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Performance Characteristics of Recombinant Enzyme Immunoassay To Detect Antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) and HIV-2 and To Measure Early Antibody Responses in Seroconverting Patients

RICHARD A. GALLI,[1]* SANTINA CASTRICIANO,[2] MARGARET FEARON,[1] CAROL MAJOR,[1] KAM WING CHOI,[1] JIM MAHONY,[2] AND MAX CHERNESKY[2]

Ontario Ministry of Health, Toronto, Ontario, Canada M9P 3T1,[1] and McMaster University Regional Virology and Chlamydiology Laboratory, St. Joseph's Hospital, Hamilton, Ontario, Canada L8N 4A6[2]

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We investigated the performance of a double-antigen sandwich recombinant enzyme immunoassay (EIA; Abbott Laboratories, North Chicago, Ill.) and compared it with that of a synthetic-peptide-based EIA (Biochem Immunosystems, Montreal, Quebec, Canada) for the detection of human immunodeficiency type 1 (HIV-1) and HIV-2 antibodies in 2,321 clinical serum samples. The results of both EIA methods and Western blot (immunoblot) were in agreement for 1,046 HIV-1 and 10 HIV-2 specimens from a panel of known positives. From a prospective panel of 1,085 specimens, 38 proved to be positive by both EIAs and Western blot, 3 were positive by the recombinant EIA only, and 9 were positive by the peptide EIA only, for calculated specificities of 99.71 and 99.04%, respectively. Of 180 specimens from a seroconversion panel collected from 77 patients, the results for 170 were in agreement by all antibody testing methods and 10 were found to be repeat reactive for HIV antibodies by the recombinant EIA only. All 10 were initial specimens of seroconverting patients; 7 were also reactive for HIV p24 antigen. An examination of four of these sera by radioimmunoprecipitation assay showed gp120 and gp160 bands in each. Analysis of the anti-Env antibody class in three of these samples showed that one consisted of immunoglobulin M (IgM) only and two contained both IgG and IgM antibodies. Although both EIA procedures were sensitive and specific in the detection of antibodies to HIV-1 and HIV-2 and both were capable of detecting early antibodies, the recombinant assay was more sensitive for antibody detection during early seroconversion.

Human immunodeficiency virus type 1 (HIV-1) remains the predominant virus associated with AIDS in North America, but HIV-2 infections have been identified in many parts of the world, including North America (12, 13). Although cases of HIV-2 infection, whether indigenous or imported, are rare (3), testing for HIV antibodies to screen the blood supply or diagnose infection should no longer be based on HIV-1 antigens alone. Because of extensive homogeneity between these two viruses, primarily in the *gag* and *pol* regions (1, 7), cross-reactions in antibody tests have been demonstrated in 28 to 93% of HIV-2 infections when employing existing HIV-1 immunoassays (17). Because of the possibility of HIV-2 infections being undetected by HIV-1 immunoassays, highly sensitive, cost-effective assays capable of simultaneous detection of specific antibodies to HIV-1 and HIV-2 have been developed (4, 15). Newer enzyme immunoassays (EIAs) utilizing synthetic peptides or recombinant proteins as antigens, either in conventional sandwich or double-antigen sandwich configurations, have been shown to have higher degrees of sensitivity and specificity than those of whole-viral-lysate-based assays (9, 18).

Although the performance characteristics of these newer assays on seroconversion panels (6, 18) and cohort subjects (2) have been well assessed, their ability to detect early antibodies to HIV in everyday clinical specimens is not well documented. Their use can result in a reduction of the window period in the laboratory diagnosis of HIV infections (2), leading to earlier intervention strategies in patient management and elimination of potentially infectious blood donations from recently infected donors. We have evaluated the performance of two HIV-1–HIV-2 combination assays, one recombinant antigen based and the other synthetic peptide based, in screening serum specimens for HIV antibodies and found that the recombinant EIA demonstrated a particular ability to detect antibodies during the early stages of seroconversion.

The serum panels assembled for this study consisted of the following: a retrospective panel of 1,046 frozen serum samples confirmed to be reactive for HIV-1 and 10 serum samples confirmed to be reactive for HIV-2 by Western blot (WB; immunoblot); a seroconversion panel of 180 frozen specimens collected from 77 patients with recent HIV infections (the collection interval of these specimens was widely variable for each patient, ranging from 2 weeks to 4 years); and a prospective panel of 1,085 fresh serum specimens collected from the voluntary testing population of Ontario.

Serum samples from each panel were tested for antibodies to HIV-1 or HIV-2 by a recombinant EIA from Abbott Laboratories, North Chicago, Ill., and a synthetic-peptide EIA from Biochem Immunosystems, both of which are commercially available in Canada. The Abbott antigen-conjugate assay uses purified recombinant antigens (rp24 and rp41 for HIV-1 and RP36 for HIV-2, expressed in *Escherichia coli* or *Bacillus megaterium*) coated onto polystyrene beads to capture antibodies to HIV-1 or HIV-2 from undiluted patient serum. The Biochem Immunosystems Detect-HIV conventional-design EIA uses three highly purified synthetic-peptide antigens, rep-

* Corresponding author. Mailing address: Ontario Ministry of Health, Laboratory Services Branch, HIV Laboratory, 81 Resources Rd., Etobicoke, Ontario, Canada M9P 3T1. Phone: (416) 235-5727. Fax: (416) 235-6194.

TABLE 1. HIV EIA and WB results for 1,085 prospective serum samples

Result[a]			No. of samples
Recombinant EIA	Synthetic-peptide EIA	WB	
+	+	+	38
+	−	−	2[b]
−	+	−	9[b]
+	+	IND	1[b]
−	−	ND	1,035

[a] +, repeat reactive; −, nonreactive (negative); IND, indeterminate; ND, not done.

[b] All specimens were negative for HIV antigen and were considered to be false positives in the respective EIAs.

resenting immunodominant epitopes of the gp41 and gp120 regions of HIV-1 and of the gp36 region of HIV-2, coated onto individual wells of a plastic, 96-well microplate to capture antibodies from diluted patient serum (1:400).

All assays of samples with absorbance readings greater than or equal to the manufacturer's cutoff value in either EIA were repeated in duplicate. Those specimens found to be repeatedly reactive were subsequently tested by an in-house WB modified from an original protocol developed by the Centers for Disease Control and Prevention, Atlanta, Ga. (11). Whole HIV-1 lysate (Organon Teknika) was separated into component proteins by sodium dodecyl sulfate–10% polyacrylamide gel electrophoresis. Separated viral proteins were then transblotted onto supported nitrocellulose paper (BAS 85; Schleicher and Schuell) overnight at 4°C. The nitrocellulose was dried, quenched in 10% skim milk, rinsed in Tris-saline, cut into individual strips (4 mm in diameter), and stored at −70°C until used in WB assay procedures. Diluted serum (1:100 in 10% skim milk powder dissolved in distilled water) was added to prepared strips; after incubation and a washing process, bound HIV antibodies were detected by using goat anti-human immunoglobulin G (IgG) and IgM conjugated with alkaline phosphatase (Jackson Immunoresearch Laboratories, Westgrove, Pa.) and a nitroblue tetrazolium–5-bromo-4-chloro-3-indolyl-1-phosphate (BCIP) substrate solution (Promega, Madison, Wis.). Individual band intensities were interpreted visually for each specimen. Results were considered to be reactive if at least one band from the *gag* (p17, p24, and p55) and one band from the *env* (gp41, gp120, and gp160) regions were visible.

Selected specimens were tested for HIV antigen by using the Abbott HIVAG-1 immunoassay system (10, 14). Four of these specimens were also sent to the Laboratoire de Sante Public du Quebec, St. Anne de Bellevue, Quebec, Canada, for radioimmunoprecipitation assay (RIPA), which was kindly performed by Micheline Fauvel. Anti-Env antibody class analysis was conducted on a series of blinded specimens by James L. Gallarda, Abbott Laboratories, by his previously published method (6).

In the retrospective study, all 1,046 HIV-1 and 10 HIV-2 WB-reactive specimens were positive in both EIAs. Signal-to-cutoff ratios were generally higher in the synthetic-peptide EIA.

Table 1 presents data for 1,085 randomly selected fresh serum specimens from routine clinical submissions. There were 38 WB-confirmed specimens detected as positive by both EIAs. Two WB-negative specimens were positive only in the recombinant EIA, and nine others were positive only in the synthetic-peptide EIA. One WB-indeterminate specimen was positive in both EIAs. For the purpose of this comparative study, in the absence of follow-up specimens and information regarding patient outcome or clinical status of these individuals, these samples were considered to be false positives in the respective EIAs. The relative specificities were calculated to be 99.71% (1,044 of 1,047) for the recombinant EIA and 99.04% (1,037 of 1,047) for the synthetic-peptide EIA. By combining the positives from the prospective and retrospective panels, both assays have sensitivities of 100% for HIV-1 (1,084 of 1,084) and HIV-2 (10 of 10).

From the seroconversion panel of 180 specimens from 77 patients, a total of 10 specimens were discrepant. All were positive by the recombinant EIA, nine of them were negative by the synthetic-peptide EIA and WB, and one was negative by the synthetic-peptide EIA and indeterminate by WB. All were initial specimens of seroconversions. An additional six specimens were positive by both EIA methods but indeterminate by WB. These samples were also initial specimens of seroconversions or early follow-up samples of an initially negative sample. The profiles of discordant results for 10 patients from the seroconversion panel are presented in Table 2. Of the initial specimens of these 10 patients (samples 1a, 2a, 3a, etc.), all were positive by the recombinant EIA yet negative by the synthetic-peptide EIA. Nine of these were also negative by WB, with only the initial specimen from patient 6 giving an indeterminate WB result. From patient 5, a specimen collected 2 weeks after the initial specimen became positive in the peptide EIA and indeterminate by WB. By 4 weeks, a third specimen was positive in all assays. From patients 2, 3, and 4, follow-up specimens from 1 to 4 years after the initial sample was collected were submitted. Since the recombinant EIA was the only assay to be positive for the initial specimens from these patients, the possibility of these results being coincidental false positives cannot be ruled out.

The two EIA methods evaluated in this study showed excellent sensitivities of 100% in the detection of HIV-1 and HIV-2 antibodies in WB-confirmed specimens. However, there were differences between the two assays in the ability to detect early HIV-1 antibodies at the time of seroconversion. The third-generation recombinant EIA from Abbott Laboratories appears to be more sensitive to early-antibody detection than is the synthetic-peptide EIA. For 10 specimens, all initial samples from patients who subsequently seroconverted, the recombinant EIA was repeatedly reactive while the synthetic-peptide EIA was negative. The WB of nine of these specimens was negative, and that of the other was indeterminate. However, these individuals were likely HIV infected at the time of sample collection, as shown by the fact that 7 of 10 were positive for HIV p24 antigen; all 10 patients became HIV antibody positive in subsequent tests of sera by WB and the synthetic-peptide EIA.

It has been demonstrated that IgM antibodies are present in serum during the early phases of HIV seroconversion (5, 10). In a study using the Abbott recombinant HIV-1–HIV-2 EIA for early detection of HIV-1 antibodies (6), the authors provided evidence of peak IgM levels during the initial stages of antibody development, which were then detected by the antigen-conjugate assay but not by conventional antibody-conjugate assays. Of the 10 specimens positive in the recombinant EIA but negative in the synthetic-peptide EIA in our study, 3 were available to be analyzed for anti-Env immunoglobulin classes. All showed elevated levels of IgM compared with those of follow-up specimens; two of the three showed initial low-level IgG activity as well. These three samples were positive for p24 antigen and demonstrated detectable gp120 and gp160 antibodies in RIPA, suggesting that the double-antigen sandwich recombinant EIA is also better equipped for detecting immune complexes than is the conventional synthetic-peptide

TABLE 2. HIV antibody and antigen profiles for sequential sera of 10 patients seroconverting to HIV-1

Patient sample	Collection interval	Result[a]						
		Synthetic-peptide EIA	Recombinant EIA	WB	RIPA	HIV antigen	IgG	IgM
1a		−	+	−	ND	+	ND	ND
1b	Unknown	+	+	+	ND	−	ND	ND
1c	Unknown	+	+	+	ND	−	ND	ND
2a		−	+	−	ND	−	ND	ND
2b	1 yr	+	+	+	ND	−	ND	ND
3a		−	+	−	ND	−	ND	ND
3b	1 yr	+	+	+	ND	−	ND	ND
4a		−	+	−	ND	−	ND	ND
4b	4 yr	+	+	+	ND	−	ND	ND
5a		−	+	−	ND	+	ND	ND
5b	2 wk	+	+	p24 only	ND	+	ND	ND
5c	4 wk	+	+	+	ND	+	ND	ND
6a		−	+	p24 only	ND	+	ND	ND
6b	8 mo	+	+	+	ND	−	ND	ND
7a		−	+	−	120, 160	+	−	+
7b	5 wk	+	+	+	ND	+	+	+
8a		−	+	−	120, 160	+	+	+
8b	4 mo	+	+	+	ND	−	+	+
8c	5 mo	+	+	+	ND	−	+	+
9a		−	+	−	120, 160	+	+	+
9b	10 mo	+	+	+	ND	−	+	+
10a		−	+	−	120, 160	+	ND	ND
10b	3 yr	+	+	+	ND	−	ND	ND

[a] +, repeat reactive; also for HIV antigen, >50% reduction in signal was achieved with blocking antibody; −, negative; ND, not done.

EIA. It is also not surprising that these sera, which were positive in the recombinant EIA composed of rp24 and rp41 HIV-1 antigens, reacted to gp120 and gp160 in RIPA. The antigen-conjugate (recombinant) EIA has been shown to be particularly sensitive to anti-gp41 antibodies (6). It is not unreasonable to expect this antibody to react with gp120 and gp160 envelope proteins in RIPA but not in WB, since RIPA is known to be more sensitive to anti-Env antibodies than is WB.

Both EIAS were more sensitive than was WB for the detection of early HIV antibodies in this study. In addition to the discordant results presented in Table 2, a total of six initial or early specimens from patients in the process of seroconversion were positive by both EIAs but indeterminate by WB. An earlier study (18) comparing third-generation EIAS with WB also found these EIAs to be more sensitive. Peptide-based EIAs have also shown high degrees of specificity (16), although in our study higher specificity was achieved with the recombinant EIA. The enhanced performance characteristics of these third-generation HIV assays emphasizes the need for the development of improved confirmatory procedures or alternative testing strategies employing EIAs such as the ones evaluated in this study. Holloman et al. (8) proposed several algorithms incorporating a combination HIV-1–HIV-2 EIA as an initial screen test, designed for high- and low-risk populations. In Canada, similar algorithms are already in use in several federal and provincial laboratories. They offer a cost-effective method to identify specific HIV-2 antibodies without compromise to the sensitivity and specificity of HIV-1 detectability. Use of these highly sensitive combined-antibody detection EIAs either for screening or as supplemental tests could result in the development of testing algorithms utilizing one EIA for initial screening and a different EIA for supplemental testing. As seen in this study, although a reactive result in both EIAs is highly indicative of HIV infection and in most cases would require no further confirmation, care should be exercised in the determination of which EIAs are to be used in tandem. An assessment of the differences in sensitivity of EIA procedures, as demonstrated in Table 2, should become an important part of the selection process. In addition, the impact of these third-generation EIAs on confirmatory strategies needs to be explored. Since these assays are more sensitive than is WB, perhaps the addition of p24 antigen and/or HIV nucleic acid detection techniques to algorithms employing these EIA methods along with WB should be considered.

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