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Suitability of a Rapid Immunochromatographic Test for Detection of Antibodies to Human Immunodeficiency Virus in Ghana, West Africa

S. AIDOO,[1] W. K. AMPOFO,[1] J. A. M. BRANDFUL,[1] S. V. NUVOR,[1] J. K. ANSAH,[2] N. NII-TREBI,[1] J. S. BARNOR,[1] F. APEAGYEI,[3] T. SATA,[4] D. OFORI-ADJEI,[1] AND K. ISHIKAWA[1,4]*

Noguchi Memorial Institute for Medical Research, University of Ghana,[1] National Blood Transfusion Service, Ministry of Health, Korle-Bu,[2] and Hygiene Wing, Military Hospital,[3] Accra, Ghana, and National Institute of Infectious Diseases, Tokyo, Japan[4]

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In West African countries such as Ghana, efficient human immunodeficiency virus (HIV) testing is a priority in the fight against AIDS. A new immunochromatographic rapid test, Determine HIV-1/2 (Abbott Diagnostics, North Chicago, Ill.), that detects antibodies against HIV type 1 (HIV-1) and/or HIV-2 was evaluated using Ghanaian blood samples. Two hundred four serum and/or plasma specimens were tested. HIV screening was done by a particle agglutination test and confirmed by a Western blot (WB) test as the "gold standard." The results revealed 125 HIV-seropositive AIDS patients, 75 HIV-seronegative healthy individuals, and 4 individuals for whom the HIV-1 result was indeterminate. The results obtained by the Determine HIV-1/2 assay and Diagnostic HIV SPOT (Genelabs), which is currently widely used in many districts in Ghana, were compared with those of the WB test, excluding the four HIV-1-indeterminate samples. The sensitivity of the Determine HIV-1/2 assay was 100%, compared with 98.0% for the HIV SPOT assay. The specificity was 100% for both tests. Determine HIV-1/2 is a single-step assay and was found to be rapid and easy to perform without any special equipment. It was highly sensitive and specific. The kit can be applied without electricity and water supplies, making it suitable for the detection of HIV antibodies especially in the rural areas of Ghana, West Africa.

Despite intensive efforts to prevent new human immunodeficiency virus (HIV) infections, the Joint United Nations Programme on HIV/AIDS and the World Health Organization (WHO) now estimate that 36.1 million people worldwide were living with HIV infection and/or AIDS at the end of the year 2000. Some 21.8 million people have died of AIDS, with a cumulative total of 4.3 million children having died before reaching age 15 years. Sub-Saharan African countries remain the epicenter of the pandemic, with nearly 25.3 million men, women, and children infected with HIV (6). It is estimated that between 5 and 10% of all HIV infections worldwide have been acquired through transfusion of contaminated blood and blood products (4, 8).

In Ghana, from 1986 to the end of December 1999, a cumulative total of 37,298 AIDS cases had been reported to the Ministry of Health. From this, it is estimated retrospectively that over 55,000 AIDS cases have existed and that about 600,000 Ghanaians are living with HIV infection and/or AIDS. The age distribution of these HIV-seropositive persons shows that the most sexually active age group (20 to 39 years) makes up about 70% of the total number of cases. Nearly 90% are in the economically productive age group (20 to 49 years), and this has serious implications for the future social and economic development of the country (12).

As part of the efforts to reduce the transmission of HIV, there is the need for a simple, rapid, sensitive, and specific HIV test which would be suitable for rural areas where electricity and water may not be readily available. In sub-Saharan Africa, improving nutritional and health standards and controlling malaria in children is difficult. This results in a high incidence of chronic anemia and increases the requirement for consequent transfusions, emphasizing the need for safe blood supplies (8). In addition, the risk of mother-to-child transmission of HIV can be reduced for mothers who know they are infected through voluntary counseling and testing (VCT) facilities (7).

Serological tests such as the enzyme-linked immunosorbent assay (ELISA), particle agglutination (PA) assay, and Western blot (WB) assay for the detection of HIV antibodies are routinely utilized for the screening and confirmation of HIV infection in urban areas in Ghana. Although ELISA and WB assay are very sensitive, they require relatively complex instrumentation. The PA method is easy and simple but consists of several steps with about 2 h required to achieve results, making it inappropriate, especially for emergency use, compared with simple rapid tests (2, 10, 11, 13). The cost of the individual simple rapid test may be higher than that of an ELISA, but when accurate cost assessment is done, using specificity, reliability, and reproducibility, the use of a simple rapid test is seen to be more cost-effective (18).

A new rapid test, Determine HIV-1/2 (Abbott Diagnostics, North Chicago, Ill.), for the rapid detection of HIV type 1 (HIV-1) and/or HIV-2 antibodies based on lateral flow immunochromatography has been developed (14). This test requires no laboratory infrastructure or highly skilled personnel and requires only 10 min to obtain the result. Therefore, the test can be used on site for screening of HIV infection, facilitating the process of VCT in rural areas. The Determine HIV-1/2 assay has been evaluated previously with whole blood, serum, and plasma samples from Thailand and Cote d'Ivoire. The test showed 100% specificity and sensitivity for HIV-1 and HIV-2

* Corresponding author. Mailing address: Noguchi Memorial Institute for Medical Research, Virology Unit, University of Ghana, P.O. Box LG. 581, Legon, Accra, Ghana. Phone: 233-21-501178/9. Fax: 233-21-502182. E-mail: kishikaw@nih.go.jp.

TABLE 1. Sensitivities and specificities of tests for detection of antibodies to HIV-1 and HIV-2 with samples confirmed as positive or negative by WB assay

Test	Sensitivity					Specificity				
	Serum samples (n = 65)		Plasma samples (n = 60)		Total sensitivity (%) (n = 125)	Serum samples (n = 40)		Plasma samples (n = 35)		Total specificity (%) (n = 75)
	No. negative[a]	Sensitivity (%)	No. negative	Sensitivity (%)		No. positive[b]	Specificity (%)	No. positive	Specificity (%)	
Determine HIV-1/2	0	100	0	100	100	0	100	0	100	100
HIV SPOT	0	100	2	96.8	98.4	0	100	0	100	100
Innotest HIV-1/2	0	100	1	98.4	99.2	1	97.6	1	97.2	97.4

[a] Number of samples (serum and plasma) detected as false negatives.
[b] Number of samples (serum and plasma) detected as false positives.

(1). We therefore studied the suitability of the Determine HIV-1/2 assay in Ghana for the detection of antibodies to HIV.

MATERIALS AND METHODS

Blood samples. The specimens used in this study were plasma or serum samples collected during 1998 and 1999 from pregnant women, persons suspected of having HIV, and blood donors from different locations in Ghana. These samples were selected to represent the type of population to which the rapid assay would be applied. The blood samples were centrifuged, and the serum or plasma samples were collected into vials and stored.

Sample classification. The HIV antibody status of these samples was determined by screening first with a Serodia HIV-1/2 PA assay (Fujirebio, Tokyo, Japan). In the screening procedure, the PA assay was performed according to the manufacturer's instructions. The final results were read after 2 h of incubation. This was followed by differentiation into HIV-1-positive, HIV-2-positive, dual positive, or negative categories by immunoblotting with PEPTI LAV 1-2 (Sanofi Diagnostics Pasteur, Marnes-La-Coquette, France). PEPTI LAV 1-2 is based on an immunoenzymatic strip method using two synthetic peptides specific for HIV-1 (GP41) and HIV-2 (GP36). Further confirmation was achieved by the WB assay (New LAV-blot 1 and New LAV-blot 2; Sanofi Diagnostics Pasteur), in which reactivity against HIV-1 and HIV-2 proteins indicated the presence of antibodies in the specimens.

Evaluation. Based on the serological results obtained, we selected all of the 125 HIV-positive specimens and 75 HIV-negative specimens for the evaluation of the Determine HIV-1/2 assay (Abbott Diagnostics). The four specimens that were indeterminate for HIV-1 by WB assay were also used in the rapid tests, but their results were not used for the evaluation.

Determine HIV-1/2, an immunochromatographic rapid test, is based on a sandwich immunoassay technique with HIV-1 (group M and O subtypes) and HIV-2 recombinant antigens plus HIV-1 and HIV-2 envelope peptides. The test uses a nitrocellulose strip with a conjugate to selenium colloid and a capture site containing HIV-1 and HIV-2 antigens. If a sample contains HIV-1 or HIV-2 antibodies, the antibodies first react with the antigen-selenium colloid conjugates. As the antibody-antigen-selenium colloid complex flows past the capture site, the antibodies react with the antigens at the site, with the formation of a visible red line within 10 min. The test also contains a procedural control site, which confirms the validity of the assay by the formation of a visible red line. In the test procedure, 50 μl of serum or plasma was placed on the sample application pad, and the result was read within 10 min.

An immunodot assay, the HIV SPOT test (Genelabs Diagnostics, Singapore), and an enzyme immunoassay, Innotest HIV-1/2 Ab (Innogenetics N.V., Ghent, Belgium) were also employed because they are at present routinely used in Ghana. The HIV SPOT test involves trapping of antibodies to HIV-1 and/or HIV-2 by the capture reagents, which are adsorbed on a porous membrane. The conjugate binds to the adsorbed HIV antibodies to form a red color (spot) on the membrane. This involves five steps and takes less than 10 min for the results to be read. The Innotest HIV-1/2 Ab is a simple ELISA method where virus-specific antibodies to HIV-1 (including group O) or HIV-2, if present in the sample, will bind to the solid-phase antigens. Subsequent addition of affinity-purified rabbit anti-human immunoglobulin G labeled with the enzyme horseradish peroxidase and substrate produces a blue color. The reaction is stopped by the addition of sulfuric acid, and optical densities are read with a microplate reader equipped with a 450-nm filter. The Innotest HIV-1/2 Ab assay takes about 4 h for completion.

Statistical analysis. The sensitivities and specificities of the tests were determined for the evaluation of the utility of the tests. The following formulas were used to calculate test indices (9): sensitivity = [TP/(TP + FN)] × 100 and specificity = [TN/(TN + FP)] × 100 (where TP = number of true positives, TN = number of true negatives, FN = number of false negatives, and FP = number of false positives).

RESULTS

The result of the WB assay showed 125 HIV-seropositive specimens, 75 HIV-seronegative specimens, and 4 HIV-1-indeterminate specimens. However, 200 samples, comprised of 125 HIV-seropositive and 75 HIV-seronegative specimens and excluding the 4 HIV-1-indeterminate specimens, were used for the evaluation. The positive samples consisted of 107 HIV-1-positive, 12 HIV-2-positive, and 6 dual positive samples.

Table 1 shows the sensitivities and specificities of the tests determined using the HIV-seropositive and -seronegative samples, respectively. Determine HIV-1/2 demonstrated 100% sensitivity for the detection of HIV-1 and HIV-2 with the WB and PEPTI LAV assays. Innotest HIV-1/2 Ab did not detect one confirmed antibody-positive specimen obtained from an asymptomatic individual, indicating a sensitivity of 99.2%. The HIV SPOT assay showed 98.4% sensitivity, missing two HIV-1 antibody-confirmed seropositive specimens. Both samples were from individuals asymptomatic for HIV infection or AIDS and displayed faint glycoprotein bands by WB assay.

All of the tests showed 100% specificity except Innotest HIV-1/2 Ab, which registered two false positives (specificity, 97.4%). These two samples detected by Innotest HIV-1/2 Ab as positive displayed mean optical density values of 1.20 and 0.90, and the cutoff values were 0.25 and 0.24, respectively, when the ELISA was repeated.

The results for samples that were negative by PEPTI LAV 1-2 but were neither positive nor negative by WB assay are presented in Table 2. All four had at least one glycoprotein band (GP160, GP120, or GP41) present. According to the criteria recommended by WHO for interpreting results from WB assays, they were classified as indeterminate (15).

The Determine HIV-1/2 assay detected HIV antibodies in all four indeterminate specimens, while the Innotest HIV-1/2 recorded three positives and one negative and the HIV SPOT indicated two positives and two negatives. The indeterminate samples, however, were excluded from the analysis of the sensitivities and specificities of the kits.

TABLE 2. Results of the assays on samples indeterminate for HIV-1 by WB assay[a]

Sample	Result for the following HIV-1 band by WB assay:									Result of:				
										WB assay		Determine HIV-1/2	HIV SPOT	Innotest HIV-1/2
	P18	P25	P34	GP41	P52	P55	P66	GP120	GP160	HIV-1	HIV-2			
MHC267	+	+	−	−	−	+	+	−	+	Ind.	Neg.	Pos.	Neg.	Neg.
MHC361	+	+	−	−	−	+	−	−	+	Ind.	Neg.	Pos.	Pos.	Pos.
MHC262	+	+	+	−	−	+	+	−	+	Ind.	Neg.	Pos.	Neg.	Pos.
MHC264	+	+	+	±	−	+	+	+	−	Ind.	Neg.	Pos.	Pos.	Pos.

[a] ±, very faint band; Ind., indeterminate; Pos., positive; Neg., negative.

DISCUSSION

The Determine HIV-1/2 assay showed 100% sensitivity and 100% specificity for the detection of HIV-1 and HIV-2 with serum and plasma samples. The results completely agreed with the PEPTI LAV 1-2 assay and the WB 1 and 2 assays used as the "gold standard" in the present study. Furthermore, the four specimens indeterminate for HIV-1 by WB assay were all found positive by the Determine HIV-1/2 assay, indicating its sensitivity as a supplemental screening assay. However, the indeterminate results were not used in statistical analysis for calculating sensitivity and specificity due to the limitation by the formula (9).

In Ghana, the HIV-1 subtypes currently circulating are A, D, and G, with subtype A predominating (3, 5). It has been reported that the Determine HIV-1/2 assay can detect all of the presently identified HIV-1 subtypes as well as HIV-2 subtypes A and B (14). This makes the Determine HIV-1/2 assay ideal for HIV antibody screening of the prevailing HIV subtypes in Ghana. However, other diagnostic kits may have detected subtypes which are not specified.

Diagnosis of HIV infection and surveillance activities in sub-Saharan Africa are a great challenge. This can be attributed to the fact that many clinics or health care centers are poorly equipped and lack diagnostic capabilities and equipment needed to perform a standard confirmatory assay, such as WB assay. The problem is compounded in rural areas, where most often electricity and refrigeration for storage of diagnostic test kits and reagents may be interrupted frequently or are not available.

In the other assays examined, procedures require many steps to achieve results, but the Determine HIV-1/2 assay is a rapid, one-step procedure for plasma and/or serum specimens. In addition, the Determine HIV-1/2 assay can be used for a batch or single use, since the strips can be separated into single units. Another feature is the lack of a requirement for any equipment or specific instrument for its application. In this assay, the sample and conjugate migrate by capillary flow, eliminating the need for prior centrifugation of test samples. All of these features of the Determine HIV-1/2 make it suitable for screening to detect HIV-1 and HIV-2 antibodies in remote and rural areas without electricity and complex laboratory equipment.

Current VCT programs worldwide have had moderate success in increasing access to HIV antibody testing. For instance, many who agreed to take an HIV test did not return to collect results (16). Tests with prompt results have the potential to change this situation if their use is incorporated into existing health care programs.

UNAIDS and WHO recommend that countries consider testing strategies for HIV antibody detection that use ELISA and/or simple rapid assays rather than ELISA and WB assay. According to strategy I of the recommendation, all serum or plasma samples should be tested with one ELISA or simple rapid assay. Reactive serum is considered HIV antibody positive, and nonreactive serum is considered HIV antibody negative. This strategy should preferably be a combined HIV-1–HIV-2 assay, which is highly sensitive when applied for safeguarding the blood supply. In strategy II, the test sample (serum or plasma) is first tested with one ELISA or simple rapid assay, and if it is reactive, it is retested with a second ELISA or simple rapid assay based on a different antigen preparation and/or a different test principle. The sample is considered HIV antibody positive if it is reactive in both tests. If there is a discordant result or both test results are reactive, then strategy III, which requires a third test of different antigen preparations and/or different test principles from those used in strategy II, should be considered. Serum reactive in all three of these tests is considered HIV antibody positive (17).

A combination of the HIV SPOT test and the Determine HIV-1/2 assay would satisfy strategy II and should prove cost-effective, since the Determine HIV-1/2 assay results are comparable to those of the WB assay. We conclude that this test is suitable for the screening of blood and for VCT programs in developing countries such as Ghana.

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REFERENCES

1. **Arai, H., B. Petchclai, K. Khupulsup, T. Kurimura, and K. Takeda.** 1999. Evaluation of a rapid immunochromatographic test for detection of antibodies to human immunodeficiency virus. J. Clin. Microbiol. **37:**367–370.
2. **Behets, F., A. Disasi, R. W. Ryder, K. Bishagara, P. Piot, M. Kashamuka, M. Kamenga, N. Nzila, M. Laga, G. Vercanteren, V. Batter, C. Brown, and T. Quinn.** 1991. Comparison of five commercial enzyme-linked immunosorbent assays and Western immunoblotting for human immunodeficiency virus antibody detection in serum samples from Central Africa. J. Clin. Microbiol. **29:**2280–2284.
3. **Brandful, J. A. M., W. K. Ampofo, W. Janssens, Y. Adu-Sarkodie, F. Apeagyei, F. Anyomi, S. Aidoo, N. Yamamoto, K. Ishikawa, T. Sata, and T. Kurata.** 1998. Genetic and phylogenetic analysis of HIV type 1 strains from southern Ghana. AIDS Res. Hum. Retroviruses **14:**815–819.
4. **Curran, J. W., H. W. Jaffe, A. M. Hardy, W. Morgan, R. M. Selik, and T. Z. Dondero.** 1998. Epidemiology of HIV infection and AIDS in the United States. Science **239:**610.
5. **Ishikawa, K., W. Janssens, J. Brandful, L. Heyndrickx, Y. Takebe, W. Ampofo, T. Sata, S. Yamazaki, M. Osei-Kwasi, N. Yamamoto, Y. Koyanagi, G.**

Van der Groen, and T. Kurata. 1996. Genetic and phylogenetic analysis of HIV type 1 env subtypes in Ghana, West Africa. AIDS Res. Hum. Retroviruses **12:**1575–1577.
6. **Joint United Nations Programme on HIV/AIDS.** 2000. AIDS epidemic update, p. 3–5. Joint United Nations Programme on HIV/AIDS and the World Health Organization, Geneva, Switzerland.
7. **Joint United Nations Programme on HIV/AIDS.** 1998. Discussion document. Emerging issues and challenges for women, young people and infants, p. 8. Joint United Nations Programme on HIV/AIDS, Geneva, Switzerland.
8. **Joint United Nations Programme on HIV/AIDS.** 1997. Technical update; blood safety and HIV, p. 3–5. Joint United Nations Programme on HIV/AIDS and the World Health Organization, Geneva, Switzerland.
9. **Karan, J.** 1995. Humoral immune responses and detection during HIV infection, p. 191–209. *In* D. Rickwood and B. D. Hames (ed.), HIV, a practical approach, vol. 1. Virology and immunology. Oxford University Press Inc., New York, N.Y.
10. **Malone, J. D., E. S. Smith, J. Sheffield, et al.** 1993. Comparative evaluation of six rapid serological tests for HIV-1 antibody. J. Acquir. Immune Defic. Syndr. **6:**115–119.
11. **Mortimer, P. P.** 1991. The fallibility of HIV Western blot. Lancet **11:**286–287.
12. **National AIDS/STD Control Programme, Ministry of Health, Ghana.** AIDS surveillance, Jan. to Dec. 1999. Ministry of Health, Accra, Ghana.
13. **Riggin, C. H., G. A. Beltz, C. H. Hung, R. M. Thorn, and D. J. Marciani.** 1987. Evaluation of passive particle agglutination test for antibody of human immunodeficiency virus. J. Clin. Microbiol. **25:**1433–1437.
14. **Vallari, A. S., R. K. Hickman, J. R. Hackett, J. R. Catherine, A. Brennan, V. A. Varitek, J. R. Sushil, and G. Devare.** 1998. Rapid assay for simultaneous detection and differentiation of immunoglobulin G antibodies to human immunodeficiency virus type 1 (HIV-1) group M, HIV-1 group O, and HIV-2. J. Clin. Microbiol. **36:**3657–3661.
15. **World Health Organization.** 1990. Acquired immunodeficiency syndrome (AIDS): proposed W. H. O. criteria for interpreting results from Western blot assays for HIV-1, HIV-2 and HTLV-1/HTLV-2. Wkly. Epidemiol. Rec. **37:**281–283.
16. **World Health Organization.** 1992. Recommendations for the selection and use of HIV antibody tests. Wkly. Epidemiol. Rec. **67:**145–149.
17. **World Health Organization.** 1997. Revised recommendations for the selection and use of HIV antibody tests. Wkly. Epidemiol. Rec. **12:**83–86.
18. **World Health Organization.** 1998. The importance of simple/rapid assays in HIV testing. Wkly. Epidemiol. Rec. **73:**321–326.