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Evaluation and Diagnostic Usefulness of Domestic and Imported Enzyme-Linked Immunosorbent Assays for Detection of Human Immunodeficiency Virus Type 1 Antibody in India

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Diagnosis of human immunodeficiency virus (HIV) infection is important for patient management and prevention of new infections. The number of test kits available for the detection of HIV antibodies is unprecedented. In order to identify appropriate test kits, we evaluated a variety of commercial kits manufactured abroad as well as in India. The plasma and serum specimens ($n = 264$) were collected from individuals attending the Voluntary Counseling and Testing Centre at the YRG Centre for AIDS and Education. The specimens were used to evaluate six commercially available HIV test kits: Enzaids HIV 1+2, HIV-CheX, Murex HIV-1.2.0, Genscreen HIV 1/2 version 2, Vironostika HIV Uni-Form II Ag/Ab, and CombAids RS Advantage. High sensitivities and specificities (≥99%) were observed for the Enzaids, Murex, Vironostika, and CombAids assays. HIV-CheX showed the highest number of false-positive and false-negative results. The Genscreen test also gave many false positives. The study indicated that the Enzaids, Murex, and Vironostika enzyme-linked immunosorbent assay kits and the CombAids RS Advantage rapid assay could be used to achieve acceptable results for the detection of HIV antibodies. A combination of two tests is recommended to optimize the efficiency of HIV antibody testing algorithms, especially when evaluation with an HIV Western blot confirmatory test is not possible.

Infection with human immunodeficiency virus (HIV) has become pandemic since its first documentation in 1981 and is a major public health concern (11). HIV antibody testing is critical for the diagnosis and counseling of HIV-infected persons, the monitoring of trends in HIV prevalence, and the evaluation of the effectiveness of HIV prevention programs (5, 12). An unprecedented number of tests for the detection of HIV antibodies are available. In some kits, improved sensitivity is frequently accompanied by a decreased specificity. This has been of particular concern with the introduction of test kits that detect all isotypes of antibodies, such as those based on antibody capture by antigens on a solid phase with labeled antigens as the detecting reagents (4, 8).

In resource-poor developing countries, the surveillance and diagnosis of HIV infection are major challenges (15). The conventional algorithm for HIV diagnostic testing consists of screening with enzyme immunoassays followed by confirmation with a Western blot test. Moreover, a double enzyme-linked immunosorbent assay (ELISA) without Western blotting has been accepted as the customary screening assay for HIV infection (18). Because of the high cost of the Western blot test, it has not been affordable in a number of laboratories in developing countries (1). Rapid screening for HIV infection performed on-site with tests that do not require expensive laboratory infrastructure or highly skilled personnel helps with the diagnoses of patients in emergencies (13). The present study has been designed to evaluate five different commercially available diagnostic ELISA kits, and also a rapid test kit, for their performance in diagnosing HIV infection.

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MATERIALS AND METHODS

This study was carried out at the Y. R. Gaitonde Centre for AIDS Research and Education (YRG CARE) in Chennai, India; it is a referral center for voluntary counseling and testing (VCT) in South India. A total of 264 specimens (plasma and serum) collected from VCT clients were tested using various commercial HIV ELISA kits, and the positive specimens were confirmed by Western blot analysis (Genetic Systems HIV-1 Western blot; Bio-Rad Laboratories, Redmond, WA). The following commercially available ELISA kits were employed in this study: Enzaids HIV 1+2 (Span Diagnostics Ltd., Surat, India), HIV-CheX (Xcyton Diagnostics Ltd., Bangalore, India), Murex HIV-1.2.0 (Murex Biotech Limited, Dartford, United Kingdom), Genscreen HIV 1/2 version 2 (Bio-Rad Laboratories, France), and Vironostika HIV Uni-Form II Ag/Ab (BioMérieux, The Netherlands). Along with these, a rapid test kit, CombAids RS Advantage (Span Diagnostics Ltd., Surat, India), was also evaluated. A double-blind format was adopted in order to conceal patient information from the testing personnel. One staff member generated duplicate numbers for specimens at the specimen processing section; a second staff member generated plate maps and performed the tests. Finally, the results were analyzed by both personnel. The kits were stored under cold conditions at all times, and all of the tests were performed according to the manufacturer's instructions. An optical density higher than the cutoff value, obtained per the manufacturer's instructions, was considered a positive result, and an optical density lower than the cutoff value was considered a negative result. Sensitivity, specificity, predictive values, and efficiency were calculated using the Western blot results as the standard. A Western blot was considered positive for HIV type 1 (HIV-1) if any two of the following viral proteins were present: p24, gp41, and gp120/160 (per the manufacturer's instructions). We have calculated the performance characteristics of all the kits using formulae given elsewhere (17).

The kits were also evaluated with the following known specimens: 100 Western blot-confirmed HIV-positive specimens (Genetic Systems HIV-1; Bio-Rad Laboratories), 100 HIV-negative specimens (U.S. FDA-approved ELISA; Genetic Systems HIV-1/2 PLUS O), 4 HIV-II-positive specimens (confirmed by NEW LAV BLOT II; Bio-Rad Laboratories, France), and 9 in-house seroconversion specimens. Six specimens from Boston Biomedica, Inc. (BBI panel), Boston, Mass., were tested with only two kits (Enzaids and Murex) due to insufficient

TABLE 1. Performance of kits with known specimens

Specimen (total no.)	No. of specimens identified correctly by:				
	Enzaids	Murex	Genscreen	HIV-CheX	Comb Aids
Western blot confirmed, HIV-I positive (n = 100)	100	100	100	89	100
HIV-I negative, in FDA-approved ELISA (n = 100)	100	100	100	100	100
BBI panel (n = 6)	4[a]	6	ND[b]	ND	ND
NEW LAV BLOT II confirmed, HIV-II positive (n = 4)	4	4	4	4	4
In-house seroconversion panel (n = 9)	9	9	9	7	9

[a] The two initial specimens, from 0 and 9 days, were not detected.
[b] ND, not done.

specimen volume. Of the six BBI panel specimens, four were 0, 9, 11, and 20 days old, and the others were known positive specimens for HIV-2 and HIV-1 group O, respectively. We were unable to include the Vironostika kit due to the unavailability of funding.

RESULTS

As far as the known specimens are concerned (Table 1), all of the specimens which contained antibodies to HIV-1 were correctly identified by all of the kits except HIV-CheX, which was unable to detect 11 HIV Western blot-positive specimens, thus resulting in reduced sensitivity. All of the kits correctly identified negative specimens. All of the nine in-house seroconversion specimens were identified as positive by all of the kits except HIV-CheX, which recorded two specimens as negative. All of the in-house specimens of HIV-2 were identified as positive by all of the kits. From the BBI seroconversion panel, Murex detected all six specimens, while Enzaids did not detect the two initial specimens (i.e., 0 and 9 days). Both kits showed positive results for the HIV-2 and HIV-1 group O specimens from the BBI panel.

The results of the 264 VCT specimens used for the evaluation of commercially available kits for the diagnosis of HIV infection are summarized in Table 2. It is clearly evident that the Enzaids, Murex, Vironostika, and CombAids test kits gave uniform results with good sensitivity and specificity. All four of these kits exhibited one false positive (Table 3). The results of the HIV-CheX and Genscreen tests were not satisfactory due to their poor performance characteristics. Of the 264 specimens tested by Western blotting, one was indeterminate in exhibiting the p24 band, positive in the Murex kit, and negative in all of the other kits, and this specimen was removed from the analysis due to the unavailability of PCR results. A total of 19 specimens were found to be discordant with the Western blot results, which can be considered an inherent limitation of the respective test kits (Table 3). Many false positives and false negatives were observed in the Genscreen and HIV-CheX tests, respectively.

DISCUSSION

In the present study, we have evaluated the performance of two domestic and three imported HIV ELISA kits. We have also included a domestic rapid HIV kit with the ELISA kits.

TABLE 2. Performance characteristic of six HIV antibody tests used for comparative evaluation

Antibody test and result	Western blot results[a]		Performance characteristic result (%)[b]				
	POS	NEG	Sensitivity (95% CI)	Specificity (95% CI)	PPV	NPV	Efficiency
Enzaids HIV 1+2			100 (100–100)	99.3 (97.8–100)	99.2	100	99.6
Positive	127	1					
Negative	0	135					
Murex HIV-1.2.0			100 (100–100)	99.3 (97.8–100)	99.2	100	99.6
Positive	127	1					
Negative	0	135					
HIV-CheX			96.9 (93.9–99.8)	96.3 (93.1–99.6)	96.1	97	96.5
Positive	123	5					
Negative	4	131					
Vironostika			100 (100–100)	99.3 (97.8–100)	99.2	100	99.6
Positive	127	1					
Negative	0	135					
Genscreen HIV 1/2			100 (100–100)	94.9 (91.0–98.7)	94.8	100	97.3
Positive	127	7					
Negative	0	129					
CombAids			100 (100–100)	99.3 (97.8–100)	99.2	100	99.6
Positive	127	1					
Negative	0	135					

[a] Total number of samples, 264 (one sample was removed from the analysis; see text for the details). POS, positive; NEG, negative. Values shown are numbers of samples with indicated result.
[b] CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

TABLE 3. Samples found to be discordant with Western blot results

Sample	Results with[a]:						
	Western blot	Enzaids	Murex	Genscreen	HIV-CheX	CombAids	Vironostika
1	—[b]	TN	NK	TN	TN	TN	TN
2	TN	TN	TN	FP	TN	TN	TN
3	TP	TP	TP	TP	FN	TP	TP
4	TP	TP	TP	TP	FN	TP	TP
5	TN	TN	TN	TN	FP	TN	TN
6	TN	TN	TN	TN	FP	TN	TN
7	TP	TP	TP	TP	FN	TP	TP
8	TN	TN	TN	TN	FP	TN	TN
9	TN	TN	TN	TN	FP	TN	TN
10	TN	TN	TN	FP	FP	TN	TN
11	TP	TP	TP	TP	FN	TP	TP
12	TN	TN	FP	TN	TN	TN	TN
13	TN	TN	TN	TN	TN	TN	FP
14	TN	TN	TN	FP	TN	TN	TN
15	TN	FP	TN	FP	TN	TN	TN
16	TN	TN	TN	FP	TN	TN	TN
17	TN	TN	TN	FP	TN	TN	TN
18	TN	TN	TN	FP	TN	TN	TN
19	TN	TN	TN	TN	TN	FP	TN

[a] TP, true positive; FP, false positive; FN, false negative; TN, true negative; NK, status unknown.
[b] Sample was indeterminate with p24 and was removed due to the unavailability of PCR results.

Poor performance was seen with the Genscreen and HIV-CheX kits. The Murex test detected antibodies in all of the specimens in the BBI panel, and this result coincided with those of other studies (2, 7). Concordant results were obtained with the in-house seroconversion specimens with all of the kits except HIV-CheX. In other studies, the Genscreen kit demonstrated worse performance characteristics than other kits, as it could not detect either HIV-1 group M, subtypes A and B, or HIV-1 group O antigens in the range tested (10 to 125 pg/ml) (7, 19, 20). This result matches the poor performance shown in our study, as many false positives were detected.

Even though Western blot analysis is a confirmatory test (3, 9), the combination of two or more antibody screening assays, which use different antigenic bases, may be more sensitive and specific than Western blotting (3, 8–10, 16, 18). According to our study, for double ELISA combinations, the recommended kits are the Enzaids, Murex, and Vironostika kits. Using the HIV-CheX or Genscreen kit with any other kit would be an ineffective combination. One notable issue here is the discordance between the results of two assays (6, 8, 14), which can be resolved if better kits are used. Hence, this kind of evaluation gains importance for determining a better choice of kits. It is also noted that the sera of up to 40% of uninfected persons gave indeterminate Western blot results (3, 8, 9).

As far as patients are concerned, discordant results may have the consequence of inducing psychological stress while the patient is waiting for confirmatory testing and may also result in additional expenses. False-positive results could cause considerable unnecessary anxiety and mental trauma for the patients. This outcome may also lead to the unnecessary waste of valuable blood in blood banks. In this evaluation, we found a large number of false positives with the Genscreen kit. The damage caused by false-negative test results is also a serious problem, as a person who is falsely tested to be negative may continue the transmission of the virus. The possibility of transfusing blood after such a false-negative test also underlines the need for high-quality, sensitive ELISAs. In this evaluation, we found false-negative results with the HIV-CheX kit, leaving the population under the threat of silent HIV transmission.

Even though this study did not concentrate on a rapid HIV detection assay, we included the CombAids rapid assay in order to evaluate its comparative performance. The performance of this rapid assay was found to be comparable to that of ELISA in terms of sensitivity and specificity. Therefore, the CombAids assay can be a useful tool for the prompt screening of patient specimens at point-of-care facilities, reference laboratories (15), prenatal clinics, and emergency rooms located in regions where laboratorial infrastructure is either not present or not well outfitted. We do not have the clinical characteristics of the patients, such as concomitant infections or use of chemotherapeutic or recreational drugs, to compare with our results. Due to insufficient quantities of specimens, we used the seroconversion panel only for the Murex and Enzaids tests. One test specimen, found to be positive by only the Murex HIV-1.2.0 test, was indeterminate by Western blotting with the p24 band. Due to the lack of availability of PCR assay and/or follow-up specimen results for this individual, we were unable to resolve the conflicting serology for this specimen; hence, it was removed from the analysis. Also due to unavailability of funds, we did not use the Vironostika kit to test known specimens. These are some of the limitations of this study.

To conclude, as demonstrated by the data presented in this report, the domestic Enzaids kit exhibits a high level of performance that equals that of the imported Murex and Vironostika kits. The same performance was also observed with the domestic CombAids rapid assay, while poor performance was observed with the domestic HIV-CheX kit and the imported Genscreen kit. The other advantages of the Enzaids kit are a short run time, comparative cost-effectiveness, and incubation of samples at room temperature. Hence, the domestically developed Enzaids kit is sufficiently accurate to screen for HIV-1

infection and can be widely used in India where people cannot afford to test with imported kits.

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REFERENCES

1. **Asihene, P. J., R. L. Kline, M. W. Moss, A. V. Carella, and T. C. Quinn.** 1994. Evaluation of rapid test for detection of antibody to human immunodeficiency virus type 1 and type 2. J. Clin. Microbiol. **32:**1341–1342.
2. **Bürgisser, P., F. Simon, M. Wernli, T. Wüst, M.-F. Beya, and P. C. Frei.** 1996. Multicenter evaluation of new double-antigen sandwich enzyme immunoassay for measurement of anti-human immunodeficiency virus type 1 and type 2 antibodies. J. Clin. Microbiol. **34:**634–637.
3. **Genesca, J., J. W. K. Shih, B. W. Jett, I. K. Hewlett, J. S. Epstein, and H. J. Alter.** 1989. What do Western blot indeterminate patterns for human immunodeficiency virus mean in EIA-negative blood donors? Lancet **ii:**1023–1025.
4. **Gonzalez, L., R. W. Boyle, M. Zhang, J. Castillo, S. Whittier, P. Della-Latta, L. M. Clarke, J. R. George, X. Fang, J. G. Wang, B. Hosein, and C. Y. Wang.** 1997. Synthetic-peptide-based enzyme-linked immunosorbent assay for screening human serum or plasma for antibodies to human immunodeficiency virus type 1 and type 2. Clin. Diagn. Lab. Immunol. **4:**598–603.
5. **Kannangai, R., S. Ramalingam, S. Pradeepkumar, K. Damodharan, and G. Sridharan.** 2000. Hospital-based evaluation of two rapid human immunodeficiency virus antibody screening tests. J. Clin. Microbiol. **38:**3445–3447.
6. **Lin, H. J., L. E. Myers, B. Yen-Lieberman, F. B. Hollinger, D. Henrard, C. J. Hooper, R. Kokka, S. Kwok, S. Rasheed, M. Vahey, M. A. Winters, L. J. McQuay, P. L. Nara, P. Reichelderfer, R. W. Coombs, and J. B. Jackson.** 1994. Multicenter evaluation of quantification methods for plasma human immunodeficiency virus type 1 RNA. J. Infect. Dis. **170:**553–562.
7. **Ly, T. D., L. Martin, D. Daghefal, A. Sandridge, D. West, R. Bristow, L. Chalouas, X. Qiu, S. C. Lou, J. C. Hunt, G. Schochetman, and S. G. Devare.** 2001. Seven human immunodeficiency virus (HIV) antigen-antibody combination assays: evaluation of HIV seroconversion sensitivity and subtype detection. J. Clin. Microbiol. **39:**3122–3128.
8. **Matter, L., and D. Germann.** 1995. Detection of human immunodeficiency virus (HIV) type 1 antibodies by new automated microparticle enzyme immunoassay for HIV types 1 and 2. J. Clin. Microbiol. **33:**2338–2341.
9. **Midthun, K., L. Garrison, M. L. Clements, H. Farzadegan, B. Fernie, T. Quinn, and the NIAID AIDS Vaccine Clinical Trials Network.** 1990. Frequency of indeterminate Western blot tests in healthy adults at low risk for human immunodeficiency virus infection. J. Infect. Dis. **162:**1379–1382.
10. **Mortimer, P. P.** 1991. The fallibility of HIV Western blot. Lancet **337:**286–287.
11. **National AIDS Control Organization.** 2004. An overview of the spread and prevalence of HIV/AIDS in India. NACO, Ministry of Health and Family Welfare, Government of India. [Online.] http://www.nacoonline.org/facts_overview.htm.
12. **Nkengasong, J. N., C. Maurice, S. Koblavi, M. Kalou, D. Yavo, M. Maran, C. Bile, K. N′guessan, J. Kouadio, S. Bony, S. Z. Wiktor, and A. E. Greenberg.** 1999. Evaluation of HIV serial and parallel serologic testing algorithms in Abidjan, Côte d'Ivoire. AIDS **13:**109–117.
13. **Palmer, C. J., J. M. Dubon, E. Koenig, E. Perez, A. Ager, D. Jayaweera, R. R. Cuadrado, A. Rivera, A. Rubido, and D. A. Palmer.** 1999. Field evaluation of the Determine rapid human immunodeficiency virus diagnostic test in Honduras and the Dominican Republic. J. Clin. Microbiol. **37:**3698–3700.
14. **Pyra, H., J. Boni, and J. Schupbach.** 1994. Ultrasensitive retrovirus detection by a reverse transcriptase assay based on product enhancement. Proc. Natl. Acad. Sci. USA **91:**1544–1548.
15. **Ribeiro-Rodrigues, R., L. F. da Silva Pinto Neto, C. B. Cunha, V. P. Cabral, and R. Dietze.** 2003. Performance characteristics of a rapid new immunochromatographic test for detection of antibodies to human immunodeficiency virus. Clin. Diagn. Lab. Immunol. **10:**303–307.
16. **Sato, P. A., W. J. Maskill, H. Tamashiro, and D. L. Heymann.** 1994. Strategies for laboratory HIV testing: an examination of alternative approaches not requiring Western blot. Bull. WHO **72:**129–134.
17. **Schochetman, G., and J. R. George.** 1992. Serodiagnosis of HIV infection, p. 66–67. *In* G. Schochetman and J. R. George (ed.), AIDS testing: methodology and management issues. Springer-Verlag, New York, N.Y.
18. **UNAIDS/WHO Working Group on Global HIV/AIDS/STI Surveillance.** 2001. Guidelines for using HIV testing technologies in surveillance: selection, evaluation, and implementation. UNAIDS and WHO HIV testing strategies. Strategy II. WHO/CDS/CSR/EDC/2001.16 UNAIDS/01.22E:23 [Online.] http://www.who.int/hiv/pub/surveillance/en/guidelinesforUsingHIVTestingTechs_E.pdf.
19. **Weber, B., A. Berger, H. Rabenau, and H. W. Doerr.** 2002. Evaluation of a new combined antigen and antibody human immunodeficiency virus screening assay, VIDAS HIV DUO Ultra. J. Clin. Microbiol. **40:**1420–1426.
20. **Weber, B., L. Gürtler, R. Thorstensson, U. Michl, A. Mühlbacher, P. Bürgisser, R. Villaescusa, A. Eiras, C. Gabriel, H. Stekel, S. Tanprasert, S. Oota, M.-J. Silvestre, C. Marques, M. Ladeira, H. Rabenau, A. Berger, U. Schmitt, and W. Melchior.** 2002. Multicenter evaluation of a new automated fourth-generation human immunodeficiency virus screening assay with a sensitive antigen detection module and high specificity. J. Clin. Microbiol. **40:**1938–1946.