JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1988, p. 1496–1500
0095-1137/88/081496-05$02.00/0

Vol. 26, No. 8

Performance and Reliability of Five Commercial Enzyme-Linked Immunosorbent Assay Kits in Screening for Anti-Human Immunodeficiency Virus Antibody in High-Risk Subjects

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Received 9 November 1987/Accepted 20 April 1988

Anti-human immunodeficiency virus enzyme-linked immunosorbent assay kits marketed by Electro-Nucleonics Inc. (ENI), Genetic Systems Corp. (GSC), Organon Teknika Inc. (OTI), Ortho Diagnostic Systems Inc. (ODSI), and Wellcome Diagnostics (WD) were evaluated by using 289 randomly selected serum samples from a high-risk population and 53 serum samples likely to produce false-positive results. The radioimmunoprecipitation assay was used as the reference test. Sensitivities ranged from 96.51% (ODSI, WD) to 97.67% (ENI, GSC, OTI). Sera showing antibodies to viral glycoproteins only produced the false-negative results. Specificities ranged from 99.6% (ENI, GSC, ODSI, OTI) to 100% (WD). False-positive results were obtained with sera from patients with autoimmune disease or Epstein-Barr virus infection. Only results from GSC and OTI kits were distributed in two compact clusters well segregated on either side of the cutoff point. ODSI and GSC kits had the best intralot reproducibility. The GSC kit had the best interlot reproducibility. Cutoff values for ODSI and GSC kits were the least variable. Intraplate repeatability was good for all kits. Sample localization was not an important source of variability. Our results do not point out one outstanding kit among the five evaluated. However, the GSC kit showed the best overall results.

Anti-human immunodeficiency virus (HIV) enzyme-linked immunosorbent assays (ELISAs), first introduced in 1985 (12), are inexpensive and easy to use for mass screening compared with the radioimmunoprecipitation assay (RIPA) (1), Western blotting (immunoblotting), and immunofluorescence (IFA) (2). However anti-HIV ELISAs must be sensitive enough to detect all anti-HIV-positive individuals and as specific as possible to reduce the cost generated by confirmatory tests.

In the province of Quebec, hospitals offering HIV screening use the same type of equipment (ELISA Processor II; Calbiochem-Behring) and the same commercial ELISA anti-HIV kit to control expenses and to standardize the quality of testing. Since several kits are said to be compatible with the ELISA Processor II, an evaluation was carried out to determine the most suitable kit.

Five anti-HIV ELISA commercial kits (versions available during the first trimester of 1987) were evaluated by using current sera from the high-risk population screened in our hospitals. The RIPA was selected as the reference technique because it is more sensitive than most Western blot assays for the detection of antibodies to HIV glycoproteins (5). The relative performance of each kit compared with the RIPA, its predictive value, and its reliability were determined. Finally, the ease of use was evaluated by three experienced technologists.

MATERIALS AND METHODS

Serum samples. A new frozen sample of coded serum was used for each test performed. The number of randomly selected serum samples was determined to provide the statistical power of 95% probability of finding significant differences (type 1 error, 0.05; type 2 error, 0.05) in a population showing an anti-HIV prevalence (RIPA) of 0.25 and assuming a sensitivity of 96% for the ELISAs (3). The 342 samples (Table 1) comprised 289 randomly selected serum samples from patients screened for anti-HIV and 53 selected serum samples (Table 1) obtained from patients with infections or diseases which might give rise to false-positive results in anti-HIV ELISAs (9–11, 18). The anti-HIV antibody status of the serum was not known at the time of selection.

Enzyme immunoassays. The following anti-HIV ELISA kits were evaluated: VIRGO HTLV-III ELISA (Electro-Nucleonics Inc. [ENI]), Genetic Systems LAV EIA (Genetic Systems Corp. [GSC]), ORTHO HTLV-III ELISA Test System (Ortho Diagnostic System Inc. [ODSI]), Vironostika anti-HTLV-III Microelisa system (Organon Teknika Inc. [OTI]), and Wellcozyme anti-HTLV-III (Wellcome Diagnostics [WD]) (Table 2). Tests were carried out with the ELISA Processor II with the agreement of each of the kit suppliers. They were performed and interpreted according to the manufacturers' instructions. Sera were not heat inactivated before testing (12). The ratio of the optical density to the cutoff value (R ratio) (ENI, GSC, ODSI, OTI) or the inverse ratio (WD) was used to classify the results as negative ($R < 0.6$), high negative ($0.6 \leq R < 1.0$), low positive ($1.0 \leq R < 3.0$), moderate positive ($3.0 \leq R < 6.0$), high positive ($R > 6.0$), or not classifiable (R highly variable on repeated tests) (J. L. Sever and D. L. Madden, Clin. Immunol. Newsl. 7:137–140, 1986). R ratio allows for the comparison of results between plates, since it is a function of the cutoff value which is plate specific.

Reference techniques. The RIPA was performed on the 342 serum samples as previously described (1). HIV-infected CEM cells were labeled with 0.4 mCi of [^{35}S]cysteine (Amersham Corp.; specific activity, >1,200 Ci/mmol) per ml. Extracellular virus concentrated by ultracentrifugation

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TABLE 1. Description of sera used to evaluate the anti-HIV ELISA commercial kits

RIPA result	No. of serum samples by type[a]						
	R	CMV	EBV	RF	LE	L	Total
Positive	81	4	1	0	0	0	86
Negative	208	9	22	15	1	1	256
Total	289	13	23	15	1	1	342

[a] CMV, Selected sera positive for anti-cytomegalovirus antibody; EBV, selected sera positive for anti-Epstein-Barr virus nuclear antigen and/or anti-Epstein-Barr viral capsid antigen; RF, rheumatoid factor-positive selected sera; L, selected serum from a patient showing a lymphadenopathy of unknown origin; LE, selected serum from a patient with lupus erythematosus; R, randomly selected sera from patients screened for anti-HIV.

and lysed was used as the antigen (final concentration, 2.5×10^4 immunoprecipitable cpm per 50 μl). Sera were considered positive when reactivity to the HIV glycoproteins was detected; they were negative when no viral polypeptide was precipitated.

RIPA-positive sera were titrated by IFA by using lymphadenopathy-associated virus type 1-infected MOLT4-T4 cells and fluorescein isothiocyanate-conjugated [$F(ab')_2$] anti-human immunoglobulin G. Sera were classified as low positive (titer < 800), positive (800 ≤ titer ≤ 1,600), or high positive (titer > 1,600).

Study design. The 342 serum samples (Table 1) tested twice on 2 different days with microdilution plates from the same lot were used to evaluate the sensitivity, specificity, efficiency, predictive value, and intralot reproducibility (between-run precision) (17).

The interlot reproducibility and the repeatability (within-run precision) were evaluated by using a panel of 24 reference sera titrated by IFA (8 negative, 8 low positive, 8 high positive). They were tested six times on two microdilution plates from three different lots. One lot was tested per day by the same person in the same experimental conditions. The plate loading pattern also allowed for the evaluation of edge effect (13).

The ease of use of each kit was analyzed by the following criteria: clarity of the user's manual, number and simplicity of the steps, time to process one plate, and potential causes for errors.

Data analysis. Result status (true- or false-positive, true- or false-negative) was determined by using a 2 × 2 table (18) with the RIPA as the reference test. Sensitivity, specificity, efficiency, and predictive value were computed with the usual formulas (6, 16). Distribution of results was determined for each kit by using the mean R ratio of the four repeats for each serum (Fig. 1).

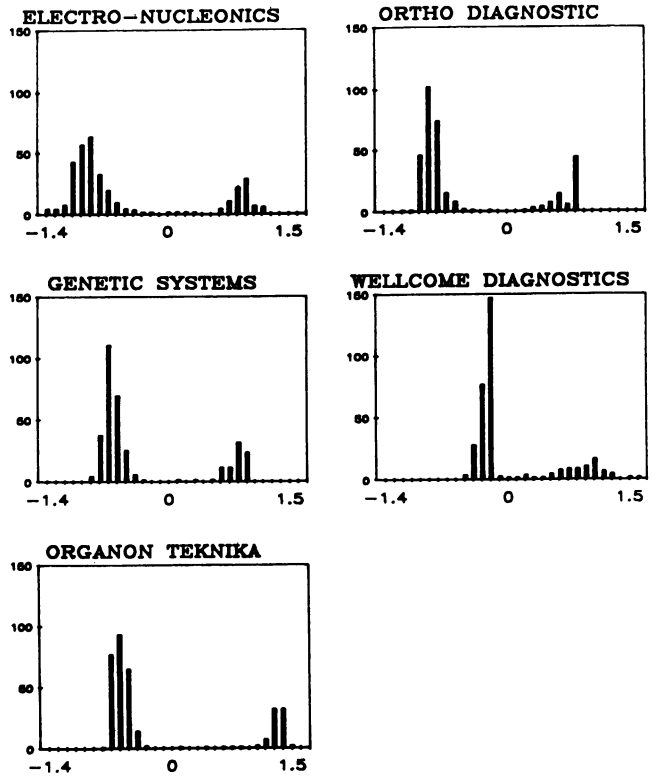

FIG. 1. Distribution patterns of R ratios (optical density to the cutoff value [ENI, GSC, ODSI, OTI]; inverse ratio [WD]) obtained with the five anti-HIV ELISA kits. The horizontal axis shows the minimum and maximum values of the R ratio expressed as $\log_{10}$ R (from −1.4 to 1.5 by 0.05). Zero represents the cutoff point where R is equal to 1 (optical density equal to the cutoff value; $\log_{10} 1 = 0$). The vertical axis shows the number of results obtained for each interval of R ratio.

Two intralot qualitative reproducibility parameters were computed. Status reproducibility indicates the percentage of sera yielding the same result (positive or negative) on four repeats. Class reproducibility represents the percentage of sera having four repeats within the same class as determined by the value of the R ratio (see above). Three intralot quantitative reproducibility parameters were computed. The interplate correlation (Pearson's coefficient of correlation [4]) indicates the agreement between paired results on two

TABLE 2. Characteristics of anti-HIV ELISA kits[a]

Kit	Type of assay[b]	No. of steps	Wells (bottom)	Virus antigen	Cell line	Substrate[c]	H_2SO_4 concn	Wavelength (nm)		Final serum dilution	Cutoff value[d]
								Reading	Reference		
ENI	A	12	Round	HTLV-III	H9	OPD	2 N	492	600 or 650	1:100	(PC + NC)/3
GSC	A	12	Flat	LAV-1[e]	CEM	TMB	3 N	450	615 or 630	1:400	NC + 0.255
OTI	A	12	Flat	HTLV-III	H9	OPD	4 N	492	600 or 650	1:100	(4NC + PC)/5
ODSI	A	11	Flat	HTLV-III	H9	OPD	4 N	492	650	1:20	NC + 0.25
WD	C	9	Round	HTLV-III	CEM	TMB	2 M	450	615 or 630	1:4	CO × 1.1

[a] All kits used microtiter plates, horseradish peroxidase, and H_2SO_4 as the stop solution.
[b] A, Antiglobulin assay; C, competitive assay.
[c] OPD, Orthophenylenediamine; TMB, tetramethylbenzidine.
[d] PC, NC, and CO, Mean optical density of positive control serum, negative control serum, and cutoff control serum, respectively.
[e] LAV-1, Lymphadenopathy-associated virus 1.

TABLE 3. Sensitivity, specificity, efficiency, and predictive values of anti-HIV ELISA kits

Kit	Sensitivity (%)	Specificity (%)	Efficiency (%)	Predictive value[a] (%)			
				Positive		Negative	
				P = 0.01	P = 0.2	P = 0.01	P = 0.2
ENI	97.67[b]	99.60[c]	99.12	71.15	98.38	99.97	99.41
GSC	97.67[b]	99.60[c]	99.12	71.15	98.38	99.97	99.41
OTI	97.67[b]	99.60[c]	99.12	71.15	98.38	99.97	99.41
ODSI	96.51[d]	99.60[c]	98.83	70.90	98.36	99.96	99.13
WD	96.51[d]	100.0[e]	99.12	100.00	100.00	99.96	99.13

[a] P, Prevalence of anti-HIV seropositivity in the population studied.
[b] Raw value: 84 of 86.
[c] Raw value: 255 of 256.
[d] Raw value: 83 of 86.
[e] Raw value: 256 of 256.

plates (two repeats, same localization on two different plates from the same lot, two different testing days). Student's *t* test was performed on each interplate coefficient of correlation to determine the strength of the straight-line relationship between results from repeats. The interplate coefficient of variation (4), computed from nonpaired results, expresses the variability due to differences between plates within a lot. The coefficient of variation of the cutoff values, computed from results with control sera supplied by the manufacturer, indicates the variability of the cutoff point used to interpret results.

The interlot reproducibility was evaluated by using the pooled standard deviation (8) and the coefficient of variation. The interlot standard deviation is an assessment of the absolute variation due to differences between lots. A small value indicates a good interlot reproducibility. The interlot variability is computed by subtracting the average coefficient of variation of the lots from the coefficient of variation of the kit (computed from the pooled standard deviation). It indicates the relative importance of the interlot standard deviation in relation to the mean value of all the results within a kit for a given sample.

The intraplate coefficient of correlation was used to evaluate the repeatability of the kits. It represents the average coefficient of correlation between paired results on the same plate (two repeats on the same plate, same testing day, single person using the same sample).

The edge effect (13) was computed by subtracting the intraplate variability of repeats of sera located at the center of the plate from the variability of repeats of the same sera located on the edge of the plate.

Computer analysis was performed by using a relational database management system (REFLEX 1.1; Borland International/Analytica Inc.) interfaced with electronic spreadsheet templates (LOTUS 1-2-3 1A; LOTUS Development Corp.), both softwares running on an IBM-PC/XT microcomputer.

RESULTS

Performance of the kits. Sensitivities, specificities, efficiencies, and predictive values are given in Table 3. False-negative results were obtained with three sera from group R (Table 1) showing weak RIPA reactivity to HIV glycoproteins only (proportion of sera detected as positive: 1/3 [ENI, GSC, OTI]; 0/3 [ODSI, WD]). False-positive results were obtained with sera from patients with lupus erythematosus (ENI, ODSI, OTI) or Epstein-Barr virus infection (GSC).

The distribution of results with GSC and OTI kits indicated a good separation between reactive and nonreactive results (Fig. 1). The distribution of results from ENI, ODSI, and WD kits was less segregated.

Reliability of the kits. The intralot reproducibility parameters are given in Table 4. The interlot reproducibility parameters are given in Table 5. The intraplate coefficient of correlation was high for all the kits (0.974 [WD], 0.990 [ODSI], 0.998 [ENI, GSC, OTI]). This and a slope of 1 indicate a positive, directly proportional, straight-line relationship. The variabilities due to edge effect were −1.06% (ODSI), 0.73% (WD), 1.72% (OTI), 2.50% (GSC), and 3.75% (ENI).

Ease of use of the kits. The time required to process one microdilution plate from serum dilution to reading of results was from 3 to 4 h (3 h [ENI], 3.25 h [OTI], 3.5 h [WD], and 4 h [GSC, ODSI]). The GSC kit was the easiest to use. The GSC user's manual was clear with well-detailed protocols. The preparation of the different buffers was identical. The buffers were color coded, and reagent volumes added to each well were constant, thereby decreasing the risk of errors. The four remaining kits were also relatively easy to use. However, each of them lacked some of the features that made the GSC kit easier to use.

DISCUSSION

The highest sensitivity was obtained with the ENI, GSC, and OTI kits (97.67%). The false-negative results were obtained with sera showing weak RIPA reactivity to HIV

TABLE 4. Intralot reproducibility of the anti-HIV ELISA kits[a]

Kit	Qualitative data (% reproducibility)		Quantitative data		
	Status[b]	Class[c]	Interplate correlation[d]	Variability	
				Interplate	Cutoff
ENI	100.00	95.32	0.964	12.540	20.868
GSC	100.00	96.78	0.991	9.786	3.678
OTI	100.00	99.12	0.974	12.083	8.867
ODSI	100.00	97.95	0.989	7.029	2.880
WD	99.60[e]	75.14	0.939	7.610	15.089

[a] Qualitative data are discontinuous results expressed qualitatively such as positive or negative (status) or negative, high negative, low positive, moderate positive, or high positive (class). Quantitative data are continuous results expressed as numbers, such as R ratio.
[b] Percentage of sera with four repeats with identical status.
[c] Percentage of sera with four repeats within the same class.
[d] Student's *t* test ($P < 0.001$).
[e] One serum sample gave three low-positive results and one high-negative result on repeats. This sample was ultimately scored as positive.

TABLE 5. Interlot reproducibility of the anti-HIV ELISA kits[a]

Kit	Interlot SD				Interlot variability			
	N	LP	HP	All sera	N	LP	HP	All sera
ENI	0.005	0.595	0.489	0.436	23.30	8.35	3.79	11.81
GSC	0.009	0.073	0.018	0.033	3.07	2.65	1.16	1.16
OTI	0.013	0.219	0.396	0.251	3.61	27.17	33.81	21.81
ODSI	0.005	0.014	0.048	0.017	9.03	6.57	6.34	7.31
WD	0.026	0.003	0.006	0.015	2.23	10.96	18.17	10.17

[a] N, Negative sera (RIPA and IFA negative); LP, low-positive sera (RIPA positive, IFA titer of <800); HP, high-positive sera (RIPA positive, IFA titer of >1,600); All sera, analysis of results without reference to the status of the sera.

glycoproteins (gp) only. This weak reactivity to HIV gp only was also confirmed by Western blot with a gp160 recombinant antigen, performed by an independent laboratory (Viral Diagnostic Services Div., Bureau of Microbiology, Laboratory Centre for Disease Control, Ottawa, Canada). Since these anti-gp are the only HIV antibodies detectable in the serum of some patients (early and terminal cases of acquired immunodeficiency syndrome) (5, 14), screening tests for anti-HIV seropositivity should be able to detect them (14). Our results show that the five commercial kits evaluated were equally unable to detect most of the sera showing gp reactivity only. Similar results were obtained in one other study of five different kits (15), indicating that most of the licensed anti-HIV ELISA are not able to detect sera showing gp reactivity only. These results show that their sensitivity range must be improved to permit the detection of positive sera from recently infected individuals.

The highest specificity (100%) was obtained with the WD kit with HTLV-III antigen grown in CEM cells which are free of class II human leukocyte antigens (10). These antigens, present in the H9 cell line, are known to react with antibodies produced in autoimmune diseases such as lupus erythematosus (11). The false-positive reactions obtained with ENI, OTI, and ODSI kits which use virus grown in H9 cells (Table 2) may be due to the presence of antibodies reacting with contaminating cellular antigens. False-positive reactions with sera from patients with EBV infection have been previously reported (12). Since antibody to lymphocytes may be induced during EBV infection (9), these may react with cellular antigenic determinants present in the wells.

Reactive and nonreactive ELISA values should be distributed in two compact clusters well segregated on either side of the cutoff point to decrease the risk of misinterpretation due to overlapping (7). Two kits (GSC, OTI) had this type of distribution, indicating that they should produce fewer misinterpreted results due to R ratios in the high-negative/low-positive region ($0.6 < R < 3$). Most of the negative results from the WD kit are in the high-negative region (Fig. 1); this is characteristic of a competitive assay.

The ODSI and GSC kits had the best intralot reproducibility, and the GSC kit had the best interlot reproducibility. The cutoff values for these two kits were also the least variable (<4%). The ENI, GSC, and OTI kits had the best repeatability.

The variabilities due to the edge effect were small, indicating that sera localization was not an important source of variability in our experimental conditions.

Errors or invalidated tests due to technical difficulties are rare when using a well designed, easy to use kit. Thereby the cost of using such a kit is kept to a minimum. Our observations, even if partly subjective, indicate that the GSC kit was the easiest to use in our experimental conditions.

Our results do not point out one outstanding kit among the five kits evaluated. However, the GSC kit had the best overall results when performance, reliability, and ease of use were taken into account.

ACKNOWLEDGMENTS

Serum samples were provided by the following hospitals: Sainte-Justine, Maisonneuve-Rosemont, General de Montreal, Hotel-Dieu de Montreal, Royal Victoria, Saint-Luc, and Centre Hospitalier Universitaire Laval. We thank Denise Jutras-Dupaul, Lise Leblanc, and Micheline Pelletier for their excellent technical assistance.

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