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Comparison of Two Commercially Available Anti-HIV ELISAs: Abbott HTLV III EIA and Du Pont HTLV III-ELISA

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Serum specimens were tested for HIV antibodies by two commercially available ELISAs (Abbott HTLV III EIA and Du Pont HTLV III-ELISA). The specificity and sensitivity of these assays were determined by comparison with indirect immunofluorescence and Western blot analysis. Specificity ranged from 94.3% in the Abbott assay to 97.9% in the Du Pont-ELISA. The sensitivity was 100% in the Abbott-ELISA and 99% in the Du Pont test. With both tests, false-positive results occurred predominantly in sera from patients with immunological disorders (kidney transplant recipients, lymphoma, Stevens-Johnson syndrome, etc.), whereas symptomatic AIDS-patients, patients with ARC, and persons with a defined risk for HIV infection could be diagnosed unequivocally. Specificity and sensitivity of anti-HIV ELISAs seemed to depend not only on definition of the cutoff value but also on other factors, such as antigen preparation and inactivation measures. Testing of ELISA-reactive sera by confirmatory tests remains necessary.

Key words: LAV/HTLV III-ELISAs, specificities, sensitivities, Western blot, immunofluorescence

INTRODUCTION

Since the first description of enzyme-linked immunosorbent assay (ELISA) for detection of antibodies to human T-lymphotropic virus type III (LAV/HTLV III), now called human immunodeficiency virus (HIV) [Sarngadharan et al, 1984; Cheingsong-Popov et al, 1984], this test system has become the principal diagnostic tool to search for HIV infections. Furthermore, the World Health Organization (WHO, 1985) and Public Health Organizations in many countries have issued recommendations for screening of blood donors and persons at risk of HIV infection [CDC, 1985; Velimirovic, 1985]. The serious implications of an HIV infection require that these assays have a high specificity and sensitivity. Several attempts have been made to determine the rate of false-positive and false-negative results of various anti-HIV assays [Weiss et al, 1985; Mortimer et al, 1985; Pizzocolo et al, 1986; Reesink et al, 1986]. In our study we compared two commercially available ELISAs (Abbott HTLV III EIA and Du Pont HTLV III-

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TABLE I. Definition of Results in Anti-HIV ELISAs

Result	Symbol	Abbott	Du Pont
Positive	+	value $\geq 3 \times$ cutoff[a]	value $\geq 1.5 \times$ cutoff[b]
Weak positive	(+)	value $< 3 \times$ cutoff $\geq$ cutoff	value $< 1.5 \times$ cutoff $\geq$ cutoff
Weak negative	(−)	value < cutoff $\geq 0.75 \times$ cutoff	value < cutoff $\geq 0.5 \times$ cutoff
Negative	−	value $< 0.75 \times$ cutoff	value $< 0.5 \times$ cutoff

[a]Cutoff value Abbott assay: $NC\bar{x}$ (mean of negative control values) + $0.1 \times PC\bar{x}$ (mean of positive control values).
[b]Cutoff value Du Pont assay: $0.5 \times PC\bar{x}$ (mean of positive control values).

ELISA), both as to their specificity and sensitivity with each other, and with an immunofluorescence and a Western blot test.

MATERIALS AND METHODS

Sera

Serum specimens (503) that had been sent to our laboratory for determination of anti-HIV were tested. Physicians' notes indicated that 151 sera were from risk group patients such as homosexuals, drug abusers, hemophiliacs, and prostitutes. Two of these sera were from patients with manifest AIDS, two others originated from patients with persistent generalized lymphadenopathy.

Twenty-six sera came from medical staff and 23 sera were from kidney transplant recipients. Transfusion recipients were represented by 18 sera. The majority of serum specimens was sent without information about specific risks or clinical data. From the relatively high rate of positive results, we presume that most of these sera were from persons at risk.

Assays

The two commercially available ELISAs were performed according to the manufacturers' instructions. All reactive and borderline results were retested at least once.

Both assays use antigen (HTLV III) prepared from an H9/HTLV III_B-T-lymphocyte cell line. In the Abbott assay the antigen is coated on 6 mm polystyrene beads; the Du Pont assay uses 12-well microtiter strips. Preparation and inactivation of the antigen is different in the tests (Abbott: detergent and sonication; Du Pont: psoralen, ultraviolet light and detergent). Washers and ELISA readers were rented as required.

The immunofluorescence assay (IFA) was performed with an H9/HTLV III-T-lymphocyte cell line coated onto slides and inactivated with acetone at −20°C. Each serum specimen was also tested on a noninfected H9-T-lymphocyte cell line treated like the infected cells. Serum dilutions were done 1:16 and 1:32 in phosphate-buffered saline (including 7% bovine albumine, 0.02% sodium azide). Western blot analysis was done with antigen obtained from Dr. Habermehl, Deutsche Vereinigung zur Bekämpfung von Viruskrankheiten (DVV), West Berlin. Antigen separation was performed with polyacrylamide gel electrophoresis followed by electro transfer to nitrocellulose paper (method recommended by the DVV). The nitrocellulose strips were incubated with a 1:200 serum dilution (incubation buffer: 0.5 M NaCl, 20 mM phosphate buffer pH 7.4, 0.5% bovine albumine, 3% milk powder, 0.3% Triton X-100 in aqua bid). Bound antibodies were

TABLE II. Evaluation of Immunofluorescence and Western Blot Tests

Result	Symbol	Criteria
Positive	+	Specific reaction with infected cells or virus specific bands
Equivocal	?	No clear-cut determination of positive and negative result possible
Negative	–	No reaction with infected cells or no virus specific bands
Control antigen	cag	Reaction with control antigen (immunofluorescence only)

detected with a peroxidase-conjugated Coombs-serum (American Qualex, 1:1,000–1:2,000).

Evaluation

The results of the ELISA assays were expressed as optical density (OD) values and related to a cutoff value calculated from control OD values according to the manufacturers' instructions. OD values above cutoff indicated reactivity. Borderline results were classified by us as "weak positive" and "weak negative" for both ELISAs by definition of additional cutoff values as described in Table I. This gradation allowed better differentiation in comparing the ELISAs.

Immunofluorescence and Western blot results were considered positive or negative by visual inspection. In a few cases the results were equivocal. Sometimes a reaction with uninfected control cells occurred in immunofluorescence assays. In such cases the sera were defined cag (= reaction with control antigen; see Table II).

Calculations and Statistics

Specificity and sensitivity rates of the ELISAs were calculated by standard methods:

$$\text{Sensitivity} = \frac{\text{number of true-positive results}}{\text{number of true-positives} + \text{number of false-negatives}}$$

$$\text{Specificity} = \frac{\text{number of true-negative results}}{\text{number of true-negatives} + \text{number of false-positives}}$$

True-positive result = Positive in ELISA (Table I) and positive in IFA. Positive or weak positive in ELISA (Table I) and positive in Western blot (cases of discrepancy between ELISA and IFA).

True-negative result = Negative in ELISA (Table I) and negative in IFA. Negative or weak negative in ELISA (Table I) and negative in Western blot (cases of discrepancy between ELISA and IFA).

Significance levels were calculated by chi-square test. Probability of error 2α was 0.05.

RESULTS

Comparison of Abbott- and Du Pont-ELISA

Initially, 503 serum specimens were tested once with the Abbott HTLV III EIA and with the Du Pont HTLV III-ELISA. In Table III the results of both assays are presented;

TABLE III. Comparison of Du Pont- and Abbott-ELISA 503 Sera

		Abbott-ELISA			
		+	(+)	(−)	−
Du Pont-ELISA	+	92	1		1
	(+)		2	1	3
	(−)			2	5
	−		19	5	370

95 sera (18.9%) reacted positive in both tests, which corresponds to the percentage of anti-HIV positive sera in our complete serum collection up to now and shows that the specimens sent to us for anti-HIV determination must be strongly selected for risk groups, even when the physicians did not relay this information. Sera determined negative by both ELISAs numbered 382. To confirm positive or negative results with an independent test system, all sera were tested by IFA.

Using the manufacturers' definition of cutoff values, discrepancies between the two ELISAs were found initially in 26 cases (5.2%, Table III). Fifteen sera initially reacted weak positive/negative in one or both ELISAs, but not discrepant. Most of these sera were retested with both ELISAs and, if necessary, with IFA. In addition, they were tested at least once by Western blot analysis.

Table IV is a synopsis of all results with ELISA-discrepant and weak positive/negative sera (including serum 42, see below). Probably nearly all sera positive in Abbott but negative in the Du Pont assay (sera 1 to 21) were false-positive. Serum 18 reacted equivocal and in a second attempt positive in the Western blot test (three virus specific bands). Thus, this serum apparently reacted false-negative in the Du Pont-ELISA. All sera at first negative in the Abbott but positive in the Du Pont test (sera 22 to 26), were apparently false-positive in the Du Pont assay. We did not find any false-negative result with the Abbott assay.

Two sera weak positive in the Abbott and positive or weak positive in the Du Pont test (sera 27 and 28) were interpreted as false-positive in both ELISAs, whereas serum 29 reacted weak but true-positive in both ELISAs. Sera 30 to 41 could always be confirmed as negative.

No discrepant, weak positive, or weak negative reactions occurred in sera from the few patients with manifest AIDS or lymphadenopathy syndrome. Most of the "problem sera" came from hospitalized patients with severe diseases (marked with x in Table IV): 12 sera were from kidney transplant recipients, 7 sera from patients with lymphoma, pseudolymphoma, necrotizing lymphadenitis, atypical pneumonia, fever, Stevens-Johnson syndrome, and sideroachrestic anemia. Only 7 ELISA-discrepant or weak positive/negative sera were drawn from apparently healthy persons (personnel, blood donors). Five sera were sent without any notes. Eleven problem sera belonged to persons at risk for HIV-infection (marked with * in Table IV).

Comparison of ELISA and Western Blot Analysis

Eighty-seven of 503 serum samples were tested by Western blot analysis. All ELISA-discrepant and weak positive/negative sera were included. We also included 46

TABLE IV. Synopsis of ELISA-Discrepant and Weak Positive/Negative Sera

			Du Pont			Abbott			IFA			Western blot	
Serum			A	B	C	A	B	C	A	B	C	A	B
1	x		(−)	(+)	(+)	+	+		?	−		−	−
2		*	(−)	−	−	+	+		−	−	−	−	−
3			−	−		(+)	(+)		−			−	
4			−			(+)	−		−			−	
5		*	−	−		(+)	(+)	(+)	−	−		−	
6		*	−			(+)	(+)		−	−		−	
7	x		−	−	−	(+)	(+)		+	−		−	
8	x		−	−		(+)	−		?	−		−	
9			−	−		(+)	(+)	+	−			−	
10	x		−			(+)	−		−			−	
11			−			(+)	−		−			−	
12	x		−	−		(+)	(+)		−			−	
13	x		−	−		(+)	(+)		−	−		−	
14	x		−	−	−	(+)	+		cag	cag		−	−
15	x		−	−	−	(+)	(+)		−	−		−	
16	x		−	−	−	(+)	(+)		−	−		−	−
17			−	(−)	(−)	(+)	(+)		−	−		−	
18	x		−	−	−	(+)	(+)		−	−		?	+
19	x		−			(+)	−		−			−	
20			−			(+)	(−)		−			−	
21		*	−	−	−	(+)	−	−	+	−		−	
22		*	+	−	−	−	−		−	−		?	−
23	x		(+)	(−)	(−)	(−)	(−)		−			−	
24	x		(+)	+	+	−	(+)		+	−		−	
25	x		(+)	(−)	(+)	−			−			−	
26		*	(+)	(+)	(+)	−	−		−	−		−	
27			+	+	+	(+)	(+)		?	?		?	−
28	x		(+)	+	+	(+)	(−)	−	−			−	
29	x		(+)	(+)	(+)	(+)	+		−	−		+	
30		*	(−)	(−)	(+)	(−)			−			?	−
31		*	(−)	(−)	−	(−)	−		−			−	
32			(−)	(−)	(−)	−			−			−	
33	x		(−)	(−)	(−)	−			−			−	
34		*	(−)	−	−	−	−	−	?	?	−	−	−
35	x		(−)	−	−	−			−			−	
36		*	(−)	−	−	−			−				
37			−			(−)	−		−				
38			−			(−)	−		−				
39	x		−			(−)			?			?	−
40			−			(−)	(+)		−	−		−	
41			−	−	−	(−)	−	(+)	−	?		−	
42		*	+	+	+	+			+			−	−

A = Initial testing results.
B, C = Results of retesting.
x = Sera from hospitalized patients with severe disease not thought to be related to HIV.
* = Sera from persons at risk for HIV-infection.

TABLE V. Specificity and Sensitivity Rates of Abbott and Du Pont ELISA

	Specificity	Sensitivity
Abbott	94.3%	100%
Du Pont	97.9%	99%

serum specimens that did not show discrepant or weak positive/negative results with either ELISA technique. In this serum collection, one serum was found positive by both ELISAs, but negative by the Western blot test (serum 42 in Table IV). In the remainder of ELISA-concordant sera tested by Western blot analysis, the ELISA-results were always confirmed (data not shown). Including sera 27, 28, and 42, the number of false-positive ELISA cases evaluated in Table III increased from 5 to 8 sera in the Du Pont assay and from 20 to 23 sera in the Abbott assay.

Specificity and Sensitivity

False-positive results reduce the specificity rating of an assay. The specificity of the Abbott assay (94.3%) was significantly lower than that of the Du Pont assay (97.9%) ($2\ \alpha = 0.05$, Table V). The sensitivity rate of the Abbott assay was 100%, the rate for the Du Pont assay was 99% (Table V), due to the one false-negative serum described earlier. The difference was not statistically significant ($2\ \alpha = 0.05$).

The sensitivities of the various tests were also assessed by titration of an anti-HIV positive standard serum (Dr. Habermehl, Deutsche Vereinigung zur Bekämpfung von Viruskrankheiten, Lot-Nr. 116302): titers ranged from 1:6,400 in the immunofluorescence assay and the Du Pont-ELISA to 1:204,800 in the Abbott-ELISA. In our system the Western blot analysis was nearly as sensitive (1:128,000) as the Abbott-ELISA (Table VI).

DISCUSSION

The two commercially available anti-HIV ELISAs (Abbott HTLV VIII EIA and Du Pont HTLV III-ELISA) follow the same pattern: inactivated HTLV III antigen coated to a solid phase, "serum incubation," detection of antibodies to HIV by an enzyme-linked, anti-human IgG. Nevertheless, significant differences in specificity and sensitivity between the two ELISAs are obvious: in our study the Abbott assay had a significantly lower specificity (94.3%) than the Du Pont ELISA (97.9%). These results are compatible with those of other authors [Mortimer et al, 1985; Pizzocolo et al, 1986] who reported specificities in the range of 95.3% to 97.5% for the Abbott assay. Recently, Reesink et al [1986] found a specificity of 99.6% for the Abbott assay in a collection of blood donor samples. In the same study this specificity declined to 91.5% in a "tricky" serum collection. We also found that the lack of specificity of the Abbott assay was particularly evident with sera drawn from patients with immunological abnormalities, such as kidney transplant recipients, lymphoma patients, and patients with allergic manifestations like Stevens-Johnson syndrome. In such cases the Abbott-ELISA tended to give false-positive results (see Results).

The sensitivity of the Abbott assay was 100%, and this high sensitivity could be confirmed by titration of the standard serum. Others found the Abbott assay to be the

TABLE VI. Sensitivity as Determined by Titration of a Standard Serum[a]

Assay	Positive reaction of standard serum at dilution[b]
Abbott HTLV III EIA	1:204,800
Western blot	1:128,000
Du Pont HTLV III ELISA	1:6,400
IFA	1:6,400

[a]Standard serum: Deutsche Vereinigung zur Bekämpfung von Viruskrankheiten (DVV) Lot No. 11 63 02.
[b]Serum dilutions were done in the respective test buffers.

most sensitive out of 7 [Mortimer et al, 1985] or 3 [Pizzocolo et al, 1986] anti-HIV assays (RIAs and ELISAs) tested. In the study of Reesink et al [1986], the Abbott assay was one of 3 tests with a 100% sensitivity.

The specificity of the Du Pont assay was 97.9%, and its sensitivity was reduced by one false-negative serum specimen to 99%. Three of 5 false-positive sera came from kidney transplant recipients. Thus, sera from patients with immunological disorders seemed to be responsible for reduced specificity in both assays. The molecular basis for false results in such "problem sera" is not known.

Differences in specificity and sensitivity of ELISAs are often attributed to the different cutoff calculations. In our study simple increase of cutoff (3-fold) in the Abbott-ELISA would have led to elimination of 20 of 23 false-positive results (see Table IV), but this in turn would have resulted in 2 false-negative results. A 1.5-fold increase in cutoff in the Du Pont-ELISA would have reduced false-positive results from 8 to 3, but this would have produced one additional false-negative specimen. On the other hand, a 0.5-fold reduction of cutoff in the Du Pont-ELISA would not have eliminated the one false-negative result. Thus, it is obvious that a different cutoff definition does not eliminate differences between the assays. Probably, differences in antigen preparation and inactivation measures are responsible for discrepancies in these cases.

The few sera from patients with manifest AIDS and lymphadenopathy syndrome were clearly positive by the commercially available ELISAs and by our immunofluorescence assay. With the exception of 11 serum specimens (out of 151 sera), samples from asymptomatic patients with a defined risk of HIV infection could be clearly determined as positive or negative.

Our study again illustrates that specificity and sensitivity are not easily achieved at the same time in anti-HIV assays. A very high sensitivity has the advantage that very low antibody titers are detected, but this is usually accompanied by a loss of specificity. On the other hand, a highly specific test led to a false-negative result, thus reducing its sensitivity. The dilemma between high specificity but reduced sensitivity and vice versa in anti-HIV-ELISAs is so far unresolved. However, future assays with "antigen cocktails" produced in vitro by gene cloning may achieve higher specificities. At present, retesting all ELISA-reactive sera by an independent test system and checking the result with a second serum specimen from the same patient are necessary.

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REFERENCES

Centers for Disease Control (1985): Provisional Public Health Service inter-agency recommendations for screening donated blood and plasma for antibody to the virus causing acquired immunodeficiency syndrome. Morbidity and Mortality Weekly Report 34/1:5–7.

Cheingsong-Popov R, Weiss RA, Dalgleish A, Tedder RS, Shanson DC, Jeffries DJ, Ferns RB, Briggs EM, Weller IVD, Mitton S, Adler MW, Farthing C, Lawrence AG, Gazzard BG, Weber J, Harries JRW, Pinching AJ, Craske J, Barbara JAJ (1984): Prevalence of antibodies to human T-lymphotropic virus type III in AIDS risk patients in Britain. Lancet II:477–480.

Mortimer PP, Parry JV, Mortimer JY (1985): Which anti-HTLV III/LAV assays for screening and confirmatory testing? Lancet II: 873–877.

Pizzocolo G, Gussago A, Barbieri U, Callegaro L, Palla M (1986): Comparison of three commercially available kits for the determination of antibodies to HTLV III. International Symposion on Monoclonals and DNA-Probes in Diagnostic and Preventive Medicine: Abstract 204.

Reesink HW, Huisman JG, Gonsalves M, Winkel IN, Hekker AC, Lelie PN, Schaasberg W, Aaij C, van der Does JA, Desmyter J, Goudsmit J (1986): Evaluation of six enzyme immunoassays for antibody against Human Immunodeficiency Virus. Lancet II: 483–486.

Sarngadharan MG, Popovic M, Bruch L, Schüpbach J, Gallo RC (1984): Antibodies reactive with human T-lymphotropic retroviruses (HTLV III) in the serum of patients with AIDS. Science 224: 506-508.

Velimirovic B (1985): AIDS, Blut und Blutderivate. Bundesgesundheitsblatt 28/1:13–19.

Weiss SH, Goedert JJ, Sarngadharan MG, Bodner AJ, The AIDS seroepidemiology collaborative working group, Gallo RC, Blattner WA: Screening test for HTLV III (AIDS agent) antibodies. JAMA 253/2:221–225.

WHO (1985): The acquired immunodeficiency syndrome (AIDS): Memorandum from a WHO meeting. Bulletin of the World Health Organization 63/4:667–672.