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Confirmation and Differentiation of Antibodies to Human Immunodeficiency Virus 1 and 2 with a Strip-Based Assay Including Recombinant Antigens and Synthetic Peptides

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We evaluated the use of the INNO-LIA HIV-1/HIV-2 Ab test (LIA HIV; Innogenetics) for the confirmation of antibodies to human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2). The test includes three recombinant HIV-1 proteins: p24 (*gag*), p17 (*gag*), and endonuclease (p31; *pol*), in combination with two synthetic peptides derived from the *env* gene of HIV-1 and one synthetic peptide selected from the *env* gene of HIV-2. Analysis of 450 sera from blood donors, 220 sera from patients with non-HIV pathology, and 28 Western blot (WB) p24-only reactive sera revealed no false-positive results, and the rate of indeterminate results was substantially lower than that with WB. Testing of 334 WB-confirmed HIV antibody-positive sera (309 HIV-1; 25 HIV-2) revealed no false-negative results. In two of seven seroconversion panels tested, LIA HIV detected the presence of HIV antibodies before WB did. In the other five panels, LIA HIV and WB confirmed the presence of HIV antibodies in the same sample. The LIA HIV assay therefore appears well suited for routine confirmation of the presence of HIV-1 and HIV-2 antibodies.

Additional Keyphrases: *gene probes · recombinant proteins and peptides · Western blot compared · confirmatory testing · acquired immunodeficiency syndrome*

Human immunodeficiency virus type 1 (HIV-1) is the etiologic agent of the acquired immunodeficiency syndrome (AIDS) (*1–4*).[3] A related retrovirus, HIV-2, is prevalent in West Africa and has been found occasionally in Europe (*5–8*). Tests for antibodies to HIV have been widely used in screening blood donors and others to ascertain the absence of prior infection by the virus. Most routinely used screening tests are enzyme-linked immunosorbent assays (ELISAs), which detect both anti-HIV-1 and anti-HIV-2 antibodies. Because false-positive reactions in these present-day screening tests can occur (*9–11*), at least one confirmation test, usually Western immunoblotting (WB), is therefore needed (*12–14*). These confirmatory tests must be not only sensitive, but also very specific and reproducible, and must allow discrimination between HIV-1 and HIV-2.

The presence of human cellular antigens in the HIV preparations used to make WBs for HIV is known to give rise to indeterminate and atypical WB patterns (*10, 15*). Virion preparation may also vary significantly in the amount of certain viral antigens included, which may lead to underestimation of the antibodies directed against these antigens. In addition, the interpretative criteria may vary substantially between laboratories.

In this study, we examined the sensitivity and specificity of the INNO-LIA HIV-1/HIV-2 Ab assay (LIA HIV; Innogenetics, Ghent, Belgium) for the confirmation of HIV antibodies by using the LIA (line immunoassay) HIV-specific criteria. In the LIA HIV test, purified recombinant HIV-1 antigens p24 (*gag*), p17 (*gag*), and endonuclease (p31; *pol*) are used in combination with synthetic peptides derived from gp41 (*env;* HIV-1) and gp36 (*env;* HIV-2). To discriminate between HIV-1 and HIV-2 antibodies, the test involves specific peptides from the transmembrane glycoproteins of both viruses. The antigens are applied as parallel lines on a test strip. In addition to the specific HIV antigen lines, several control lines are included on the strip: three human IgG lines, to monitor the test procedure and to serve as internal references for rating purposes, and goat anti-human IgG, to verify the addition of a sample containing human IgG.

Materials and Methods

LIA HIV

The test strips (5 × 0.4 cm) consist of a plastic backing covered with a chemically activated membrane that allows covalent binding of proteins through free amino groups. The purified antigens are applied directly onto the strips as parallel lines. The general layout of the LIA HIV strips and the position of the antigens applied are illustrated in Figure 1.

Three *Escherichia coli*-produced recombinant proteins and one mixture of two synthetic peptides are used as HIV-1 antigens. For expression of the p24 equivalent, a restriction fragment encoding the HIV-1 *gag* amino acids 120 through 436 was inserted into an *E. coli* expression vector (pIGAL10) to obtain a 41-kDa fusion protein with the vector-encoded leader peptide (*16*). The expression of p17 and endonuclease information was achieved by introducing of the relevant gene fragments into the expression vector pIGLH23δ2. The p17 information expressed comprises the amino acids 1 through 119 encoded by the HIV-1 *gag* gene, whereas the endo-

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[3] Nonstandard abbreviations: AIDS, acquired immunodeficiency syndrome; HIV-1, -2, human immunodeficiency virus types 1 and 2; LIA, line immunoassay; WB, Western blot (immunoblot); ELISA, enzyme-linked immunosorbent assay; and ASTPHLD, Association of State and Territorial Public Health Laboratory Directors.
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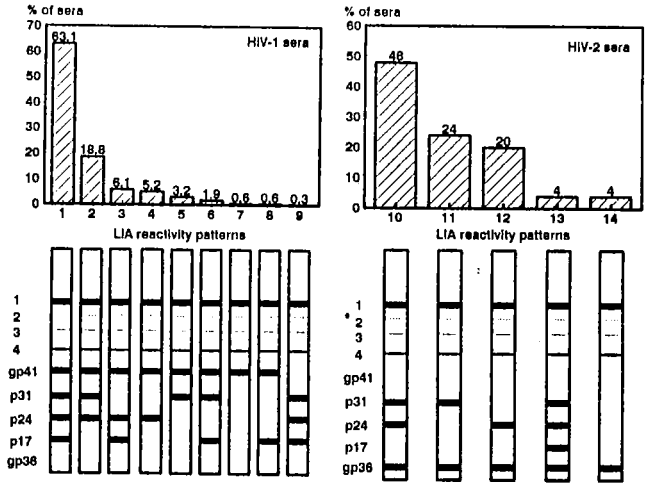

Fig. 1. Reactivity of WB-confirmed HIV-1 (n = 309) and HIV-2 sera (n = 25) with the LIA HIV strips

Layout of the LIA HIV test strip: The *upper* part of the strip contains a set of control lines (*lines 1–4*). This group of human IgG lines is used as internal references to check the test procedure and serves as internal standards for rating purposes. An anti-human IgG line (*line 4*) is used to check the addition of samples containing human IgG. The second group of lines contains HIV-1 antigens: synthetic gp41 and *E. coli*-produced recombinant proteins equivalent to HIV-1 endonuclease (p31), p24, and p17. The third part of the strip contains a synthetic peptide derived from the HIV-2 transmembrane antigen (gp36)

nuclease information starts at a methionine residue located 135 amino acids before the end of the *pol* gene. In both cases, the HIV proteins produced are not fused to any foreign peptide sequences. The gp41 antigen consists of a mixture of two synthetic peptides that were selected by immunoscreening for their ability to react with HIV-1 and not with HIV-2 sera. Both peptides are localized between amino acids 588 and 620 of the glycoprotein precursor of HIV-1.

The synthetic peptide used as the HIV-2-specific antigen was selected by immunoscreening for its ability to react with HIV-2 and not with HIV-1 sera. This HIV-2 peptide corresponds to amino acids 584 to 603 of the glycoprotein precursor.

The LIA HIV test was performed according to the instructions in the insert. In summary, each LIA HIV strip is incubated in a plastic trough (6.5 × 0.6 cm) containing 1 mL of sample diluent to which 10 μL of sample is added. Specific antibodies, if present in the sample, bind to their respective antigens on the strip. The standard sample incubation period is 1 h. Samples with indeterminate results are repeated by overnight sample incubation. Subsequently, an affinity-purified goat anti-human IgG labeled with alkaline phosphatase (EC 3.1.3.1) is added. The labeled antibody binds to any HIV-antigen/antibody complex previously formed. Incubation with substrate solution (5-bromo-4-chloro-3-indolyl phosphate *p*-toluidine salt, nitroblue tetrazolium) produces a dark brown precipitate. Color development is stopped by washing the strip with diluted sulfuric acid.

Positive or negative results are determined by visually comparing the intensity of an antigen line with the intensities of the cutoff (line 2) and the control lines (lines 1, 3, and 4). An antigen is nonreactive (0) with a particular serum if the intensity of the antigen line is less than the intensity of line 2 (see Figure 1). An antigen is considered weakly reactive (±) if the intensity of that antigen line is equal to the intensity of line 2, but less than the intensity of line 3. An antigen is considered reactive (1+) if the intensity of the antigen line is equal to the intensity of line 3 or between the intensities of lines 3 and 4, strongly reactive (2+) if the intensity of the antigen line is equal to the intensity of line 4 or between the intensities of lines 4 and 1, very intensely reactive (3+) if the intensity of the antigen line is equal to the intensity of line 1, and maximally reactive (4+) if the intensity of the corresponding antigen line is greater than the intensity of line 1.

Western Blot Assays

For immunoblot assays we used WB tests for HIV-1 (Du Pont, Wilmington, DE) and HIV-2 (Du Pont; or New LAV blot II, Diagnostics Pasteur, Paris, France) according to the instructions of the manufacturers. Positive results were established according to the criteria proposed by the Association of State and Territorial Public Health Laboratory Directors (ASTPHLD) Third Consensus Conference on HIV Testing (*17*): i.e., reactive with two of the three major bands of diagnostic significance—gp160 or gp120, gp41, and p24. On the basis of this set of rules, a negative serum shows no reactivity with any HIV-1 protein, and indeterminate samples display bands that do not meet the criteria necessary for positive interpretation. The presence of HIV-2 antibodies is considered confirmed if one envelope protein plus any other virus-specific band is present, or if two envelope proteins are revealed; a negative serum shows no reactivity on the HIV-2 WB; and an indeterminate serum reveals bands that do not meet the criteria for positive interpretation.

Quantification of LIA HIV Strips and WB Assays

Quantitative information about the intensity of the signals on both LIA HIV and WB strips was obtained by reflectance densitometry (TLC scanner II equipped with CATS software 3.03; Camag, Muttenz, Switzerland) at 550 nm (tungsten or mercury lamp) at a speed of 5 mm/s. The sensitivity was set automatically (a span of 7), the offset adjusted to 10%, and the monochromator bandwidth placed at 10 nm, in combination with a slit size of 0.025 × 0.25 mm. Signal integration was performed with baseline correction, and the peak-height threshold was set at 2 mV, with use of 10-point filtering and a data selection factor of 1. The results are expressed as voltage differences between the baseline and the top of the peak, measured in millivolts by the photomultiplier.

Sera

The specificity of the LIA HIV test was evaluated by using sera from healthy subjects (n = 450) and from subjects with non-HIV pathologies (n = 220). In addition, we examined the specificity of the LIA HIV by using 28 sera with atypical WB patterns. WB-confirmed (ASTPHLD criteria) HIV-1 (n = 309) and HIV-2 (n = 25)

sera were used to analyze the HIV antibody reactivity patterns observed on the LIA HIV strips. A panel of low-titer HIV-1 sera (panel B01; Boston Biomedica Inc., W. Bridgewater, MA) was used to estimate analytical sensitivity, and the sensitivities of the LIA HIV and the WB for detecting seroconversion were compared by assaying seven commercially available HIV-1 seroconversion panels (Boston Biomedica).

Results

Specificity: The specificity of the LIA HIV was evaluated by using the 670 sera from seronegative individuals and 28 sera that were WB-reactive with p24 only. None were found positive according to the criteria of the LIA HIV (Table 1). The 670 samples from seronegative individuals included sera from 450 blood donors, 45 patients with autoimmune disease, 60 with IgM antibodies to Epstein–Barr Virus, 10 with above-normal concentrations of C-reactive protein, 13 with increased concentrations of rheumatoid factor, 10 with IgM-toxoplasma antibodies, 10 positive for hepatitis B virus surface antigen, 10 positive for hepatitis B virus core antibodies, 10 positive for hepatitis B virus surface antibodies, 10 positive for anti-nuclear factor, five with IgM-hepatitis A virus antibodies, 11 with above-normal concentrations of human placental lactogen, six with increased concentrations of human choriogonadotropin, 10 hyperlipemic sera, and 10 hemolytic sera. Four sera (three blood donors, one hemolytic serum) were reactive only with p24 in the LIA HIV, three of which were also p24-reactive in WB (Table 2). Of 28 sera previously found to be reactive only to p24 in WB, 21 (75%) did not show any reactivity on the LIA HIV strips. Of the remaining seven sera, three showed a positive reaction to p24 (1+), one was positive to p17 (1+), and three were borderline positive to p24 (±). No reactivity patterns that might be attributable to interference from human anti-*E. coli* antibodies were observed, and incubation of the LIA HIV strips with rabbit anti-*E. coli* antiserum did not reveal any reactivity.

Sensitivity: Using the LIA HIV criteria, we could confirm each of the 309 WB HIV-1-positive sera and each of the 25 WB HIV-2-positive sera (Table 2). The distribution of the LIA HIV reactivity patterns of HIV-1 and HIV-2 sera is shown in Figure 1. Antibodies against at least two different viral proteins could be detected in 99.4% of the HIV-1 sera; gp41 was the single reactive antigen in only 0.6% of the sera.

One serum (patient Y.S.) was not reactive with the gp41 epitopes on the LIA HIV strip. This observation illustrates the importance of the criterion p31 + p24 and (or) p17, because Y.S. was clearly WB-positive for all major bands, including gp41. The Y.S. serum is unique by not reacting with the immunodominant epitopes included on the test strip, which are recognized by all other HIV-1 sera tested.

All of the HIV-2 sera tested reacted strongly with the HIV-2 synthetic peptide on the LIA HIV, and all but one (4%) contained antibodies cross-reactive with p24 (72%) and (or) p31 (76%). None of the WB-confirmed HIV-1 sera showed reactivity with the HIV-2 peptide in the LIA HIV, and no cross-reactivity was observed between the *env* peptides of HIV-1 and HIV-2. Only one of the HIV-2 sera (4%) cross-reacted with the HIV-1 p17 antigen, in contrast to 71.6% of the HIV-1-positive sera that contained antibodies directed against this antigen.

Each of the 15 members of the HIV-1 low-titer panel was reactive with at least gp41 and p24 in the LIA HIV.

To compare the sensitivity of the LIA HIV and the WB, we analyzed seven seroconversion panels by both methods. The results are summarized in Table 3. For panels C and G, seroconversion could be detected earlier with the LIA HIV than with the WB. For panels A, D, H, I, and J, seroconversion was detected by both tests at the same time. For all seven panels, reactivity on the LIA HIV gp41 line could be detected before a reaction was observed on the WB gp41 antigen. For panels C and G, LIA HIV gp41 reactivity became evident one sample before gp160 was found reactive in the WB; in the other panels, the sample showing the first LIA HIV gp41 reactivity coincided with the first reactivity to gp160 in the WB. In each of the HIV-1 seroconversions, the first antibodies detected by the LIA HIV were directed against gp41 and p24. In panels A, D, H, and J (Table 3), antibodies against p17 were also detected early in the seroconversion. Antibodies against p31, on the other

Table 1. Criteria for Confirmation of the Presence of HIV Antibodies in Human Serum Determined with the LIA HIV Test

	Antigen				
Result	gp41	p31	p24	p17	gp36
HIV-1	X	(X)	(X)	(X)	0
HIV-1	0	X	X	(X)	0
HIV-1	0	X	(X)	X	0
Indeterminate	0	0	X	(X)	0
Indeterminate	0	0	(X)	X	0
Indeterminate	0	X	0	0	0
Dually reactive	X	(X)	(X)	(X)	X
HIV-2	0	(X)	(X)	(X)	X
Negative	0	0	0	0	0

X = reactivity required, (X) = reactivity optional, 0 = no reactivity.

Table 2. Reactivity of Sera from Different Patient Groups with the LIA HIV

	n	Positive	Indeterminate	Negative
WB-negative				
Blood donors	450	0	3[a]	447
Non-HIV pathology	220	0	1[b]	219
WB-indeterminate				
WB p24 only	28	0	7[c]	21
WB-positive				
HIV-1	309	309	0	0
HIV-2	25	25	0	0

[a] Two sera, 1+ for p24 (both p24 pos.), and one serum, ± for p24.
[b] One serum, 1+ for p24 (WB neg.).
[c] Three sera, 1+ for p24; one serum, 1+ for p17; and three sera, ± for p24.

Table 3. Sensitivity of LIA HIV and WB HIV Tests at Seroconversion

	LIA					HIV-1 Du Pont WB								
	gp41	p31	p24	p17	gp36	gp160	gp120	p66	p55	p51	gp41	p31	p24	p17
Panel A														
05/04/81	−	−	±	−	−	−	−	−	−	−	−	−	±	−
07/08/81	−	−	±	−	−	−	−	−	−	−	−	−	±	−
07/29/81	−	−	±	−	−	−	−	−	−	−	−	−	±	−
08/19/81	++	−	++	+	−	+	−	−	−	−	−	−	++	−
09/02/81	++	−	++	+	−	++	±	−	±	−	−	−	+++	−
09/09/81	++	−	++	++	−	+++	+	−	±	−	−	−	+++	±
09/16/81	++	−	++	++	−	+++	+	−	±	−	−	−	+++	+
09/23/81	++	−	++	++	−	+++	+	−	±	−	−	−	+++	+
10/14/81	++	−	++	++	−	+++	+	−	±	−	−	−	+++	+
Panel C														
07/16/85	−	−	−	−	−	−	−	−	−	−	−	−	−	−
07/23/85	−	−	−	−	−	−	−	−	−	−	−	−	−	−
07/25/85	±	−	±	−	−	−	−	−	−	−	−	−	±	−
07/30/85	+	−	+	−	−	±	−	−	−	−	−	−	+	−
08/01/85	+	−	+	−	−	±	−	−	−	−	−	−	+	−
08/06/85	+	−	+	−	−	+	−	−	−	−	−	−	++	−
08/08/85	++	−	++	±	−	+	−	−	−	−	−	−	++	−
08/13/85	++	−	++	±	−	+	−	−	−	−	−	−	++	−
08/15/85	++	−	++	+	−	+	−	−	−	−	−	−	+++	−
08/20/85	++	±	+++	+	−	++	±	+	−	−	±	−	+++	−
08/27/85	+++	+	+++	+	−	++	+	++	+	+	+	−	+++	−
08/29/85	+++	+	+++	+	−	+++	+	++	+	+	+	±	+++	−
09/10/85	+++	+	+++	+	−	+++	++	++	++	++	++	+	+++	±
09/12/85	+++	++	+++	+	−	+++	++	++	++	++	++	+	+++	±
09/17/85	+++	++	+++	++	−	+++	++	++	++	++	++	+	+++	+
09/19/85	+++	++	+++	++	−	+++	++	++	++	++	+++	+	+++	+
09/24/85	+++	++	+++	++	−	+++	++	++	++	++	+++	+	+++	+
09/26/85	+++	++	+++	++	−	+++	++	++	++	++	+++	+	+++	+
Panel D														
04/29/81	−	−	−	−	−	−	−	−	−	−	−	−	−	−
05/20/81	−	−	−	−	−	−	−	−	−	−	−	−	−	−
06/17/81	−	−	−	−	−	−	−	−	−	−	−	−	−	−
07/30/81	+	−	+++	++	−	+	+	−	+	±	±	±	++	+
08/06/81	+	−	+++	++	−	+	+	−	+	±	±	±	++	+
Panel G														
11/14/88	−	−	−	−	−	−	−	−	−	−	−	−	−	−
11/17/88	−	−	−	−	−	−	−	−	−	−	−	−	−	−
11/21/88	±	−	−	−	−	−	−	−	−	−	−	−	−	−
11/25/88	++	−	++	−	−	±	−	−	−	−	−	−	+	−
11/28/88	++	−	++	−	−	±	−	−	−	−	−	−	+	−
12/02/88	++	−	++	−	−	±	−	−	−	−	−	−	++	−
12/08/88	++	−	++	−	−	+	−	−	−	−	−	−	++	−
12/12/88	++	−	+++	−	−	++	±	−	±	−	−	−	++	−
12/16/88	++	−	+++	−	−	++	±	−	±	−	−	−	++	−
12/19/88	++	±	+++	±	−	++	±	−	±	−	−	−	++	±
Panel H														
01/13/89	−	−	−	−	−	−	−	−	−	−	−	−	−	−
01/25/89	−	−	−	−	−	−	−	−	−	−	−	−	−	−
01/25/89	−	−	−	−	−	−	−	−	−	−	−	−	−	−
02/01/89	−	−	−	−	−	−	−	−	−	−	−	−	−	−
02/08/89	−	−	−	−	−	−	−	−	−	−	−	−	−	−
02/10/89	+++	+	++	+	−	+++	+	−	−	−	−	±	++	+
Panel I														
01/23/89	−	−	±	−	−	−	−	−	−	−	−	−	−	±
01/30/89	−	−	±	−	−	−	−	−	−	−	−	−	−	±

(*Continued on next page*)

Table 3. Continued

	LIA					HIV-1 Du Pont WB								
BBI	gp41	p31	p24	p17	gp36	gp160	gp120	p66	p55	p51	gp41	p31	p24	p17
02/06/89	+	–	++	–	–	+	–	–	–	–	–	–	+	±
02/08/89	+	–	++	±	–	+	–	–	±	–	–	–	+	±
02/13/89	++	–	++	±	–	+	–	–	+	±	±	–	+	+
02/15/89	++	–	++	±	–	+	±	–	+	±	±	–	++	+
02/20/89	++	–	++	±	–	+	±	–	+	±	±	–	++	+
02/22/89	++	–	++	±	–	+	±	–	+	±	±	–	++	+
Panel J														
05/11/89	–	–	–	–	–	–	–	–	–	–	–	–	–	–
05/25/89	–	–	–	–	–	–	–	–	–	–	–	–	–	–
06/06/89	++	–	+	±	–	+	–	±	±	±	–	–	+	–
06/08/89	++	–	++	±	–	+	–	±	±	±	–	–	+	–
06/13/89	++	–	++	±	–	+	±	±	±	±	–	–	+	±
06/15/89	++	–	++	±	–	+	±	±	±	±	–	–	+	±
06/20/89	++	–	++	+	–	+	±	±	±	±	–	±	+	±

Underlined dates indicate seroconversion according to the LIA HIV criteria.

hand, were detected later or remained absent throughout the panel. No reactivity was observed with the peptide originating from the HIV-2 transmembrane antigen.

Quantification: The results of the LIA HIV were interpreted not only by visually comparing the reference IgG lines with the antigen lines, but also by quantifying the intensity of the lines by reflectance densitometry. Figure 2 presents the results of densitometric analysis of the LIA HIV strips and the WB strips for seroconversion panel C. The LIA HIV detected a slightly increased reactivity (but still below the cutoff value) on the gp41 and p24 lines on day 9, whereas no reactivity was observed in the WB of this sample. On day 14, the LIA HIV confirmed the presence of HIV antibodies directed against gp41 and p24, whereas WB detected only p24 antibodies. The first sample to be confirmed for the presence of HIV antibodies by WB was that taken on day 16, which showed antibodies directed against gp160 and p24. From day 16 on, all samples were confirmed by both methods.

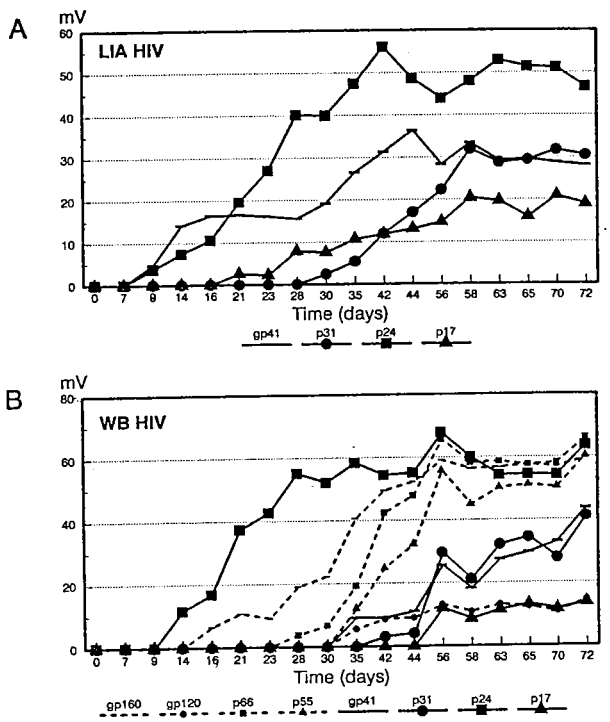

Fig. 2. Antibody reactivity of seroconversion panel C (Table 3) measured by densitometric scanning: (*A*) LIA HIV, (*B*) WB

Day 0 is the day of the first serum sample of the seroconversion panel. The other serum samples are identified with reference to this first sample

Using reflectance densitometry, we analyzed the interlot variability of the WB and the LIA. For the LIA HIV we found a mean (±SD) CV of 8.4% (2.6%) for gp41, 6.1% (2.8%) for p31, 6.4% (1.7%) for p24, 8.5% (3.1%) for p17, and 8.1% (2.8%) for the HIV-2 line. These data were obtained by testing five HIV-1 sera and two HIV-2 sera with three different LIA HIV test lots. It was not considered relevant to calculate a coefficient of variation for the WB because the interlot variability was extremely high. For example, when using WB lot C9244-084, we observed a signal of 13 mV on gp120, 25.6 mV on gp41, and 29.6 mV on p31 for a given serum (day 56 of seroconversion panel C). Using WB lot C9244-232, we observed for the same serum a signal of 41.6 mV on gp120, 8 mV on gp41, and 7.7 mV on p31. The signals measured for gp160 (59 and 58.3 mV), p66 (65.7 and 63.8 mV), p55 (55.7 and 38.8 mV), p24 (67.8 and 66.8 mV), and p17 (11.9 and 0 mV) indicated a better WB interlot reproducibility.

Processed LIA HIV strips, when stored protected from direct sunlight, did not show any significant decrease in intensity over a two-year period. In contrast, WB bands show a steady decrease in color intensity with time, which interferes with quantitative evaluation of older strips.

Discussion

The use of the WB as a confirmation test in HIV diagnosis has been hampered by the presence of human

cellular antigens in the virion preparations, which may induce atypical reactivity patterns (*10*) and lead to ambiguity in the interpretation of test results. In the LIA HIV, the use of synthetic peptides and recombinant proteins expressed in *E. coli* avoids this problem. In our study, the LIA HIV gave no reactivity patterns attributable to the interference of human anti-*E. coli* antibodies—an indication that the recombinant antigens had been purified sufficiently to prevent anti-*E. coli* antibodies from being detected.

Several reports indicate that indeterminate WB results may be obtained with sera from patients with various non-HIV-related diseases (*15, 18*). Using the LIA HIV, we found a substantially lower number of sera reactive to p24 only, in comparison with those found by WB. The issue of p24-only reactive sera has not yet been resolved. It has been hypothesized that these atypical WB patterns arise from cross-reacting antibodies against HIV core proteins and core proteins from picornaviruses (*19*) or other retroviruses (*20*). In WB, the anti-p24 reactivity can persist for several months without progression to reactivity against envelope antigens or other HIV-1-specific antigens (*15*). This is in contrast to true seroconversions, where at least anti-envelope antibodies (i.e., anti-gp160/120 or anti-gp41) become detectable within this time period.

Another potential drawback of the WB assay is the inability to discriminate between a negative WB strip (the result of an incubation with an HIV-negative serum) and a WB strip to which by accident the sample has not been added. In our opinion, important benefits would be derived from the detection of this kind of technical error. In the LIA HIV, the addition of an IgG-containing sample can be verified via the fourth control line (anti-IgG).

Confirmation of antibodies to HIV by using recombinant antigens in an ELISA has been described by Chiang et al. (*21*) and Ng et al. (*22*). However, the ELISA format is not able to discriminate between specific binding of immunoglobulins and nonspecific interactions between immunoglobulins and blocking agents within a single well. In the LIA HIV system, as well as in the WB, specific reactivity patterns are required to confirm the presence of HIV antibodies, thereby eliminating false positives caused by background reactivity.

Although only a few antigens are used in the LIA HIV assay, this test confirmed the presence of HIV antibodies with specificity and sensitivity exceeding those of WB. Within the limits of this study, no false positives were reported by the LIA HIV, and fewer indeterminate results were obtained in the group of seronegative individuals when the LIA HIV and the WB were compared; moreover, all of the seropositive individuals were correctly identified. In two of seven seroconversion panels analyzed, the LIA HIV confirmed the presence of HIV antibodies before the corresponding WB did. From these data, we conclude that the sensitivity at seroconversion of the gp41 used in the LIA HIV is superior to the reactivity of the gp41 in the WB. In addition, the LIA HIV had a better interlot reproducibility than the WB, where interlot variability is an inherent problem because of the variability of the virion preparations.

The interpretation criteria used with the LIA HIV are simple, allowing a straightforward classification of the samples as negative, indeterminate, or positive. In 99.4% of the WB-confirmed HIV-1 sera, the LIA HIV detected antibodies directed against at least two distinct gene products (*env* + *gag*, *env* + *pol*, *pol* + *gag*). In 0.6% of the HIV-1-containing sera, the LIA HIV permitted the detection only of antibodies directed against gp41; in each case, this corresponded to a WB-positive HIV-1 serum without antibodies to p24 and p31. The criteria of gp41-only reactivity can be used in the LIA HIV because of the high specificity of this antigen. Only one serum was found to be nonreactive to LIA HIV gp41 (p31, p24, and p17 reactive), although it was clearly WB-positive, including gp41 reactivity. This serum is unique in that it did not react with the immunodominant epitopes expressed on the synthetic peptides used in the LIA HIV. The epitopes of the transmembrane antigen used in the LIA HIV were selected not only because of their high specificity and sensitivity towards HIV-1 sera, but also because these epitopes allow discrimination between HIV-1 and HIV-2. We could find no additional epitopes that allowed discrimination between HIV-1 and HIV-2 and also reacted with the Y.S. serum. The present data indicate that no additional HIV antigens need to be added to the LIA HIV to confirm all WB-positive HIV-1 and HIV-2 sera.

In each of the seroconversion panels analyzed, the amount of HIV antibodies increased significantly within a short time. Indeterminate LIA HIV reactivity patterns were observed in only a few samples (panel C 07/25/85, panel G 11/21/88). Within one week, the indeterminate samples were followed by a clear positive reaction. These data emphasize the need to request a new sample from patients with an indeterminate reactivity pattern in a confirmatory assay.

Because most present-day HIV-antibody screening tests allow the detection of antibodies against both HIV-1 and HIV-2, WB confirmation of HIV infection requires separate analysis for both HIV-1 and HIV-2. Until recently, an ELISA-positive sample was tested with an HIV-1 WB; if found positive (even if only just meeting the criteria for confirmation), it was classified as HIV-1. However, in a substantial number of sera from patients infected with the HIV-2 virus, HIV-1 cross-reacting antibodies are present (*23–25*). Therefore, some HIV-2 sera may previously have been misclassified as HIV-1, because most reference laboratories did not perform a WB for HIV-2, which would have shown a more extensive reactivity than the WB for HIV-1. Using the LIA HIV test, one can discriminate between HIV-1 and HIV-2 sera, because synthetic peptides specific for both HIV-1 and HIV-2 transmembrane antigens have been used. Also on the LIA HIV strip, in accordance with WB data, we observed HIV-2 antibodies cross-reacting with HIV-1 p31 and p24.

Using the LIA HIV with sera originating from West Africa, we recently found antibodies reacting with both the HIV-1- and HIV-2-specific synthetic peptides (data not shown). The significance of this observation is being investigated.

Quantification of results on immunostrips is meaningful only if the production and quality control of the strips are reproducible. If the WB strips are specially made to quantify the protein bands and if special control techniques are used (*25*), WB strips can provide quantitative data (*24, 26–28*). However, we observed a high WB interlot variability, in particular with regard to the important antigens gp120 and gp41; therefore, commercial WB strips do not appear suitable for quantification. This poor reproducibility probably reflects the complex production procedures for manufacturing WB strips (*28*).

Another methodological problem with the WB is that the elimination of interference from background noise may create an underestimate of low-amplitude peaks (*29*). This problem does not apply to the LIA HIV strip because all antigen lines are clearly separated from one another (Figure 1). In addition, we observed no decrease in intensity of the lines on the LIA HIV strips over a two-year period. As did Schiavini et al. (*28*), however, we detected a significant decrease in the color intensity of the WB strips.

Because the exact amount of each antigen applied to the LIA HIV strip is known, densitometric scanning analysis can yield more meaningful quantitative data than can a WB strip, where the amount of each individual antigen is unknown and largely dependent on the variabilities in the growth of the virus strain and in the subsequent production procedures. Given both the high reproducibility of the LIA HIV production process and the way the antigens are applied to the membrane, a more quantitative evaluation and interpretation of the data is possible through densitometric scanning of the processed strip. This allows efficient and unambiguous storage and processing of the data in a digital format. Quantification becomes particularly important if a test is to be used for the follow-up of HIV antibody-positive subjects. It has been demonstrated that a decline of p17 (*30, 31*) or p24 (*32, 33*) antibody titers is of predictive value in the evolution toward the AIDS and AIDS-related complex stages of HIV infection. The ratio of gp41 to p24 antibodies has also been suggested to be of predictive value (*34*). Reflectance densitometric scanning of the LIA HIV strips may be considered for monitoring each of these prognostic markers.

For epidemiological studies it may often be inconvenient to collect blood for serological tests. However, antibodies to HIV can be detected in eluates from whole-blood-impregnated filter-paper discs (*35, 36*). The feasibility of using eluates from dried blood spots in the LIA HIV has been demonstrated by Kestens et al. (*37*). Alternatively, HIV antibodies can also be detected in other body fluids such as saliva (*38*) and urine (*39, 40*). Using the LIA, Van Renterghem et al. (*41*) detected HIV antibodies in saliva of HIV-infected patients. We are currently exploring the possibility of using urine samples with the LIA HIV test. Finally, the present data are in good accordance with the results obtained by the World Health Organization (*42*) and K. Fransen et al. (Department of Microbiology, Institute for Tropical Medicine, Antwerp, Belgium; personal communication), who, testing 2110 sera, found an overall specificity of 100% and a sensitivity of 99.77% for the LIA HIV in comparison with the WB. We conclude that the LIA HIV combines high specificity, sensitivity, and reproducibility, and therefore provides a convenient and reliable alternative to WB for the confirmation of HIV antibodies.

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