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Evaluation of a Rapid Immunochromatographic Test for Detection of Antibodies to Human Immunodeficiency Virus

HIROYASU ARAI,[1]* BENCHA PETCHCLAI,[2] KALAYANEE KHUPULSUP,[2] TAKASHI KURIMURA,[3] AND KATSUMICHI TAKEDA[1]

Research and Development Department, Dainabot Co., Ltd., Chiba,[1] and Research Institute for Microbial Diseases, Osaka University, Osaka,[3] Japan, and Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Bangkok, Thailand[2]

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A new immunochromatographic rapid test, Determine HIV-1/2, for the detection of antibodies to human immunodeficiency virus type 1 (HIV-1) and HIV-2 in human whole blood, serum, and plasma was evaluated. Determine HIV-1/2 is a sandwich immunoassay and uses a nitrocellulose strip with a capture site for the patient's results and a procedural control site to confirm the validity of the assay. The results can be read visually, and a positive result is indicated by the formation of a red line within 15 min after sample application. The test showed 100% sensitivity for HIV-1 with 102 whole-blood, 152 serum, and 144 plasma samples obtained from Ramathibodi Hospital, Bangkok, Thailand. The sensitivity of the test for HIV-2 was 100% with 100 serum or plasma samples obtained from Ivory Coast. The sensitivity of the test with 4 anti-HIV-1 seroconversion panels from Boston Biomedica Inc. was equivalent to or better than those of another agglutination assay with serum or plasma and the enzyme immunoassay licensed by the U.S. Food and Drug Administration. The specificity was 100% with 367 sets of whole-blood, serum, and plasma samples from Ramathibodi Hospital. This method had an analytical sensitivity for the detection of HIV-1 equivalent to or better than that of another agglutination assay with serum or plasma. This test had an analytical sensitivity for the detection of HIV-1 better than that of another immunochromatographic test with whole blood. This evaluation demonstrated the excellent performance of this immunochromatographic test with EDTA-anticoagulated whole-blood, serum, and plasma samples. We conclude that this test is suitable for use in emerging countries and is an excellent alternative to HIV antibody testing at remote sites, as well as in traditional laboratories.

AIDS, which is caused by at least two types of human immunodeficiency virus (HIV), HIV type 1 (HIV-1) (1, 10, 13) and HIV-2 (6, 7), has spread rapidly, and most new infections are acquired in developing countries by transmission through blood transfusions, sexual contact, or intravenous drug use (3, 8, 9, 11). Despite intense local and international efforts to prevent new HIV infections, more than 10 million people have already become infected, and the World Health Organization estimates that by the year 2000 the cumulative number of HIV infections worldwide will be approximately 40 million (15, 16).

Serological tests such as enzyme immunoassays (EIAs), particle agglutination assays (PAs), and Western blotting for the detection of anti-HIV antibodies have been useful in the diagnosis of and screening for HIV infection (2, 4, 5, 12, 14). Although EIAs are widely used because of their excellent sensitivities, they are expensive, require complex instrumentation, and are too complex for use in the field. The PA methods are also widely used since they do not require complex instrumentation. However, the PA methods need 2 h to achieve results, according to the manufacturer's instructions (Serodia HIV PA and Serodia HIV-1/2 PA; Fujirebio, Tokyo, Japan) and therefore are not appropriate for rapid emergency use. Considering the current worldwide emergency and the spread of HIV, the limitations of the methods described above warrant a rapid, simple, low-cost, sensitive, and specific test for the detection of anti-HIV antibodies.

A method for the detection of anti-HIV-1 and anti-HIV-2 antibodies based on immunochromatography, specifically, Determine HIV-1/2 (Abbott Laboratories, Abbott Park, Ill.), has been developed. We evaluated Determine HIV-1/2 with whole-blood, serum, and plasma samples from patients in Ramathibodi Hospital, Bangkok, Thailand, to determine the sensitivity, specificity, and clinical utility of this test in emerging and developing countries.

MATERIALS AND METHODS

Clinical samples. The specimens used in this study were 470 fresh paired samples from HIV-1-infected patients and blood donors, 92 frozen serum and plasma samples from HIV-1-infected patients, 111 specimens from HIV-2-positive patients, and 4 commercial seroconversion panels.

Fresh sets of samples (EDTA-anticoagulated whole blood, EDTA-anticoagulated plasma and serum) were collected from 102 HIV-1-infected patients and were used within 72 h. Frozen serum ($n = 50$) and plasma ($n = 42$) samples from different HIV-1-infected patients were also examined. All patients seen at the AIDS clinic in Ramathibodi Hospital were positive for anti-HIV antibodies by EIA (Abbott HIV-1/2 3rd Gen. Plus; Abbott Laboratories, Delkenheim, Germany) and were confirmed to be positive by Western blotting (LAV Blot 1; Fujirebio, Tokyo, Japan). Five serum and five whole-blood specimens from the HIV-1-positive patients were also used to study the analytical sensitivity. Anti-HIV-1 seroconversion panels (Boston Biomedica Inc., West Bridgewater, Mass.) AC (PRB928), AD (PRB929), AE (PRB930), and AF (PRB931) were also evaluated. Fresh sets of samples (EDTA-anticoagulated whole blood, EDTA-anticoagulated plasma, and serum) were collected from 368 blood donors at Ramathibodi Hospital and were used within 72 h. All of the serum samples from the blood donors were negative for anti-HIV antibodies by the EIA. One hundred plasma or serum samples that were serologically positive for HIV-2 antibody and that were confirmed to be positive by Western blotting (Biotech HIV-2; Cambridge Biotech Limited, Galway, Ireland) were obtained from a blood center in the Ivory Coast and were evaluated in this study.

Determine HIV-1/2. Determine HIV-1/2 (Abbott Laboratories), an immunochromatographic rapid test, was evaluated. The assay is based on the sandwich immunoassay technique with HIV-1 and HIV-2 recombinant antigens as well as peptides of HIV-1 and HIV-2 envelope antigens. The test uses a nitrocellulose strip with a conjugate site containing HIV-1 and HIV-2 antigens conjugated to

* Corresponding author. Mailing address: Research and Development, Dainabot Co., Ltd., 344 Minoridai, Matsudo, Chiba 271, Japan. Phone: 81-47-362-4335. Fax: 81-47-363-5145. E-mail: Hiroyasu.Arai@Abbott.com.

TABLE 1. Sensitivity and specificity of tests for detection of antibodies to HIV-1 and HIV-2 with samples positive and negative for HIV-1 by EIA[a]

Test	Serum			Plasma			Whole blood			Serum			Plasma			Whole blood		
	No. of HIV-1-positive serum samples positive by EIA		Sensitivity (%)	No. of HIV-1-positive plasma samples positive by EIA		Sensitivity (%)	No. of HIV-1-positive whole-blood samples positive by EIA		Sensitivity (%)	No. of HIV-1-negative serum samples negative by EIA[a]		Specificity (%)	No. of HIV-1-negative plasma samples negative by EIA		Specificity (%)	No. of HIV-1 negative whole-blood samples negative by EIA		Specificity (%)
	Positive	Negative		Positive	Negative		Positive	Negative		Positive	Negative		Positive	Negative		Positive	Negative	
Determine HIV-1/2	152[b]	0	100	144[c]	0	100	102	0	100	0	368[d]	100	0	368[d]	100	0	368	100
PA	152	0	100	144	0	100	NA[e]	NA	NA	0	368	100	ND[f]	ND	ND	NA	NA	NA
Latex agglutination	152	0	100	144	0	100	NA	NA	NA	0	368	100	ND	ND	ND	NA	NA	NA

[a] Abbott HIV-1/2 3rd Gen. Plus.
[b] One hundred two of 152 samples were paired with the 102 whole-blood samples.
[c] One hundred two of 144 samples were paired with the 102 whole-blood samples.
[d] All samples were paired with the whole-blood samples.
[e] NA, not applicable.
[f] ND, not done.

TABLE 2. Sensitivities of tests for detection of antibodies to HIV-1 and HIV-2 with commercial seroconversion panels[a]

Panel	No. of days since first bleed	Determine HIV-1/2 result	PA result	Latex agglutination test result	S/CO[b]	
					Abbott HIV-1/2	Gen Sys HIV-1/2
PRB928-01	0	−	−	−	0.2	0.3
PRB928-02	111	+	+	−	7.8	0.3
PRB928-03	120	+	+	+	15.1	1.9
PRB928-04	125	+	+	+	15.6	2.5
PRB928-05	130	+	+	+	16.4	3.6
PRB929-01	0	−	−	−	0.2	0.1
PRB929-02	4	−	−	−	0.2	0.1
PRB929-03	14	−	−	−	0.2	0.1
PRB929-04	18	−	−	−	0.2	0.1
PRB929-05	21	−	−	−	0.9	0.1
PRB929-06	25	+	+	+	>16.3	0.1
PRB929-07	28	+	+	+	>16.3	1.2
PRB930-01	0	−	−	−	0.1	0.2
PRB930-02	3	−	−	−	0.1	0.2
PRB930-03	7	+	−	−	10.2	0.3
PRB930-04	10	+	+	+	>18.3	2.2
PRB931-01	0	−	−	−	0.1	0.1
PRB931-02	2	−	−	−	0.1	0.1
PRB931-03	7	−	−	−	0.1	0.1
PRB931-04	9	−	−	−	0.1	0.1
PRB931-05	15	−	−	−	0.1	0.1
PRB931-06	28	+	+	+	6.0	0.4
PRB931-07	33	+	+	+	>18.7	1.1
PRB931-08	35	+	+	+	>18.7	1.9
PRB931-09	42	+	+	+	>18.7	4.0

[a] Seroconversion panels were from Boston Biomedia Inc.
[b] Data were provided by Boston Biomedica Inc. S/CO, specimen absorbance (optical density)-to-cutoff ratios. Samples with ratios of ≥1.0 are considered positive.

selenium colloid and a capture site containing HIV-1 and HIV-2 antigens. If a sample contains anti-HIV-1 or anti-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 15 min. For serum or plasma, 50 μl is placed on the sample application pad. For an EDTA-anticoagulated whole-blood sample, 50 μl is placed on the pad, followed by the addition of 1 drop of buffer. The test also contains a procedural control site which confirms the validity of the assay by the formation of a visible red line. Test devices stored at room temperature (30°C) for 12 months had sensitivity equivalent to that of test devices stored at 2 to 8°C. No difference in sensitivity was observed when the results were generated at temperatures ranging from 15 to 40°C. This study was done at approximately 25°C in the clinical laboratory of Ramathibodi Hospital.

PA. The PA method (Serodia HIV; Fujirebio Inc.) was evaluated with the same specimens used for the evaluation of Determine HIV-1/2 and was performed according to the manufacturer's instruction. PA was performed with sensitized gelatin particles coated with purified inactivated HIV-1 antigens and unsensitized gelatin particles. The particles were added in 25-μl aliquots to serial twofold dilutions of the serum sample in serum diluent (potassium phosphate [monobasic], sodium chloride, and sodium azide solution) up to a 1:8 dilution for the unsensitized particles and a 1:16 dilution for the sensitized particles. The mixtures were incubated at room temperature for 2 h, followed by reading of the samples for particle agglutination. The result for a sample showing no agglutination with unsensitized particles and agglutination with sensitized particles was interpreted as positive. The result for a sample showing no agglutination with both particles was interpreted as negative.

Latex agglutination test. The Capillus HIV-1/HIV-2 (Cambridge Biotech Limited) assay was performed according to the manufacturer's instruction. One drop of latex reagent coated with recombinant HIV-1 and HIV-2 antigens is placed on the mixing well of a plastic slide, followed by application of the serum sample with the calibrated dropper included in the kit. After mixing with the dropper, the mixture is moved to the opening of the sample channel and is allowed to flow on the slide. The mixture flows into the viewing window within 3 to 7 min. If latex agglutination is observed, the result for the sample is interpreted as positive. If no agglutination is seen, the result for the sample is interpreted as negative.

TABLE 3. Analytical sensitivity of tests for detection of antibodies to HIV-1 and HIV-2 with samples positive for HIV-1 by EIA[a]

Test	Analytical sensitivity									
	Serum samples					Whole-blood samples				
	2P032S	2P043S	3P001S	3P003S	3P019S	2P033W	2P044W	3P002W	3P004W	3P020W
Determine HIV-1/2	2^{13}[b]	2^{12}	2^{12}	2^{12}	2^{14}	2^{13}	2^{11}	2^{11}	2^{11}	2^{12}
PA	2^{12}	2^{12}	2^{14}	2^{11}	2^{14}	NA[c]	NA	NA	NA	NA
Latex agglutination	2^{4}	2^{4}	2^{5}	2^{3}	2^{4}	NA	NA	NA	NA	NA
Hemo-Strip HIV-1/2	NA	NA	NA	NA	NA	2^{1}	2^{2}	2^{4}	2^{4}	2^{5}

[a] Abbott HIV-1/2 3rd Gen. Plus.
[b] The endpoint was detected by the twofold dilution method with a negative serum sample for the serum samples and a negative whole-blood sample for the whole-blood samples.
[c] NA, not applicable.

Hema-Strip HIV-1/2. Another immunochromatographic test, Hema-Strip HIV-1/2 (Saliva Diagnostic Systems, Singapore), for the detection of HIV-1 and HIV-2 antibodies in whole blood was also performed according to the manufacturer's instruction. After a finger was stuck with the lancet included with the kit, whole blood was sampled by touching the blood with the tip of the sampler followed by pressing of the sampler into the buffer vial. The buffer fluid travels up the strip inside the sampler, and the results are visible within 5 to 15 min. If only the control line appears, the result for the sample is interpreted as negative. If two bars appear (control and test line), the result for sample is interpreted as positive. This test was used for comparison of analytical sensitivities.

Evaluation of the results. The sensitivity and specificity of Determine HIV-1/2 were evaluated with the clinical samples and were compared with those of the PA and the latex agglutination test. The analytical sensitivity of Determine HIV-1/2 was examined by using serial twofold dilutions of the sera from the clinical samples, with HIV-negative serum used as a diluent, and was compared with those of the PA and the latex agglutination test. To determine the analytical sensitivity of Determine HIV-1/2 with whole-blood samples, serial twofold dilutions of the blood samples were made with whole blood from a blood donor (O type blood in the ABO system to prevent blood type incompatibilities). The results were then compared with those obtained with Hema-Strip HIV-1/2.

RESULTS

Determine HIV-1/2 demonstrated 100% sensitivity for the detection of HIV-1 with the 152 serum samples and the 144 plasma samples from among the clinical samples, and the results completely agreed with those of the EIA (Table 1). The PA and the latex agglutination test also showed 100% sensitivity with the serum samples. The sensitivity of Determine HIV-1/2 with 102 whole-blood samples from among the same clinical samples was 100%.

The sensitivity of the test with the anti-HIV-1 seroconversion panels was equivalent to or better than those of PA, the latex agglutination test, and the EIA licensed by the U.S. Food and Drug Administration (Table 2).

The analytical sensitivities by the twofold dilution method with five HIV-1-positive serum samples from Ramathibodi Hospital were 2^{12} to 2^{14} for Determine HIV-1/2, 2^{11} to 2^{14} for PA, and 2^{3} to 2^{5} for the latex agglutination test (Table 3). The analytical sensitivities by the twofold dilution method with five whole-blood samples were 2^{11} to 2^{13} for Determine HIV-1/2 and 2^{1} to 2^{5} for Hema-Strip HIV-1/2 (Table 3).

The test showed 100% sensitivity for the detection of HIV-2 with the 100 serum samples, and the results completely agreed with those of EIA and PA (Table 4).

The specificity of the test was 100% with 367 sets of whole-blood, serum, and plasma samples, and the results completely agreed with those of EIA, PA, and the latex agglutination test (Table 1).

DISCUSSION

We evaluated the sensitivity, specificity, and clinical utility of a new immunochromatographic test for the detection of antibodies to HIV-1 and HIV-2 in human serum, plasma, and whole blood. In comparison to the other assays evaluated in this study, Determine HIV-1/2 had several advantages. The other assay procedures required multiple steps (four to six steps) to achieve the results, but Determine HIV-1/2 is simple, requiring only one step to apply 50 μl of sample for serum or plasma and two steps to apply 50 μl of sample followed by one drop of buffer for EDTA-anticoagulated whole blood. The time to the retrieval of results by the PA method is 2 h. Determine HIV-1/2 is rapid, since results are available 15 min after sample application. Determine HIV-1/2 does not require any specific instrumentation or any skill or expertise for the reading of the results. All five technologists who evaluated Determine HIV-1/2 at Ramathibodi Hospital commented that they had no difficulty in performing Determine HIV-1/2 and in reading the results because the test was easy to perform and the signal indicating the results was clear. The test can be used for either batch or single use by separating the strip as required. In addition, the same device can be used for assays with EDTA-anticoagulated whole blood as well as serum or plasma. These features allow introduction of an anti-HIV antibody detection test in remote areas without electricity since Determine HIV-1/2 does not require a step for bound-free separation because of sample-conjugate migration by capillary flow. In addition, centrifugation is not required or necessary to prepare serum or plasma samples from whole blood.

Determine HIV-1/2 showed 100% sensitivity for the detection of HIV-1 and 100% specificity with whole blood, serum, and plasma, and the results completely agreed with those of PA, the latex agglutination test, and EIA. Since identical results were obtained with whole blood and paired serum and plasma samples from the same patients, the clinical sensitivity and specificity of the assay are considered to be equivalent with whole blood, serum, and plasma. The sensitivity of the test for the detection of antibodies to HIV-2 was also 100%, demonstrating the excellent capability of Determine HIV-1/2 to detect anti-HIV-2 antibodies.

TABLE 4. Sensitivity of tests for detection of antibodies to HIV-1 and HIV-2 with HIV-2 samples positive for HIV-2 by EIA[a]

Test	No. of HIV-2-positive plasma samples positive by EIA		Sensitivity (%)
	Positive	Negative	
Determine HIV-1/2	100	0	100
PA	100	0	100
Latex agglutination	ND[b]	ND	ND

[a] Abbott HIV-1/2 3rd Gen. Plus.
[b] ND, not done.

The analytical sensitivity of the test by the twofold dilution method was equivalent to that of PA and was better than that of the latex agglutination test with serum samples and better than that of Hema-Strip HIV-1/2 with whole-blood samples. In addition, the test detected the seroconversion on the same or earlier bleed days compared with the times of detection of seroconversion by the other tests.

Determine HIV-1/2 is a very simple, easy-to-read, and rapid test with excellent sensitivity and specificity with EDTA-anticoagulated whole-blood, serum, and plasma samples for the detection of both HIV-1 and HIV-2 antibodies with one device. Because of its unique features, which are the advantages of the test over the presently used rapid tests, we conclude that this test will allow screening of blood for anti-HIV-1 and anti-HIV-2 antibodies in developing countries and will provide an excellent alternative test for the detection of HIV antibodies at remote sites as well as in traditional laboratories.

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