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Evaluation of a Line Immunoassay for Simultaneous Confirmation of Antibodies to HIV-1 and HIV-2

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An anti-HIV-1/HIV-2 line immunoassay (LIA), using peptides and recombinant antigens was evaluated against commercially available Western blot tests for HIV-1 and HIV-2 antibodies. Two thousand one hundred and ten sera of European, African, and South American origin were used in the evaluation. The panel included 1066 sera with antibodies to HIV-1, 192 sera with antibodies to HIV-2, and 64 sera with antibodies to both. Using Western blot results interpreted according to the WHO criteria as a reference standard, the overall specificity obtained by this LIA was 100 % and the sensitivity was 99.77 % (97.51–100 % for 95 % confidence limits) when sera dually reactive in Western blot were included. Of the three sera negative in the LIA but positive in HIV-1 WB, two could be retested in a radioimmunoprecipitation assay and were negative. When dually reactive sera in the Western blot (WHO) were included, the LIA yielded 9.9 % indeterminate results as compared with 15.5 % for both assays (χ^2 = 29.30; $p < 0.001$). Although only one HIV-2 specific peptide antigen (gp36) was used, the LIA yielded a specificity of 100 % and a sensitivity of 100 % as compared with the HIV-2 Western blot assay. When indeterminate results were included, the overall agreement between the LIA and the HIV-1 and HIV-2 Western blot (WHO criteria) was 89.9 % and 90.1 % respectively. These results indicate that the LIA provides reliable simultaneous detection of antibodies to HIV-1 and HIV-2, and at a cost which is substantially lower than the cost of Western blot tests.

Introduction

Western blot (WB) analysis is the most widely used confirmatory assay. It provides information on antibody reactivity to specific viral antigens for human immunodeficiency virus (HIV) antibodies and avoids potentially hazardous handling of radioisotopes used in the radioimmunoprecipitation assay (RIPA). Since the introduction of the WB method, there has been much debate as to the suitability of the method for confirmation of routine HIV screening results.

A number of pitfalls exist for the WB confirmation of HIV screening results. HLA proteins present in most HIV preparations can simulate HIV proteins because of electrophoretic comigration leading to incorrect interpretation of HIV WB (1). Human and animal sera used in HIV WB can give false reactivity with *gag* proteins, thereby giving rise to a relatively large number of "indeterminate" WBs among HIV-negative individuals (G. Leslie et al., Vth International Conference on AIDS, Montreal, 1989, Abstract TBP 123) (2–4). Higher molecular weight molecules, especially glycosylated molecules (e.g. gp120, gp160) are less efficiently transferred to blotting membranes than lower molecular weight molecules, resulting in decreased sensitivity for *env* antibodies (5). WB procedures have not been standardized, resulting in assays with variable sensitivity and specificity (1, 6). Lot-to-lot variability and strip-to-strip variability with a single lot is often noted (G. Leslie et al., Vth International Conference on AIDS, Montreal, 1989, Abstract TBP 123), and there is a lack of consensus regarding interpretative criteria (7) which may result in erroneous reporting of serostatus (8). Finally, the WB technique is time-consuming, labor-intensive, and expensive.

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Epidemiological surveillance suggests that HIV-2 infection is still mainly associated with West Africa (9), although HIV-2 has also been observed in other parts of the world (10–16). Since most of the first and second generation anti-HIV-1 enzyme immunoassays (EIA) detect less than 80 % of anti-HIV-2 positive sera, EIAs which simultaneously detect antibodies against HIV-1 and HIV-2 have been developed (17–19). However, reactive screening results obtained with combined HIV-1/HIV-2 EIAs should be confirmed using confirmatory assays which identify and discriminate both HIV-1 and HIV-2 antibodies.

Such an assay for the simultaneous confirmation of antibodies against HIV-1 and HIV-2 was recently developed to provide more objective, reproducible, specific, and quantitative results. We report here on the evaluation of this assay, and compare its performance with that of WB assays for HIV-1 and HIV-2 antibodies.

Materials and Methods

Sera. A total of 2110 serum specimens were collected and tested, including 317 serum specimens from Europe (Belgium), 1,583 from Africa (Zaire, Gabon, Kenya, Ivory Coast, Cameroon, Gambia, Senegal, Burkina Faso, Guinea-Bissau) and 210 from South America (Brazil). The panel included 1066 sera with antibodies to HIV-1, 192 sera with antibodies to HIV-2, and 64 sera with antibodies to HIV-1 and HIV-2.

Line Immunoassay. The anti-HIV-1/HIV-2 line immunoassay (LIA; INNO-LIA HIV-1/HIV-2 Ab, Innogenetics, Antwerp, Belgium) also sold and distributed by Organon Teknika under the name LiaTek HIV1+2, was performed as previously described (20, 21).

In brief, the LIA binds antibodies to HIV-1 and/or HIV-2 in the sample on three HIV-1 recombinant proteins: *gag* (p24 and p17) and *pol* (p31) in combination with two synthetic peptides representative of the *env* gene products of HIV-1 (gp41), and one peptide of HIV-2 (gp36). A more detailed description of the antigens has been given elsewhere (20). These antigens are applied as parallel lines on a nylon membrane. In addition to the HIV antigens, on each strip, four control lines are present: one cut-off line (± HuIgG), one weak positive control (1+, HuIgG) one strong positive control (HuIgG), as well as a separate sample addition control line (anti-HuIgG). Positive and negative results are determined by visual reading, comparing the intensity of an antigen line with the control and cut-off lines. Five different reactivity ratings can be observed (nonreactive (0), weakly reactive (±), reactive (1+), strongly reactive (2+), intensively reactive (3+), maximally reactive (4+) (20).

A sample was considered reactive (positive) to HIV-1 when gp41, alone or together with other HIV-1 gene products, showed a reactivity rating of 1+ or higher; or when p31 together with at least one other HIV-1 gene product (p24, p17) showed a reactivity rating of 1+ or higher; *and* in both cases when the reactivity rating for HIV-2 gp36 was negative. A sample was reactive (positive) to HIV-2 when the HIV-2 gp36 gene product showed a reactivity rating of 1+ or higher, and the reactivity rating for HIV-1 gp41 was negative. Other HIV-1 antigens may show some cross-reaction. A sample was interpreted as being dually reactive to HIV-1 and HIV-2 when both HIV-1 gp41 and HIV-2 gp36 had a reactivity rating of 1+ or higher. A sample was considered indeterminate if reactivity was seen but did not meet the aforementioned rating criteria interpretable as being reactive. Finally, a sample was considered nonreactive (negative) for HIV antibody if all HIV antigen lines were found to be nonreactive.

Confirmatory Procedures. The presence of antibodies to HIV-1 was confirmed with either a commercially available WB (WB kit for the detection of HTLV-III antibody, Dupont de Nemours, Wilmington, DE, USA), or with an in-house HIV-1 radioimmunoprecipitation method (RIPA). RIPA was essentially performed as described by Peeters et al. (22) using radioactively labelled HTLV-III B virus in Molt 4 clone 8 cells. The presence of antibodies to HIV-2 was confirmed with WB HIV-2 (New LAV Blot II, Pasteur Diagnostics, Marnes-la-Coquette, France). Sera positive in both WB HIV-1 and WB HIV-2 were also analyzed by Pepti-LAV 1-2 assay (Pasteur Diagnostics, Marnes-la-Coquette, France).

HIV-1 WB results were interpreted as positive according to the criteria of the Consortium for Retrovirus Serology Standardization (CRSS) when banding profiles representative for HIV-1-specific *gag* and/or *pol* plus gp41 or gp120, gp160 were present (7). They were considered negative when no bands were present, and indeterminate when the band pattern did not meet CRSS criteria for positivity. HIV-1 specific WB results were also interpreted according to recently revised criteria of the World Health Organization (WHO) (23) whereby HIV-1 WBs are considered positive when at least **2** of 3 *env* bands (gp160, gp120, or gp41) are present with or without *gag* and/or *pol* bands. HIV-1 WBs were considered negative when no HIV-1 specific bands were observed, and indeterminate when profiles were different from positive or negative ones.

The criteria for interpretation of HIV-2 WB results were those of the WHO (23). According to these criteria, HIV-2 WBs are considered positive when at least 2 of 3 *env* bands (*env* precursor gp140, external gp120, and transmembrane gp36/41) are present, with or without *gag* and/or *pol* bands. HIV-2 WBs are considered negative when no HIV-2 specific bands are observed, and indeterminate when profiles are considered to be different from positive or negative ones.

Specimens showing WB patterns positive for both HIV-1 and HIV-2 were interpreted as being dually reactive and further analyzed by Pepti-LAV. This test is also a LIA using HIV-1 and HIV-2 specific peptides. Each strip also contains a control line. In this assay, a serum was considered HIV-1 positive when the intensity of the HIV-1 line was stronger or equal to the intensity of the control line, and stronger than the intensity of the HIV-2 line. A serum was considered HIV-2 positive when the intensity of HIV-2 line was stronger or equal to the intensity of the control line, and stronger than the intensity of the HIV-1 line. A serum was considered dually reactive when

the intensities of the two peptides were equal to each other and stronger or equal to the intensity of the control line.

Sera with discrepant results between HIV-1 WB and the LIA were further tested by RIPA. The RIPA was considered positive when at least one of the 3 *env* bands (gp160, gp120, or gp41) was present, negative when no HIV specific band was present, and indeterminate when the profile was different from the positive or negative reaction pattern.

Data Analysis. In this study the LIA's and WB's of all samples were read in a blind fashion. The WB results were not known to the investigator(s) interpreting the LIA. We used the WB as gold standard. The LIA test results compared to WB were evaluated as follows: WB and LIA positive was interpreted as a true positive, WB positive and LIA negative was taken to mean a false negative, WB negative and LIA positive was interpreted as a false positive, WB and LIA negative was interpreted as a true negative.

The sensitivity of the test was calculated as the number of WB-confirmed positive specimens detected as positive by LIA, divided by the total number of WB-confirmed positives multiplied by 100. The test specificity was calculated as the number of WB-confirmed negative specimens detected negative by LIA, divided by the total number of WB-confirmed negatives multiplied by 100. The 95 % confidence limits (CL) around a ratio were calculated as previously described (24). Indeterminate results in both LIA and WB were not considered in the calculation of sensitivity and specificity.

The rate ratio (RR) was calculated as the ratio between the rate of the characteristic in the test under assessment and the rate of the corresponding characteristic in the reference test.

If the statistical test (RR, χ^2) result was significant, the 95 % confidence limits were calculated using the test-based approach (25).

Results

Correlation between LIA and WB Results. The HIV-1 WB results were analyzed according to the two different interpretative criteria, the CRSS, and the recently revised WHO criteria, respectively (Table 1). When indeterminate results were excluded for both assays, the overall agreement between HIV-1 WB (WHO criteria) and LIA was 1503/1506 (99.8 %) resulting in a specificity of 100 % and a sensitivity of 99.72 % (CL 99.40–100 %). Between HIV-1 WB (CRSS criteria) and the LIA the overall agreement was 1510/1514 (99.7 %) resulting in a specificity of 100 % and sensitivity of 99.63 % (CL 99.27–99.99 %). When indeterminate results were included, the overall agreement between the LIA and the HIV-1 WB was 1660/1847 (89.9 %) (WHO criteria) and 1663/1847 (90.0 %) (CRSS criteria), respectively. With and without exclusion of indeterminate results, the overall agreement between the LIA and HIV-2 WB was 420/420 (100 %) and 421/467 (90.1 %), respectively. The LIA did not score any false-positive reactions.

Although the LIA strips do not contain the gp160 and gp120 proteins, 877 (98.8 %) specimens scored positive with the LIA out of a total of 887 HIV-1 WB (WHO) positive sera with evidence for the presence of antibodies having at least two HIV glycoproteins. Among the ten specimens with discrepant results in the LIA and glycoprotein-containing HIV-1 WB assays, three were negative and seven were indeterminate with the LIA. Out of these, two LIA negative and five LIA-indeterminate sera were retested by RIPA. The two LIA negative, WB positive sera remained negative with RIPA. Among the five LIA-indeterminate but WB positive sera, one was positive, two were indeterminate, and two were negative by RIPA.

Some of the interpretative criteria used in LIA for HIV-1 positivity (gp41 only, p31 + p24 and/or p17) and for HIV-2 positivity (gp36 positive and gp41 plus all other HIV-1 bands negative) are widely different from the WB criteria used so far. However, the LIA results obtained by application of these rather unusual criteria corresponded well with the results of HIV-1 WB by application of both CRSS and WHO criteria, as well as with the results of HIV-2 WB by application of the WHO criteria (Table 2). Only one serum positive in the LIA according to the p31 + p24 and/or p17 criterium was HIV-1 WB indeterminate using CRSS criteria, whereas four HIV-1 LIA positive sera were HIV-1 WB indeterminate when WHO criteria were used.

Out of the 1056 sera, positive for HIV-1 antibody in both the WB (WHO criteria) and the LIA tests, 882 LIA and WB's were available on which the different LIA and WB reactivity patterns were compared. Out of these 882 sera, 848 sera (96.1 %) had a gp41 band in the WB, and 868 sera (98.4 %) showed a gp41 band in the LIA ($p < 0.01$; RR = 1.02y, CL 1.01–1.04). Out of these 868 HIV-1 antibody sera positive in both the WB and LIA, only 22 sera showed a weak reactivity rating on gp41 in the LIA, while 93 sera were weak and/or doubtful on the gp41 band in the WB ($p < 0.001$; RR = 4.23y, CL 2.68–6.66).

Indeterminate LIA and WB Results. A significantly lower proportion of indeterminate sera was observed in the LIA as compared with the WB: 208/1847 (11.3 %) vs. 278/1847 (15.1 %) (p

Table 1: Comparison of INNO-LIA HIV-1/HIV-2 Ab and Western blots (WB) for HIV-1 and HIV-2 antibodies.

INNO-LIA HIV-1/HIV-2 Ab	WB HIV-1 (CRSS)[a]				WB HIV-1 (WHO)[b]				WB HIV-2[c]			
	Positive	Negative	Indeterminate	Total	Positive	Negative	Indeterminate	Total	Positive	Negative	Indeterminate	Total
Positive	1063	0	6	1069	1056	0	13	1069	192	0	0	192
Negative	4	447	119	570	3	447	120	570	0	228	46	274
Indeterminate	11	44	153	208 (11.3 %)	7	44	157	208 (11.3 %)	0	0	1	1 (0.2 %)
Total	1078	491	278 (15.1 %)	1847	1066	491	290 (15.7 %)	1847	192	228	47 (10.0 %)	467
Sensitivity[d]	99.63 %	(99.27–99.99)			99.72 %	(99.4–100.0)			100 %			
Specificity[e]	100 %				100 %				100 %			

[a] HIV-1 WB results were interpreted according to the criteria of the Consortium for Retrovirus Serology Standardization (CRSS) (7).
[b] HIV-1 WB results were interpreted according to the revised criteria of the World Health Organization (WHO) (23).
[c] HIV-2 WB results were interpreted according to the revised criteria of the WHO (23).
[d] The sensitivity of the INNO-LIA HIV-1/HIV-2 Ab (LIA) test was calculated as the number of WB-confirmed positive specimens detected as positive by LIA, divided by the total number of WB-confirmed positives multiplied by 100.
[e] The specificity of LIA was calculated as the number of WB-confirmed negative specimens detected negative by LIA, divided by the total number of WB-confirmed negatives multiplied by 100. For sensitivity and specificity calculations the WB dually reactive sera were included.

Table 2: Comparison of LIA results obtained after application of some unusual interpretative LIA criteria and WB HIV-1 and WB HIV-2 results.

INNO-LIA criteria for positivity	WB HIV-1 positive		WB HIV-1 negative		WB HIV-1 indeterminate		WB HIV-2 positive	WB HIV-2 negative	WB HIV-2 indeterminate
	(n = 1078)	(n = 1066)	(n = 491)		(n = 278)	(n = 290)	(n = 192)	(n = 228)	(n = 47)
	CRSS	WHO	CRSS	WHO	CRSS	WHO			
only gp41	6[a]	6	0	0	0	0	0	0	0
p31 + p24 and/or p17	24	21	0	0	1	4	0	0	0
only p36	0	0	0	0	0	0	21	0	0

[a] Out of a total of 1078 HIV-1 WB positive sera according to CRSS criteria, the LIA test scored 1063 positive results (Table 1), of which 6 sera showed only a positive reactivity rating on gp41, and no reaction at all on any other of the LIA antigens.

< 0.001) for HIV-1 WB using CRSS criteria, and 208/1847 (11.3 %) vs. 290/1847 (15.7 %) (p < 0.001) for the HIV-1 WB using WHO criteria (RR = 1.39y, CL 1.18–1.65).

The most common band patterns occurring in WB HIV-1 and LIA indeterminates were observed with core and matrix proteins in both test systems. Out of a total of 467 sera tested for HIV-2 by WB and LIA, 47 indeterminate results were observed in the WB HIV-2, and one indeterminate in the LIA. In decreasing order of frequency, the following band patterns in indeterminate HIV-2 WB results were observed: core (p26), 69.2 %; reverse transcriptase (p68), 10.3 %; precursor (p56), 5.1 %; p68 + p25, 5.1 %, (and others 10.4 %).

Cross-Reactivity of HIV-1 Antigen and HIV-2-Positive Sera in LIA and WB. Out of 183 specimens positive in the HIV-1 Western blot according to both the CRSS and WHO criteria which were simultaneously tested in the HIV-2 WB, 168 (90 %) cross-reacted with one or more HIV-2 antigens in the HIV-2 WB. The HIV-2 *gag* core protein (p26) and the transcriptase protein (p68) cross-reacted most frequently with HIV-1 antibody positive sera on HIV-2 WB strips. None of these 183 specimens cross-reacted with the HIV-2 gp36 peptide in the LIA. In addition, 1066 HIV-1 WB positive, 491 negative and 290 indeterminate (WHO criteria) specimens did not show a reaction to the HIV-2 gp36 peptide in the LIA.

WB HIV-1 positive sera of African origin showed a higher rate of cross-reaction (118/122 or 97 %) with HIV-2 antigens in WB HIV-2 than WB HIV-1 positive sera of European origin 14/20 (70 %) (p < 0.001; RR 1.38). On the other hand, in 90 % of the cases (73/81), HIV-2 WB positive sera cross-reacted with HIV-1 antigens on HIV-1 WB strips; in decreasing order of cross-reactivity: core p24, 83 %; endonuclease p31, 70 %; reverse transcriptase p64, 47 %; transmembrane gp41, 22 %; external gp160, 22 %, reverse transcriptase p53, 31 %; *gag* precursor p55, 31 %; matrix p17, 11 % and gp120, 9 %. These specimens were all confirmed in the LIA as HIV-2 positive, and cross-reacted with HIV-1 antigens in the LIA (75/81; 93 %) in decreasing order: p31, 74 %; p24, 52 %; p17, 17 %; gp41, 2.5 %.

Dually Reactive Sera in WB, LIA and Pepti-LAV. Out of a total of 761 sera simultaneously tested on HIV-1 WB, HIV-2 WB, and LIA, 101 (13.3 %) were dually reactive in WB according to CRSS criteria; only 64 (8.4 %) remained dually reactive in WB according to WHO criteria and only 41 (5.3 %) remained dually reactive in the LIA.

Table 3: Comparison of the intensities in Pepti-LAV with HIV-1 gp41 and HIV-2 gp36 antigen lines in INNO-LIA HIV-1/HIV-2 Ab, and WB HIV-1 and WB HIV-2 titers.

Serum no.	INNO-LIA HIV-1/HIV-2 Ab					WB[d]		Pepti-LAV		
	gp41	gp36	FR[b]	HIV-1[c] titer	HIV-2 titer	HIV-1 titer	HIV-2 titer	Peptide 1	Peptide 2	FR[e]
1	2+[a]	±	HIV-1	62500	100	≥ 312500	500	2+	±	HIV-1
2	1+	3+	DR	NT	NT	NT	NT	±	2+	HIV-2
3	–	3+	HIV-2	NT	NT	NT	NT	+	2+	HIV-2
4	1+	4+	DR	500	312500	2500	62500	+	2+	HIV-2
5	–	2+	HIV-2	NT	NT	NT	NT	+	2+	HIV-2
6	2+	1+	DR	12500	12500	62500	62500	2+	2+	DR

[a] Reactivity ratings as observed on the INNO-LIA HIV-1/HIV-2 Ab strips after comparison of the intensity of the gp41 lines with the control cut-off lines, as described in detail previously (20).
[b] Final result after application of the INNO-LIA HIV-1/HIV-2 Ab interpretative criteria.
[c] Reciprocal of the highest dilution of serum at which INNO-LIA HIV-1/HIV-2 Ab can still be interpreted as positive according the INNO-LIA criteria. Dilutions of sera were made in HIV antibody negative human serum.
[d] Reciprocal of the highest dilution of serum at which the WB HIV-1 can still be interpreted as positive according to CRSS criteria.
[e] Final result after application of the Pepti-LAV interpretation criteria.
DR: Dually reactive; FR: Final Results; NT: Not Tested.

Sixty-three WB HIV-1 and HIV-2 dually reactive sera according to CRSS criteria were analyzed in parallel with LIA and Pepti-LAV; only 31 were dually reactive in LIA and 31 dually reactive in Pepti-LAV. The dually reactive sera in LIA and Pepti-LAV were not the same.

The reactivity rating of HIV-1 (gp41) and HIV-2 (gp36) antigens in Pepti-LAV and LIA of 6 sera dually reactive in WB were compared as depicted in Table 3. Although more striking differences in reactivity rating between HIV-1 and HIV-2 antigen lines were observed in the LIA test as compared with the Pepti-LAV test, three sera in LIA and only one serum in Pepti-LAV remained dually reactive. A significantly higher titer on HIV-2 WB as compared with HIV-1 WB correlated well with a higher reactivity rating on HIV-2 antigen in both Pepti-LAV and LIA. The opposite was also true for high-titered HIV-1 WB sera (Table 3). An equally high-titered serum in HIV-1 and HIV-2 WB showed an equal reactivity rating on both HIV-1 and HIV-2 antigens in Pepti-LAV and only a 1+ difference in reactivity rating in LIA (No. 6; Table 3). On the average, the titers obtained with LIA were five-fold lower than those obtained in the WB with the exception of serum No. 4 (Table 3) for which the HIV-2 LIA titer was five times higher than the WB titer.

Discussion

The INNO-LIA HIV-1/HIV-2 Ab assay makes use of only three recombinant HIV-1 proteins (p17, p24, p31) and two synthetic HIV peptides, combined as one HIV-1 antigen (gp41), and one HIV-2 peptide (gp36) to simultaneously confirm the presence of antibodies to HIV-1 and HIV-2 in serum samples.

The smaller number of antigens used in the LIA assay as compared with the 18 antigens commonly observed in HIV-1 and HIV-2 WB reduces the possible number of nonspecific reactions and thereby results in a lower number of LIA indeterminates. This, in turn, may considerably reduce the cost of test confirmation. In other studies, the percentage of WB indeterminates may be as high as 30 % (G. Leslie et al., Vth International Conference on AIDS, Montreal, 1989, Abstract TBP 123). In addition, the use of HIV antigens produced by recombinant techniques or chemical synthesis avoids the interferences observed with viral lysate kits due to the presence of human cellular antigens. In the LIA kit evaluated, this resulted in a specificity of 100 % and a sensitivity of 99.72 % (CL 99.4–100 %), for HIV-1, and both 100 % specificity and sensitivity for HIV-2. When compared with WB results obtained by application of WHO criteria, no false positive results were observed. Out of seven WB positive sera (CRSS as well as WHO) showing the presence of gp160 and gp120 bands, only one serum was positive, four were negative, and two were indeterminate by RIPA, illustrating the existence of nonspecific reactions obtained with the precursor and external glycoproteins. With the LIA, two were negative and five specimens were indeterminate. One specimen indeterminate when using the LIA was found to be positive by RIPA. This specimen showed only a ± reactivity rating on gp41 in LIA and a weak reactivity for p24, gp41, gp120, and gp160 in HIV-1 WB.

Among 13 HIV-1 LIA positive HIV-1 WB (WHO) indeterminates, nine sera were also positive in HIV-1 WB according to CRSS criteria. This illustrates that the HIV-1 WB criteria recently revised by the WHO do increase the number of indeterminates (Table 1). The interpretative criteria used for the LIA, which differ in some respects from the classical WB criteria, generated results which fit well with those obtained according to WB CRSS and WHO criteria. To what extent the WB or the LIA results represent a true HIV infection cannot be deduced from this study, because the necessary additional clinical and virological data were not available for each individual examined.

The use of a mechanical procedure to coat the antigens onto the membrane, defined amounts of highly purified antigens in a LIA format, and control levels for estimation of the intensity, permitted reproducible quantitative (densitometric reading) measurements and objective interpretation of the results (20). With the LIA, intensities of HIV antigen lines for a specimen could be compared with a set of predetermined cut-off lines, enabling more objective interpretation by different operators. Subjective interpretations, which were routinely employed in WB assays, are avoided in LIA using the above-mentioned procedure. In particular, the HIV gp41 band was easier to observe in LIA as compared with HIV-1 WB. In the former test, gp41 was present as a clear homogenous, distinct line instead of a diffuse band as in the WB assay. The gp41 band was observed more frequently in the LIA (98.4 %) as compared with WB HIV-1 (96.1 %), and gp41 could also be detected earlier in seroconversion by LIA than by HIV-1 WB, as was described else-

where (20). Although the LIA does not contain the precursor and external glycoproteins for HIV-1 and HIV-2, no false negative results were observed when RIPA results were used as the reference to confirm WB glycoprotein-positive bands.

When compared with the Dupont HIV-1 WB and the Pasteur HIV-2 WB, the Innogenetics LIA yielded a substantially lower proportion of dually reactive sera. The LIA results on sera dually reactive in HIV-1 WB and HIV-2 WB were all confirmed by Pepti-LAV, except for three sera which remained dually reactive in LIA but were considered HIV-2 positive in Pepti-LAV. The higher number of dually reactive sera in LIA as compared with Pepti-LAV was due to different criteria used in the LIA system to declare sera dually reactive. The LIA criteria do not take into consideration the difference in reactivity rating between HIV-1 and HIV-2 antigens, whereas the Pepti-LAV criteria do. To what extent LIA results for WB dually reactive sera do in fact correspond to either HIV-1, HIV-2, a double infection or presence of a new HIV type, can only be documented by systematic virus culture and PCR in lymphocytes from these patients. The LIA was also successfully applied to confirm eluates from filter paper impregnated with HIV seropositive blood samples (L. Kestens et al., Vth International Conference on AIDS, Montreal, 1989, Abstract TBP 135).

In conclusion, the INNO-LIA HIV-1/HIV-2 Ab test is a highly sensitive and specific assay in which recombinant and synthetic peptides are used for simultaneous confirmation of the presence of HIV-1 and HIV-2 antibodies in serum samples. The LIA assay is objective and precise. Qualitative readings can be made visually, in addition to quantitative data which can be obtained by densitometric scanning.

Antibodies against HIV-1 and HIV-2 can be confirmed in a single run at a cost which is half that needed to simultaneously confirm HIV-1 and HIV-2 antibodies by WB. This type of assay should be particularly useful, when combined screening assays for simultaneous detection of HIV-1 and HIV-2 antibodies are used.

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References

1. **Blomberg J, Klasse PJ:** Specificities and sensitivities of three systems for determination of antibodies to human immunodeficiency virus by electrophoretic immunoblotting. Journal of Clinical Microbiology 1988, 26: 106–110.
2. **Ferroni P, Tagger A, Pasquali M, Profeta ML:** HIV antibody screening and confirmatory testing of Italian blood donors. Vox Sanguinis 1988, 55: 143–147.
3. **Lantini JP, Graf I, Frei PC:** Serological diagnosis of HIV infection: incidence, outcome and significance of partial reactions found in Western blot analysis. Clinical and Experimental Immunology 1989, 76: 332–336.
4. **Genesca J, Jett BW, Epstein JS:** What do Western blot indeterminate patterns for human immunodeficiency virus mean in EIA – negative blood donors? Lancet 1989, ii: 1023–1025.
5. **Britz JA, Rolon N, Hill T, Page E, Geltoski J:** Interpreting HIV ELISA reactivity: alternatives to stern blot. Journal of Clinical and Laboratory Analyses 1988, 2: 174–181.
6. **Wittwer CT, Ash KO:** Evaluation of three commercial kits for the confirmation of antibodies to human immunodeficiency virus (HIV-1). Clinical Chemistry 1988, 34: 1930.
7. Interpretation and use of the Western blot assay for serodiagnosis of human immunodeficiency virus type 1 infections. Morbidity and Mortality Weekly Report 1989, 38: 1–7.
8. **Beneson AS, Peddecord KM, Hofherr LK, Ascher MS, Taylor RN, Hearn TL:** Reporting the results of human immunodeficiency virus testing. Journal of the American Medical Association 1990, 262: 3435–3438.
9. **Romieu I, Marlinck R, Kanki P, M'Boup S, Essex M:** HIV-2 link to AIDS in West Africa. Journal of AIDS 1990, 3: 220–229.
10. **Brucker G, Brun-Vézinet F, Rosenheim M, Rey MA, Katlama C, Gentilini M:** HIV-2 infection in two homosexual men in France. Lancet 1987, i: 223.
11. **Saimot AG, Coulaud JP, Mechali D, Matheron S, Dazza MC, Rey MA, Brun-Vézinet F, Leibowitch S:** HIV-1/LAV-2 in Portugese men with AIDS (Paris 1978) who had served in Angola in 1968-1974. Lancet 1987, i: 688.
12. **Werner A, Stazsewski S, Helm EB, Stille W, Weber K, Kurth R:** HIV-2 (Western Germany, 1984). Lancet 1987, i: 868–869.
13. HIV-2 detected in UK. Nature 1988, 332: 295.
14. AIDS due to HIV-2 infection. New Jersey. Morbidity and Mortality Weekly Report 1988, 87: 33–35.
15. **Lyons SF, Clausen L, Schoub BD:** HIV-2 induced AIDS in Southern Africa. AIDS 1988, 2: 406–407.
16. **Vanderborght B, De Leys RJ, Nys P, van der Groen G, Merregaerts J, Prinsen H, Van Heuverswyn H:** Isolation of human immunodeficiency virus type-2 from a homosexual man in Belgium. European Journal of Clinical Microbiology and Infectious Diseases 1988, 7: 816–818.

17. **Denis F, Leonard G, Sangara A, Gershy-Damet G, Rey G, Soro B, Schmidt D, Mounier M, Verdier M, Baillou A, Barin F:** Comparison of 10 enzyme immunoassays for detection of antibody to human immunodeficiency virus type 2 in West African sera. Journal of Clinical Microbiology 1988, 26: 1000–1004.
18. **Peeters M, Delaporte E, Dazza MC, Brun-Vézinet F:** Detection of HIV-2 antibodies using five commercial HIV enzyme immunoassays. AIDS 1988, 2: 389–390.
19. **Ayres L, Avillez F, Garcia-Benito A, Deinhardt F, Gutler L, Denis F, Leonard G, Ranger S, Grob P, Joller-Semelka H, Hess G, Seidl S, Flacke H, Simon F, Brun-Vézinet F, Sondag D, Hampl H, Schoen R, Strames S, Troonen H:** Multicenter evaluation of a new recombinant enzyme immuno assays for the combined detection of antibody to HIV-1 and HIV-2. AIDS 1990, 4: 131–138.
20. **Pollet DE, Saman EL, Peeters DG, Warmenbol HM, Heyndrickx LM, Wouters CJ, Beelaert G, van der Groen G, Van Heuverswyn H:** Confirmation and differentiation of antibodies to human immunodeficiency virus I and II, using a strip based assay including recombinant antigens and synthetic peptides. Clinical Chemistry 1991, 37: 1700–1707.
21. **Vranckx R:** Evaluation of a line immunoassay for the differential detection of antibodies to human immunodeficiency virus. European Journal of Clinical Microbiology & Infectious Diseases 1990, 9: 674–676.
22. **Peeters M, Honoré C, Huet T, Bedjabaga L, Ossari S, Bussi P, Cooper RW, Delaporte E:** Isolation and partial characterization of an HIV-related virus occurring naturally in chimpanzees in Gabon. AIDS 1989, 3: 625–630.
23. **Acquired immunodeficiency syndrome (AIDS):** Proposed WHO criteria for interpreting results from Western blot assays for HIV-1, HIV-2 and HTLV-I/HTLV-II. Weekly Epidemiological Record 1990, 65: 281–283.
24. **Rosner B:** Fundamentals of cross statistics. Duxbury Press, Boston, 1982, p. 158.
25. **Miettinen O:** Estimability and estimation in case-referent studies. American Journal of Epidemiology 1976, 103: 226–235.