Comparison of Five Commercial Enzyme-Linked Immunosorbent Assays and Western Immunoblotting for Human Immunodeficiency Virus Antibody Detection in Serum Samples from Central Africa

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Detection by five different enzyme-linked immunosorbent assays (ELISAs) of antibody to human immunodeficiency virus (HIV) in sera from three Zairian populations consisting of 1,998 individuals with various risks for HIV infection was evaluated. Sera that were reactive by at least one assay and 10% of the nonreactive serum samples were analyzed by Western blot (immunoblot) by using U.S. Public Health Service interpretation criteria. Of sera which were positive by ELISA for detection of antibody to HIV-1 and HIV-2 and indeterminate by HIV-1 Western blot were also analyzed by HIV-2 Western blot. Overall, 443 (22.2%) serum specimens were HIV-1 Western blot positive, 390 (19.5%) had indeterminate HIV-1 Western blot patterns, and no samples were HIV-2 Western blot positive. The sensitivity of the ELISAs ranged from 97.5 to 99.8%, and the specificity ranged from 51.7 to 98.4%. By population group, the negative predictive value ranged from 97.1 to 100%, in contrast to the positive predictive value, which varied from 6.6 to 100%. Follow-up results for sera which were indeterminate for antibody to HIV-1 documented only four seroconversions (6.0%) among 67 individuals at high risk for HIV-1 infection and no seroconversions among 202 individuals at relatively low risk for HIV-1 infection. This study demonstrates the importance of evaluating commercial ELISAs with sera from appropriate geographical regions in order to select the most cost-effective and practical assay for use in that region. Furthermore, the high frequency of indeterminate Western blots for African sera emphasizes the continual need for improved confirmatory assays and interpretation criteria.

Several commercial enzyme-linked immunosorbent assays (ELISAs) for detection of antibody to human immunodeficiency virus (HIV) infection have been evaluated, mainly in the United States and Europe, with various reported sensitivities and specificities (1, 2, 5, 6, 8, 11, 12−14). Only a few studies, evaluating a limited number of ELISAs, using Central African sera have been conducted (15, 16). In addition, the evaluation methods often differ: various "gold standards," such as Western blot (immunoblot) and radioimmunoprecipitation assay, as well as different Western blot interpretation criteria, have been used. ELISAs have frequently been evaluated with selected serum panels which differ from the sera that actually will be tested in the ELISA. To determine which commercial ELISAs have the highest performance level in screening diverse groups of individuals at various degrees of risk for HIV infection in Kinshasa, Zaire, we compared five assays, using two whole-virus lysate assays or "first-generation" ELISAs (Genetic Systems [Seattle, Wash.] LAV and Organon Vironostika [Boxtel, Holland] HTLV-III), two recombinant-antigen assays or "second-generation" ELISAs (DuPont [Geneva, Switzerland] HIV-1 Recombinant and Abbott [Wiesbaden-Delkenheim, Germany] Recombinant HIV-1/HIV-2), and one viral peptide assay or "third-generation" ELISA (DuPont for HIV-1/HIV-2), with Western blot (DuPont) as the standard reference test. The choice of these commercial assays was based primarily upon local availability and price. Although HIV-2 prevalence in Kinshasa is assumed to be low, we included two ELISAs which detect antibodies to both HIV-1 and HIV-2 because systematic screening for HIV-2 might be recommended in settings such as blood banks. Sera were collected from population groups with widely varying HIV-1 seroprevalences, including groups of symptomatic and asymptomatic patients with HIV-1 infection as well as non-HIV-2-infected individuals.

MATERIALS AND METHODS

Study populations. Group 1 serum samples were collected between July 1988 and May 1989 from 500 female prostitutes consecutively seen during their first visit to a research clinic in Kinshasa. Group 2 serum specimens were obtained consecutively from 500 patients for whom a diagnosis of HIV infection was sought and who were consecutively referred by local health care providers to the Projet SIDA diagnostic laboratory between July and August 1989. Group 3 serum specimens were collected from 998 Kinshasa factory workers and their spouses between December 1988 and May 1989. Prior to testing, the 1,998 serum samples were stored at −20°C.

Laboratory methods. The Genetic Systems LAV enzyme immunoassay is a first-generation assay consisting of HIV-1
propagated in a CEM cell line. The infected cell line is cultured, and the virus is purified and inactivated, the viral concentrate is used to coat microwell plates.

The Organon Vironostika anti-HTLV-III test is also a first-generation assay in which the solid-phase antigen is purified human T-cell lymphotropic virus III.

The DuPont HIV-1 recombinant ELISA is a second-generation assay using a recombinant envelope protein which has been purified from an expressing *Escherichia coli* clone.

The Abbott recombinant HIV-1/HIV-2 enzyme immunnoassay allows simultaneous detection of antibodies to HIV types 1 and 2 by the use of HIV-1 core and envelope and HIV-2 envelope viral proteins derived by recombinant-DNA technology.

The DuPont HIV-1/HIV-2 ELISA is a third-generation assay for the detection of antibodies to HIV types 1 and 2 by using a recombinant HIV-1 envelope antigen and a purified HIV-2 synthetic envelope peptide.

**Evaluation methodology.** In carrying out the evaluation, we followed the manufacturers' instructions for each of the five commercial assays. The Vironostika test has different procedure options; we chose the 1:34 serum dilution with the cutoff formula of 0.5 times the mean of the negative control (N) plus the mean of the positive control (P): [0.5(N + P)]. A sample was considered positive by a particular ELISA when it was repeatedly reactive by that ELISA. All samples repeatedly positive by at least one ELISA, as well as 10% of randomly selected samples which were negative by all five ELISAs, were tested with an HIV-1 Western blot test (DuPont). The Western blot was considered positive for HIV-1 when antibodies against at least two of the following three virus-specific proteins were present: p24, gp41, and gp120-160 (3). Samples presenting with only two of these bands and with no other virus-specific antibodies were tested to confirm reproducibility. Any Western blot which had at least one virus-specific band but did not meet the criteria described above was recorded as indeterminate for antibody to HIV-1. Samples which were repeatedly positive by ELISA for HIV-1 and HIV-2 and which were negative or indeterminate by HIV-1 Western blot were analyzed by HIV-2 Western blot (DuPont). These samples were considered positive for HIV-2 if antibodies were reactive with at least one env protein and with either one gag or pol antigen, as recommended by the manufacturer. A sample was defined as truly negative when negative by all five ELISAs and/or negative or indeterminate by Western blot. To confirm whether an ELISA had generated a false-negative result, any ELISA that was negative on the first analysis was repeated on the samples found to be Western blot positive. Samples with a weak-positive HIV-1 Western blot pattern (i.e., presenting with the minimal band patterns) were retested at the Institute of Tropical Medicine, Antwerp, Belgium, where both Western blot and p24 antigen assays (Abbott) were performed. Serologic results for available follow-up sera were also analyzed for samples with a weak-positive or indeterminate Western blot.

**RESULTS**

**Performance of commercial ELISA kits.** Upon completion of ELISA testing, 1,065 of 1,998 serum specimens were found repeatedly reactive by at least one assay (Fig. 1A). An HIV-1 Western blot was performed by all of these samples as well as on 89 randomly selected samples which were negative by all five ELISAs. By Western blot, a total of 443 serum samples were confirmed positive, 321 were negative, and 390 were indeterminate. The distributions of the Western blot results were calculated by population group for sera which were positive with at least one ELISA (Table 1) and for sera negative by all assays (Table 2). Of the sera negative or indeterminate by HIV-1 Western blot, 320 had been repeatedly reactive by an HIV-1 and HIV-2 ELISA and were therefore tested by HIV-2 Western blot (Fig. 1B). None of these sera were confirmed positive for HIV-2; 225 serum samples showed no bands and 95 had indeterminate HIV-2 Western blot results. The sensitivities and specificities as well as the predictive values of the five ELISAs were calculated by population group (Table 3). The band patterns observed among the samples with indeterminate Western blots are described in Fig. 2.

**HIV infection and results for available follow-up sera by population group.**

(i) **Group 1.** Of 500 female prostitutes, 166 (33.2%) were Western blot positive for HIV-1 and none were positive for HIV-2. Of the 88 individuals with indeterminate Western blot results, 67 had available follow-up serum specimens. Twelve prostitutes were monitored for up to 6 months: four prostitutes seroconverted within 4 months, as documented by a distinct positive Western blot, and eight did not show evidence of seroconversion. No seroconversion was detected among the remaining 55 prostitutes who were monitored for at least 12 months.

(ii) **Group 2.** Of the 500 individuals who had been referred to the diagnostic laboratory, 259 (51.8%) were positive by HIV-1 Western blot. None were positive for HIV-2. No follow-up sera were available.

(iii) **Group 3.** Group 3 was composed of 354 (35.5%) male workers with a mean age of 38 years, 3 (0.3%) female workers with a mean age of 23 years, and 641 (64.2%) spouses of male workers with a mean age of 33 years. A seroprevalence of 1.8% was found in this group. Of the 18 seropositive serum specimens, 4 presented with weak minimal-positive Western blot patterns: 2 had p24, p64, and gp120-160 bands, 1 had p24, p53, p64, and gp120-160 bands, and 1 had p24, p55, and gp120-160 bands. These four sera were positive only by the two DuPont ELISAs and were

<table>
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<tr>
<th><strong>Table 1. Western blot results for sera reactive by at least one ELISA</strong></th>
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<td><strong>Group</strong></td>
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<tr>
<td></td>
</tr>
<tr>
<td>1. Prostitutes (n = 299)</td>
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<tr>
<td>(n = 366)</td>
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<tr>
<td>2. Referred patients</td>
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<td>3. Workers and spouses (n = 400)</td>
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**Table 2. Western blot results for sera nonreactive by all evaluated ELISAs**

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<tr>
<th><strong>Group</strong></th>
<th><strong>No. (%) of Western blots</strong></th>
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<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>1. Prostitutes (n = 20)</td>
<td>0 (0)</td>
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<tr>
<td>2. Referred patients</td>
<td>1 (7.7)</td>
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<tr>
<td>(n = 13)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>3. Workers and spouses (n = 56)</td>
<td>0 (0)</td>
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infection. However, these results, attributable to four serum samples with minimal-positive Western blot patterns, should be interpreted with caution. We followed the U.S. Public Health Service (USPHS) criteria, which have not been evaluated with Central African sera for HIV-1 Western blot interpretation. According to other Western blot criteria, such as the DuPont-Food and Drug Administration criteria, the four initial samples would not be considered Western blot positive, because they all lacked antibodies to the p31 antigen. Furthermore, the follow-up results failed to confirm seropositivity by the same USPHS criteria. Therefore, if sensitivities are recalculated for group 3 after correcting for the four weakly positive specimens according to the USPHS criteria, then the Vironostika, Genetic Systems, and Abbott assays each have a sensitivity of 100%. Thus, the current USPHS criteria for Western blot interpretation may not be applicable to African sera, given the high frequency of indeterminate bands seen in this population.

The specificities of the various ELISAs used showed significant differences, varying from 51.7 to 98.4% in the three combined groups, with the lowest values in the population group at lowest risk for HIV infection. Screening assays which lack specificity entail significant costs because of the need for additional confirmation assays.

The negative predictive values obtained for the five commercial ELISAs had a rather narrow range (97.1 to 100%), in contrast to the positive predictive values, which had an extremely wide range (6.6 to 100%). Surprisingly, one of the two first-generation assays had the highest positive predictive values.

Although we did not specifically assess for dual HIV-1 and HIV-2 reactivity, no sera were positive for HIV-2 alone; this suggests a low HIV-2 prevalence in Kinshasa at the time the sera were collected.

The lack of a perfect gold standard for HIV testing continues to complicate assay evaluations. We found 390 serum samples with indeterminate HIV-1 Western blot patterns, or 25% of our defined "true negatives." The proportion of indeterminate samples among the total negatives (i.e., samples negative by all five ELISAs and/or negative or indeterminate by Western blot) was evenly distributed in the three population groups: 26.3, 24.1, and 24.8% in groups 1, 2, and 3, respectively. In our study design, we decided to define these sera as negative. This definition was confirmed for all of the available follow-up sera of group 3. Among the 202 follow-up samples, no seroconversions were detected in samples obtained at least 12 months after the initial analysis. Even among the 67 follow-up serum specimens from the prostitute group with a known high HIV-1 infection incidence, only four (6%) seroconversions were observed after the initial indeterminate Western blot result. Among the prostitutes with an initial negative result (Western blot negative or negative by all five ELISAs), 17 (5.1%) seroconversions were documented during the same follow-up period. Other studies have shown that most individuals with indeterminate Western blots do not appear to be infected with HIV-1 (4, 7, 9, 10). This high prevalence of Western blot-reactive bands in uninfected individuals should raise the level of caution with which this assay is interpreted. Of 390 serum samples presenting with an indeterminate Western blot, 39.7% reacted against p24 (with or without other bands) and 9% reacted against gp120-160. The probability that an independent combination of these two bands reflects a nonspecific reaction rather than infection is $0.40 \times 0.09 = 0.036$, or 3.6%. When the USPHS criteria for Western blot interpretation are used in populations with a high prevalence
TABLE 3. Sensitivities, specificities, and predictive values of five commercial ELISAs for detection of HIV-1 infection in three zairian population groups

<table>
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<tr>
<th>Group</th>
<th>ELISAa (Sensitivity, Specificity, Positive predictive value, Negative predictive value)</th>
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<tr>
<td>1. Prostitutes (33.2%) (n = 500)</td>
<td>GenSys: 100 (85.6, 77.6, 100) Virono: 100 (100, 100, 100) DP-1: 100 (74.3, 65.9, 100) DP-1 and -2: 100 (86.2, 78.3, 100) AB-1 and -2: 99.4 (99.4, 98.8, 99.7)</td>
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<tr>
<td>2. Referred patients (51.8%) (n = 500)</td>
<td>GenSys: 98.5 (90.5, 91.7, 98.2) Virono: 97.3 (98.8, 98.8, 97.1) DP-1: 99.6 (67.9, 99.4) DP-1 and -2: 98.5 (81.7, 85.3, 98.0) AB-1 and -2: 97.7 (92.5, 93.4, 97.4)</td>
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<tr>
<td>3. Factory workers and spouses (1.8%) (n = 998)</td>
<td>GenSys: 83.3 (88.2, 11.5, 99.7) Virono: 77.8 (99.6, 77.8, 99.6) DP-1: 100 (74.1, 6.6, 100) DP-1 and -2: 100 (77.7, 7.6, 100) AB-1 and -2: 83.3 (99.3, 68.2, 99.7)</td>
</tr>
<tr>
<td>Overall (all groups combined) (22.5%) (n = 1,998)</td>
<td>GenSys: 98.4 (88.0, 70.0, 99.5) Virono: 97.5 (99.6, 98.4, 99.3) DP-1: 99.8 (73.5, 51.7, 99.9) DP-1 and -2: 99.1 (80.1, 58.7, 99.8) AB-1 and -2: 97.7 (98.3, 94.1, 99.4)</td>
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a GenSys, Genetic Systems LAV enzyme immunoassay; Virono, Vironostika anti-HTLV III; DP-1, DuPont HIV-1 Recombinant ELISA; DP-1 and -2, Abbott Recombinant HIV-1/HIV-2 enzyme immunoassay.

b Four samples had weakly positive Western blots, three of which were negative on follow-up. Therefore, sensitivities should be adjusted to at least 93.3% and possibly to 100% (see text).

of indeterminate Western blot patterns, some sera with minimal positive Western blot band patterns might reflect false-positive reactions rather than infection. Therefore, the criteria of the USPHS, the World Health Organization, DuPont, and others for Western blot interpretation need to be thoroughly evaluated for Central African sera, and other alternative confirmation assays should be studied.

We conclude that, in the Kinshasa populations we studied, the negative predictive values of the evaluated ELISAs did not differ much, particularly in contrast to the widely varying positive predictive values; therefore, the most practical and cost-efficient assays which generate the fewest false-positive and indeterminate results should be selected for routine screening of HIV-1 infection.

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