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Use of an Acute Seroconversion Panel To Evaluate a Third-Generation Enzyme-Linked Immunoassay for Detection of Human Immunodeficiency Virus-Specific Antibodies Relative to Multiple Other Assays

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A human immunodeficiency virus type 1 (HIV-1)/HIV-2 antibody screening assay, the Genetic Systems HIV-1/HIV-2 PLUS O EIA, was compared to several established screening or confirmatory tests using an acute HIV seroconversion panel. The HIV-1/HIV-2 PLUS O EIA showed an improved sensitivity over all tested antibody screening methods, and detected antibody in 7 of 19 specimens found to be negative by a first-generation EIA but positive for the presence of HIV RNA.

Over the course of the human immunodeficiency virus (HIV) pandemic, enzyme immunoassays (EIAs) have been the most widely utilized method of HIV antibody screening. So-called "first-generation" EIAs for the detection of HIV-specific antibodies utilize immobilized preparations of purified, lysed HIV particles for the capture of virus-specific immunoglobulin G (IgG) from tested sera. Due to their reliability and low cost, first-generation EIAs remain in very wide use in both clinical and public health laboratories (1). Widespread, long-term use of first-generation EIAs has revealed its limitations. Such assays, as provided in the United States, utilize exclusively group M-derived virus particles as the capture antigen. Hence, these EIAs are not optimally capable of capturing antibodies generated against non-group M strains of HIV; nor are they optimally capable of detecting HIV type 2 (HIV-2)-specific antibodies (5, 7, 11). An additional limitation of first-generation EIA is that they detect only IgG, which increases the length of the window period of such tests (4).

Improved EIAs for the detection of HIV-specific antibodies have been in continual development. Among them are the so-called "third-generation" EIAs. These assays, available through several vendors, include recombinant or synthetic peptide antigens derived from HIV groups M and O, in addition to HIV-2, as the capture antigens. Also included in third-generation EIAs are the abilities to detect both IgG and IgM. This capability lends greater sensitivity to early HIV antibody detection because IgM is the first immunoglobulin product of the humoral immune response, reaching detectable concentrations in the blood prior to IgG (2, 6).

We describe here the performance of one such U.S. Food and Drug Administration-approved, third-generation EIA within the context of a public health laboratory that is accustomed to screening high numbers of suspected cases of HIV infection. This assay, the Genetic Systems HIV-1/HIV-2 PLUS O EIA (Bio-Rad, Redmond, WA) utilizes a variety of antibody capture antigens, including recombinant p24 and gp160 derived from group M HIV-1, a recombinant peptide of the immunodominant region of HIV-2 gp36, and a synthetic polypeptide which mimics an HIV-1 group O-specific epitope. HIV-specific antibodies captured in this assay are detected in a sandwich format by peroxidase-conjugated forms of the HIV antigens mentioned above, allowing for IgG and or IgM to be detected. The performance of the HIV-1/HIV-2 PLUS O EIA was compared to an established, widely utilized first-generation screening EIA, the Vironostika Microelisa (bioMerieux, Durham, NC), in addition to a panel of other assays, including the OraQuick Rapid HIV-1 Antibody Test (OraSure Technologies, Bethlehem, PA), Western blotting (Cambridge Biotech HIV-1 Western Blot Kit; Calypte Biomedical, Rockville, MD), immunofluorescence assay (IFA) (Fluorognost HIV-1 IFA; Sanochemia Pharmazeutika AG, Vienna, Austria), and HIV RNA by branched DNA (bDNA, Versant 3.0; Bayer, Emeryville, CA).

This panel of assays was used to discern the antibody and viral RNA status of 19 serum or plasma specimens determined to contain measurable HIV RNA by means of branched DNA detection and yet found to be nonreactive for HIV-specific antibody as determined by the first-generation EIA Vironostika Microelisa (9). The panel of tests was also used to test follow-up specimens from 14 of the 19 specimens in an effort to further evaluate the ability of each test to detect antibodies early in the seroconversion process.

We first sought to validate the third-generation EIA, the HIV-1/HIV-2 PLUS O EIA, relative to the first-generation test currently used in our laboratory. Of 55 retrospective serum specimens that were determined to be reactive for HIV-1 antibody by Vironostika Microelisa and confirmed to be positive by IFA, the HIV-1/HIV-2 PLUS O EIA detected antibody in all 55 specimens (55 of 55 positive [100%]). The same two EIAs were used to test 100 specimens previously determined to be nonreactive by the Microelisa and that did not contain detectable HIV RNA as determined by branched DNA analysis. The HIV-1/HIV-2 PLUS O EIA did not detect HIV antibody in any of these specimens (0 of 100 positive [0%]), indicating that the third-generation assay possesses a specificity equivalent to that of the Vironostika Microelisa.

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TABLE 1. Summary of test results for acute seroconversion panel[a]

Specimen group and patient ID	Days	Vironostika S/C avg	Vironostika EIA result	IFA	WB	HIV-1/2/O S/C avg	HIV-1/2/O EIA result	RT	Viral load (copies/ml)
Initial									
A		0.351	NR		I	0.127	NR	N	5,770
B		0.602	NR		I	0.955	NR	N	>500,000
C		0.440	NR		I	≥14.658	R	N	12,183
D		0.394	NR		N	0.098	NR	N	77
E		0.368	NR		I	0.233	NR	N	6,373
F		0.329	NR		I	13.433	R	N	>500,000
G		0.317	NR		N	0.084	NR	N	12,852
H		0.338	NR		I	0.109	NR	N	14,062
I		0.646	NR		I	≥14.658	R	P	>500,000
J		0.358	NR		N	0.106	NR	N	3,921
K		0.346	NR		N	4.574	R	N	>500,000
L		0.373	NR		N	0.175	NR	N	>500,000
M		0.344	NR		N	1.5327	R	N	>500,000
N		0.337	NR		N	0.113	NR	N	1,177
O		0.301	NR		N	0.127	NR	N	>500,000
P		0.755	NR		N	≥14.658	R	N	>500,000
Q		0.311	NR		N	0.277	NR	N	43,173
R		0.642	NR		I	0.117	NR	N	30,734
S		0.406	NR		N	13.276	R	N	>500,000
Follow-up									
B	26	5.699	R	P	P	14.634	R	P	
C	26	1.334	R	P	P	11.313	R	P	
E	41	1.638	R	P	P	9.8107	R	P	
G	24	0.905	NR	I	I	≥14.658	R	P	471,503
H	25	4.456	R	P	P	≥14.658	R	P	
I	14	4.239	R	P	P	≥14.658	R	P	
K	14	3.798	R	P	P	≥14.658	R	P	
L	30	2.458	R	P	P	≥14.658	R	P	
M	14	0.673	NR	I	I	14.478	R	N	>500,000
N	14	0.533	NR	I	I	≥14.658	R	N	267,051
O	61	5.463	R	P	P	13.934	R	P	
P	225	6.603	R	P	P	≥14.337	R	P	
Q	21	5.660	R	P	P	≥14.658	R	P	
R	13	0.969	NR	N	P	≥14.658	R	P	

[a] S/C, specimen optical density/cutoff ratio; RT, rapid test; WB, Western blot; IFA, indirect immunofluorescence assay; days, days between initial and follow-up specimens; R, reactive; NR, nonreactive; P, positive; N, negative; I, indeterminate.

Since the HIV-1/HIV-2 PLUS O EIA can detect both IgM and IgG, we sought to determine how the assay would perform upon screening serum from a retrospective panel of 19 patient specimens that had been classified as positive for the presence of HIV RNA and yet negative for the presence of HIV-specific antibodies (as determined by first-generation EIA) (9). As shown in Table 1, the HIV-1/HIV-2 PLUS O EIA detected HIV-specific antibody in 7 of the 19 (37%) patient specimens (specimens C, F, I, K, M, P, and S). Confirmation of these seven reactive specimens was attempted by Western blotting, whereupon three specimens tested nonreactive and four specimens were found to be indeterminate, showing reactive p24 bands alone or, in one case, a p24 band and a p17 band (specimen M). Western blots of the remaining 12 specimens (HIV-1/HIV-2 PLUS O EIA nonreactive) were either indeterminate (4 of 12 [33%]) or negative (8 of 12 [67%]). When all 19 specimens were subject to the OraQuick rapid test format, one specimen was found to be reactive (specimen I). These data suggest that the HIV-1/HIV-2 PLUS O EIA possesses sensitivity for the detection of HIV-specific antibodies that is greater than any of the antibody tests within our panel. The HIV-1/HIV-2 PLUS O EIA yielded evidence of HIV infection in several patients whose HIV-positive status had eluded our existing antibody-screening methodology. It is notable that the viral loads for six of these seven patients were very high (>500,000 copies/ml). These data, in combination with discordant EIA results, strongly support that these patients had acquired HIV recently. Patient C, who upon the initial visit possessed a markedly lower viral load (12,183 copies/ml) along with discordant EIA results, was an exception. We hypothesize that the lower viral load of this specimen was a result either of having been very recently infected with HIV or of being in the recovery phase of acute infection, whereupon it is common to see dramatic reductions in viral load in association with seroconversion (3, 4, 8).

Follow-up specimens were obtained from 14 of the 19 RNA-positive, first-generation EIA-nonreactive patients, with collection dates ranging from 13 to 225 days after initial specimen collection (Table 1, right portion). All 14 (100%) of the follow-up specimens were found to be reactive by HIV-1/HIV-2 PLUS O EIA, whereas 8 of the 14 follow-up specimens (57%) were found to be reactive by Vironostika Microelisa. Three of the 8 specimens that tested newly reactive by Vironostika Microelisa were from patients whose initial specimens were clas-

sified as HIV-1/HIV-2 PLUS O EIA-reactive (patients C, I, and K). The 14 follow-up specimens were also subjected to Western blotting, whereupon 11 (11 of 14 [79%]) were found to be reactive and 3 (3 of 14 [21%]) were found to be indeterminate. The results of the IFA matched those of Western blot with the exception of specimen R, which was IFA negative. OraQuick rapid tests were used to assess antibody status in all 14 of the follow-up specimens. This rapid test possessed greater sensitivity than the first-generation EIA, as 10 of the specimens demonstrated reactivity (12 of 14 [86%]).

Noteworthy specimens were provided by two patients (M and N). Both the initial and the follow-up specimens of patient M were found to be reactive by HIV-1/HIV-2 PLUS O EIA. However, neither the initial nor the follow-up specimen from this patient demonstrated reactivity by any other HIV antibody test. The follow-up specimen from patient N showed reactivity using the HIV-1/HIV-2 PLUS O EIA. However, as in the case with patient M, no other HIV antibody screen test utilized in the present study demonstrated reactivity in either the initial or follow-up assessments. While the existence of viral RNA in these patients reinforces the reactive results of the third-generation EIA, RNA testing is not currently approved as a valid test for confirmation of a reactive result for an HIV antibody EIA screen.

Given that the HIV-1/HIV-2 PLUS O EIA is capable of detecting HIV-2, the discordance of the third- and first-generation EIA results within the present study raises the possibility that infection with HIV-2 may have occurred in one or more of the members of this tested panel. To explore this possibility, all follow-up specimens, in addition to primary specimens from patients F and S, were tested by Multi-Spot HIV-1/HIV-2 Rapid Test (Bio-Rad, Redmond, WA), a test that distinguishes between HIV-1 and HIV-2 specific antibodies. Neither any of the follow-up specimens in Table 1 nor the primary specimens of F and S showed any reactivity for HIV-2 by this analysis (data not shown). Moreover, none of the patients whose test results were used in the present study possessed risk factors for HIV-2 (i.e., no reported travel to or from West Africa and no sexual contact with people known to be from West Africa).

The U.S. Food and Drug Administration-approved status and heightened sensitivity of third-generation EIAs for HIV antibody detection should lead to an increase in the popularity of such assays as primary screens for HIV infection. The data in the present study support other studies that have demonstrated that third-generation EIAs possess enhanced sensitivity versus earlier tests (4, 10, 12, 13, 14, 15). One such study used the HIV-1/HIV-2 PLUS O EIA and demonstrated that this assay was capable of detecting antibodies in two patients found to be RNA positive but negative for antibodies by first-generation EIA (12). The present study confirms and expands that finding by analyzing a larger panel of RNA-positive, first-generation EIA-negative specimens. Moreover, none of the previous studies evaluated the performance of the third-generation assay relative to a wide panel of testing methods. By comparing the HIV-1/HIV-2 PLUS O EIA to such a broad array of screening and confirmatory tests, the present study revealed that the use of screening tests with high sensitivity may require careful consideration. The inability to confirm the results obtained from high-sensitivity (third- and fourth-generation) EIAs may complicate their usefulness in the short term. As the use of more sensitive antibody tests becomes widespread, existing HIV testing algorithms will need to be reexamined, with particular attention being paid to the use of RNA-based detection methods. In addition, high-sensitivity EIAs such as the third-generation test investigated here may have significant impact on efforts to investigate HIV incidence, particularly pooling studies, which aim to detect RNA-positive, antibody-negative individuals.

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