laboratories tested samples using a gelatin particle agglutination test (Fujirebio Serodia HIV). Results of "Line or Strip Immunoassay" tests such as Organon Teknika Liatek, Innogenetics INNO-LIA and Chiron RIBA were appropriately reported on the "Other Test" results form by 11 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 348 final interpretations reported for HIV-1-negative sample (Donor 2) tested by laboratories using these "Other" procedures, 3 false-positive interpretations were reported using the Murex/Abbott SUDS HIV-1 test. Four indeterminate interpretations were reported using the Fujirebio Serodia HIV particle agglutination test, and one indeterminate interpretation was reported using the Abbott PRISM.

Among the 729 interpretations reported for the HIV-1-positive samples tested by procedures other than EIA, WB, or IIF, there were 119 false-negative interpretations and 9 indeterminate interpretations. All of the indeterminate and all but 2 of the false-negative interpretations were reported for HIV-antibody weakly reactive samples from Donors 3 and 4. Of the 119 false-negative interpretations, 79 (66.4%) were reported for samples from Donor 4. One hundred sixteen (97.5%) of these 119 false-negative interpretations were reported by laboratories using the Murex/Abbott SUDS HIV-1 test. Three false-negative interpretations were reported by laboratories using the Genelabs Diagnostics HIV-Spot. 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 at least 5 minutes should be carried out prior to testing

survey samples, and that all reagents should be brought to room temperature before use.

### **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 718 laboratories that reported EIA test results, 460 (64.1%) indicated they used quality control samples other than those provided with the manufactured test kit. Of these 460 laboratories, 263 (57.2%) used samples obtained commercially, 161 (35%) used QC samples from in-house sources, and 28 (6.1%) used QC material from both commercial and in-house sources. Eight 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 281 laboratories reporting WB test results, only 93 (33.1%) laboratories used external QC samples. Of these 93 laboratories, 57 (61.3%) used samples prepared in-house, 29 (31.2%) used QC samples obtained commercially, and 4 (4.3%) 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 38 laboratories reporting IIF results, 13 (34.2%) used IIF external QC samples. Of these, 10 (76.9%) used samples from in-house sources, and 3 (23.1%) used QC samples obtained commercially. Most of the 13 laboratories included at least a weak-positive external QC sample with each set/run of slides.

Of the 170 laboratories reporting results of tests other than EIA or WB or IIF, only 38 (22.4%) indicated the use of external QC samples. Of these, 20 (52.6%) used samples from in-house sources, 14 (36.8%) used samples from commercial sources and 4 (10.5%) 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 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 (0.5%), WB (0.3%) and IIF (15.9%) with HIV-1-positive samples. False-positive EIA (0.4%) and WB (0.7%) results were reported infrequently for samples negative for HIV-1 antibody (Donor 2). 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 99.5% and 99.6%, respectively. When indeterminate and reactive WB interpretations are combined, the WB analytic sensitivity was 99.7%. If indeterminate interpretations are considered incorrect for HIV-1 antibody-negative samples, the WB analytic specificity for this survey was 98.9%. When indeterminate and reactive IIF interpretations are combined for the HIV-1-positive samples, the IIF analytic sensitivity was 84.1%. 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 99.6%, 99.6%, and 87.7% respectively.

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