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Comparison of 10 Enzyme Immunoassays for Detection of Antibody to Human Immunodeficiency Virus Type 2 in West African Sera

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The efficacies of nine enzyme-linked immunosorbent assays (EIA) for antibody to human immunodeficiency virus type 1 (HIV-1) and one EIA for antibody to HIV-2 in detecting antibody to HIV-2 were studied. The competitive EIAs for antibody to HIV-1 were less sensitive than the indirect EIAs. The overall prevalence of positive results was between 28 and 51% with the competitive EIAs and between 70 and 93% with the indirect EIAs. Most of the EIAs were less sensitive in detecting antibody to HIV-2 in sera from people with acquired immunodeficiency syndrome-like diseases than in sera from symptomless individuals. The results indicate that there is a high degree of cross-reactivity between HIV-1 and HIV-2 by EIA, indicating that serotype specificity must be determined by Western blot (immunoblot) with both sets of viral antigens. The results are relevant for discussing public health strategies, especially the screening of blood donors; competitive EIAs for antibody to HIV-1 are not sensitive enough to be used in areas where HIV-2 is prevalent (West Africa).

A category of human retroviruses has been recently identified which is related to human immunodeficiency virus type 1 (HIV-1) but is more closely related to a group of simian retroviruses (STLV3/SIV). These viruses, named HIV-2, formerly human T cell lymphotropic virus type IV (HTLV-IV) or lymphadenopathy-associated virus type 2 (LAV-2), are prevalent in West Africa and have been found occasionally in Europe (1, 4, 6, 9, 10, 11, 13, 18, 19, 21). There is a high degree of immunologic cross-reactivity between the *gag* gene products and the *pol* gene products of HIV-1 and HIV-2 (1–4, 9, 18). In contrast, the envelope glycoproteins that are the most frequently detected antigens by antibodies from exposed individuals are less cross-reactive. Knowing these cross-reactivities, we evaluated the efficacy of 10 enzyme immunoassays (EIAs) for antibody to HIV in detecting antibody to HIV-2 in sera from West African residents.

MATERIALS AND METHODS

Sera. A total of 43 sera matching the original definition of sera positive for antibody to HIV-2 were used in this study (3, 4, 18). All these sera were collected in Ivory Coast during 1986; 23 sera were from symptomless individuals belonging to different risk groups, such as prostitutes, prisoners, or individuals suffering from a sexually transmitted disease, and 20 sera were from patients with acquired immunodeficiency syndrome (AIDS)-like diseases. These patients suffered mainly from a severe respiratory infection associated with weight loss (over 10% of body weight) in 18 cases (90%), profound asthenia in 18 cases (90%), and persistent diarrhea in 9 cases (45%).

Serotype specificity. Serotype specificity was determined by differential reactivity to envelope glycoproteins of HIV-1 and HIV-2 by using the Western blot (immunoblot) procedure as already described (3, 4, 18). The Western blot analysis was performed with strips coated with either $HTLV\text{-}III_B$ (HIV-1) virions or $HTLV\text{-}IV_{P289}$ (HIV-2) virions. Sera that were reactive to gp32 (the transmembrane glycoprotein; 16, 17) of $HTLV\text{-}IV_{P289}$ were defined as HIV-2 antibody positive. Sera that reacted to gp41 of $HTLV\text{-}III_B$ were defined as HIV-1 antibody positive and not used in this study.

EIAs. Nine commercial HIV-1 test kits were used (Table 1), including six indirect EIAs (Abbott Laboratories [first- and second-generation tests]; Du Pont Co.; Organon-Teknika; Ortho Diagnostics, Inc.; and Diagnostics Pasteur [Elavia 1 with control antigen]); two competitive EIAs (Calbiochem-Behring and Wellcome Diagnostics); and a recombinant competitive EIA (Envacore; Abbott) that tests for antibody to core or envelope proteins. One commercial EIA kit for antibody to HIV-2 (Elavia 2; Diagnostics Pasteur) was also evaluated.

RESULTS

Representative Western blots of typical HIV-2-antibody-positive sera are shown in Fig. 1. In addition to the transmembrane glycoprotein gp32, a smearing protein of approximately 68 kilodaltons resembling a glycoprotein was recognized by all the HIV-2-positive sera. This 68-kilodalton protein, which appears to be highly immunogenic, remains to be identified.

Sensitivities of the 10 assays in detecting antibody to HIV-2 are shown in Table 2. The distribution patterns of responses in these EIAs, except for the Envacore from Abbott, are represented in Fig. 2. The results indicate that the competitive EIAs for HIV-1 were less sensitive than the indirect EIAs were in detecting antibody to HIV-2. Of the 43 HIV-2-positive sera, 12 (28%) and 16 (37%) were positive by the Wellcome and Calbiochem-Behring assays, respectively. The prevalence of positive sera may be increased to 51% (22 of 43) with the Calbiochem-Behring assay if a cutoff value equal to 0.7N is used (N being the mean absorbance of the

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TABLE 1. Characteristics of the 10 EIAs

EIA	Principle	Antigen	Cutoff[a]
Abbott Laboratories			
1st generation; HTLV-III	Indirect	Virus	N + 0.1P
2nd generation: recombinant HTLV-III	Indirect	Recombinant protein	N + 0.15P
Envacore; core antibody	Competition	Recombinant protein	(P + N)/2
Envacore; envelope antibody	Competition	Recombinant protein	(P + N)/2
Du Pont Co. HTLV-III ELISA[b]	Indirect	Virus	P/2
Organon-Teknika Vironostika	Indirect	Virus	0.5(N + P) 0.2(4N + P)
Ortho Diagnostics HTLV-III ELISA	Indirect	Virus	0.25 + N
Diagnostics Pasteur			
Elavia 1	Indirect	Virus (LAV-1)	ΔOD[c] = 0.3
Elavia 2	Indirect	Virus (LAV-2)	ΔOD = 0.3
Calbiochem-Behring Enzygnost anti-HIV microdilution	Competition	Virus	0.5N
Wellcome Research Laboratories Wellcozyme anti-HTLV-III	Competition	Virus	P + 10%

[a] Cutoff value calculations are presented as given by the manufacturer. N, Mean of negative controls; P, mean of positive controls.
[b] ELISA, Enzyme-linked immunosorbent assay.
[c] ΔOD, Change in optical density.

negative controls) as recommended by A. M. Couroucé et al. (12). The indirect EIAs for antibody to HIV-1 detected from 70% (Organon-Teknika) to 93% (Ortho) of the sera positive for antibody to HIV-2. Interestingly, all the EIAs for antibody to HIV-1, except for that from Ortho, were less sensitive in detecting antibodies to HIV-2 in sera collected from sick people (AIDS-like diseases) than in sera collected from symptomless individuals (Table 2). For instance, the sensitivity fell from 91% (21 of 23) in symptomless individuals to 60% (12 of 20) in patients suffering from an AIDS-like disease when the first-generation assay from Abbott was used. Similarly, the sensitivity dropped from 48% (11 of 23) to 5% (1 of 20) with the Wellcome assay.

Of the sera positive for antibody to HIV-2, 77% (33 of 43) and 56% (24 of 43) were positive for antibody to HIV-1 core proteins and for antibody to HIV-1 envelope proteins, respectively, by the Envacore assay (Table 2). A total of 20 HIV-2-positive sera (47%) were positive for both envelope and core recombinant proteins of HIV-1 (Fig. 3), and 13 (30%) were positive only for antibody to HIV-1 core recom-

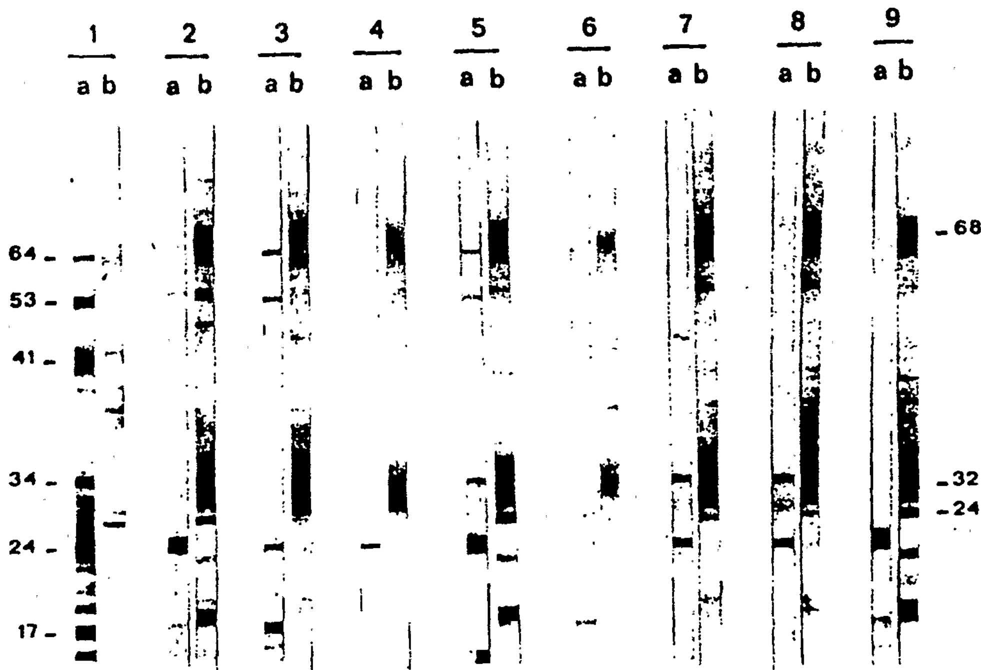

FIG. 1. Representative strips of Western blot assays against HIV-1 (lanes a) and HIV-2 (lanes b). Panels: 1, sera positive for antibody to HIV-1; 2 to 9, sera positive for antibody to HIV-2. The molecular sizes of the proteins (in kilodaltons) are indicated for HIV-1 (left side) and HIV-2 (right side).

binant protein. Four sera (9%) were positive only for antibody to HIV-1 envelope recombinant protein. This assay was also less sensitive in detecting cross-reacting antibodies in sera from patients with AIDS-like diseases than in sera from symptomless individuals (Table 2).

The Elavia 2 assay that uses LAV-2 as the viral antigen detected 98% (42 of 43) of sera positive for antibody to HIV-2. One serum collected from a sick person was found repeatedly negative (absorbance value cutoff, 0.8). This serum contained only antibody to HIV-2 glycoproteins (Fig. 1, lane 6). However, it was weakly positive in seven of the nine EIAs for antibody to HIV-1.

DISCUSSION

This study shows that commercial HIV-1 antibody test kits are not equally efficient in detecting HIV-2-antibody-positive sera. Particularly, competitive EIAs are less sensitive than indirect EIAs. This conclusion confirms preliminary observations (7, 14) which indicated that cross-reacting HIV-2 antibodies do not have enough affinity to compete with the anti-HIV-1 conjugate. HIV-2-positive AIDS patients were reported not to have serological cross-reactivity which HIV-1 (8, 9). This is not confirmed by the present extensive study. Our study indicates that the indirect EIAs for antibody to HIV-1 detected from 50% (Organon-Teknika) to 95% (Ortho) of HIV-2-antibody-positive sera collected from patients with AIDS-like diseases. The competitive EIAs detected only from 5% (Wellcome) to 20 to 40% (Calbiochem-Behring) of the same HIV-2-antibody-positive sera. However, it is clear that most of the EIAs for antibody to HIV-1 were less sensitive in detecting antibody to HIV-2 in sera collected from sick people than in sera collected from

TABLE 2. Efficacies of the 10 EIAs in detecting antibody to HIV-2 in 43 positive sera

EIA	No. (%) of positive results in individuals with: No symptoms (n = 23)	No. (%) of positive results in individuals with: AIDS-like diseases (n = 20)	Total no. (%) of positive results (n = 43)
Abbott Laboratories			
1st generation	21 (91)	12 (50)	33 (77)
2nd generation	21 (91)	13 (65)	34 (79)
Core antibody	21 (91)	12 (60)	33 (77)
Envelope antibody	14 (61)	10 (50)	24 (56)
Core + envelope antibodies	22 (96)	15 (75)	37 (86)
Du Pont Co.	22 (96)	16 (80)	38 (88)
Organon-Teknika			
0.5(N + P)	17 (74)	5 (25)	22 (51)
0.2(4N + P)	19 (83)	11 (55)	30 (70)
Ortho Diagnostics	21 (91)	19 (95)	40 (93)
Diagnostics Pasteur			
Elavia 1	18 (78)	13 (65)	32 (74)
Elavia 2	23 (100)	19 (95)	42 (98)
Calbiochem-Behring			
0.5N	12 (52)	4 (20)	16 (37)
0.7N	14 (61)	8 (40)	22 (51)
Wellcome Research Laboratories	11 (48)	1 (5)	12 (28)

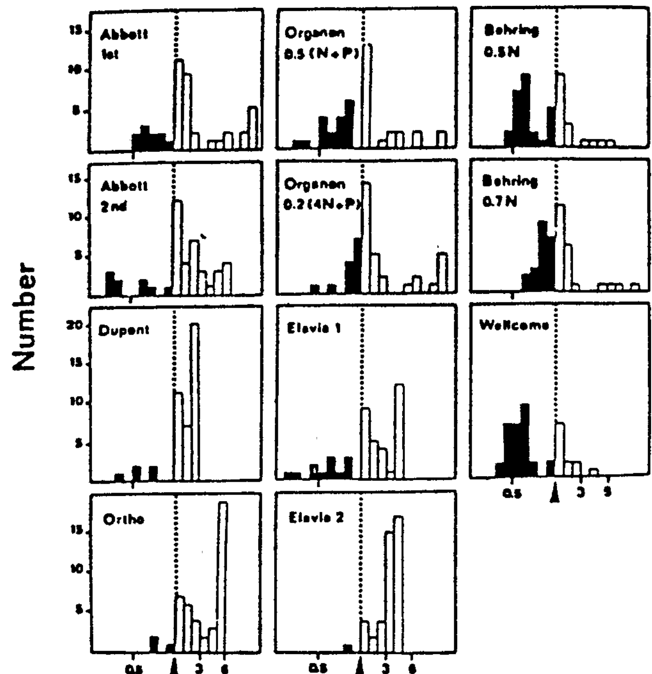

FIG. 2. Distribution patterns of responses in the EIAs for 43 sera positive for antibody to HIV-2. Results are expressed as the ratios of optical density to cutoff value, except for the Calbiochem-Behring and Wellcome assays, for which results are expressed as the ratios of cutoff value to optical density.

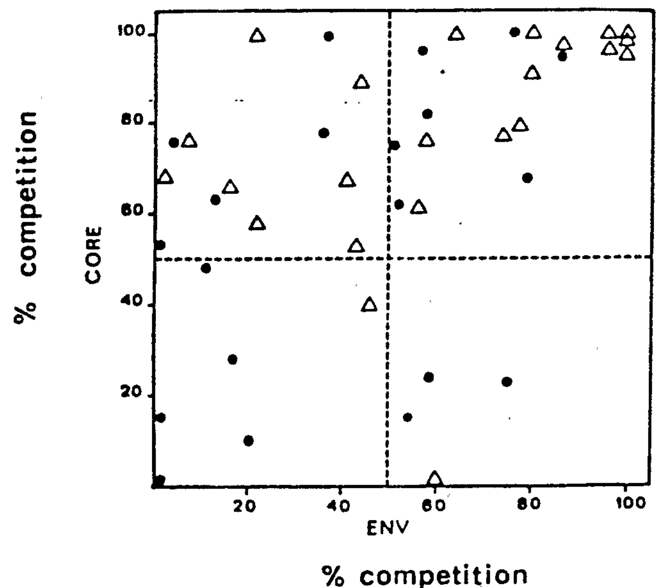

FIG. 3. Distribution of competition values obtained in the recombinant competitive EIA that tests for antibody to core or envelope proteins of HIV-1 (Envacore). Results are expressed as percentages of competition calculated as follows: % competition = $\{1 - [(A - P)/(N - P)]\} \times 100$, with A being the sample absorbance value, P being the positive control mean absorbance value, and N being the negative control mean absorbance value. Samples with competition values above 50% were considered positive. Symbols: △, symptomless individuals; ●, patients with AIDS-like diseases.

symptomless individuals. This may be ascribable to low antibody titers or to an absence of antibody to the core proteins that are also common in patients with HIV-1-associated AIDS (5, 23), the disappearance or absence of antibody to the core proteins of HIV-2 reflecting a lack of major cross-reacting antibodies.

It has been shown by radioimmunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (RIP/SDS-PAGE) that HIV-1 and HIV-2 share epitopes in all the major viral antigens (18). The cross-reactivity is prominent for the *gag*-encoded proteins, but it must be noted that some human sera may recognize both envelope glycoproteins with differential efficacy on the basis of RIP/SDS-PAGE analyses. In the present study, we demonstrate that 56% of people exposed to HIV-2 developed antibodies that cross-reacted with a recombinant-DNA-derived HIV-1 antigen from the *env* region containing all amino acids of gp41 as well as a portion of gp120. This indicates that the Envacore assay did not allow a serotype-specific diagnosis based on the recombinant envelope protein of HIV-1. This observation has important implications. First, it confirms that antigens encoded by the *env* genes of HIV-1 and HIV-2 are serologically cross-reactive when nondenatured proteins, even those produced by recombinant technology, are used. Further characterization of these cross-reactive epitopes in the *env*-encoded proteins may be important for evaluation as subunits for an AIDS vaccine. Second, care should be taken in interpreting serological diagnosis of HIV infections. A sensitive assay able to discriminate between antibodies to HIV-1 envelope proteins and HIV-2 envelope proteins must be used. In this respect, Western blot seems to be very useful; antibody-positive sera give a strong signal with the transmembrane glycoprotein of the corresponding serotype alone. RIP/SDS-PAGE is more able to detect cross-reactive antibodies to the *env*-encoded gp120 and gp160 (18), indicating that epitopes associated with the primary structure of the transmembrane proteins are weakly conserved if at all between HIV-1 and HIV-2. However, some amino acid residues important for the secondary and tertiary structures of the envelope proteins are conserved. Therefore, common or cross-reactive conformational epitopes, detected by RIP/SDS-PAGE but not by Western blot, are conserved; they are present in *env* gene products of both viruses (16, 17). It has been shown recently that it is possible to discriminate between antibodies to HIV-1 and antibodies to HIV-2 by using synthetic peptides located within the transmembrane glycoproteins of these related viruses (15, 20, 22). These studies suggest that peptides of both conserved and nonconserved epitopes of the envelope glycoproteins of related HTLVs may be useful for specific diagnostic purposes.

Our study presents data on the efficacy of 10 EIAs in detecting antibody to HIV-2. The results indicate that competitive EIAs for antibody to HIV-1 are not sensitive enough to be used in areas where HIV-2 is prevalent (West Africa). In these areas, serotype specificity must be determined by immunoblot with both sets of viral antigens whether an indirect EIA for HIV-1 or for HIV-2 is used as a screening procedure.

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