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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

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A rapid immunodiagnostic test that detects and discriminates human immunodeficiency virus (HIV) infections on the basis of viral type, HIV type 1 (HIV-1) group M, HIV-1 group O, or HIV-2, was developed. The rapid assay for the detection of HIV (HIV rapid assay) was designed as an instrument-free chromatographic immunoassay that detects immunoglobulin G (IgG) antibodies to HIV. To assess the performance of the HIV rapid assay, 470 HIV-positive plasma samples were tested by PCR and/or Western blotting to confirm the genotype of the infecting virus. These samples were infected with strains that represented a wide variety of HIV strains including HIV-1 group M (subtypes A through G), HIV-1 group O, and HIV-2 (subtypes A and B). The results showed that the HIV genotype identity established by the rapid assay reliably (469 of 470 samples) correlates with the HIV genotype identity established by PCR or Western blotting. A total of 879 plasma samples were tested for IgG to HIV by a licensed enzyme immunoassay (EIA) (470 HIV-positive samples and 409 HIV-negative samples). When they were tested by the rapid assay, 469 samples were positive and 410 were negative (99.88% agreement). Twelve seroconversion panels were tested by both the rapid assay and a licensed EIA. For nine panels identical results were obtained by the two assays. For the remaining three panels, the rapid assay was positive one bleed later in comparison to the bleed at which the EIA was positive. One hundred three urine samples, including 93 urine samples from HIV-seropositive individuals and 10 urine samples from seronegative individuals, were tested by the rapid assay. Ninety-one of the ninety-three urine samples from HIV-seropositive individuals were found to be positive by the rapid assay. There were no false-positive results (98.05% agreement). Virus in all urine samples tested were typed as HIV-1 group M. These results suggest that a rapid assay based on the detection of IgG specific for selected transmembrane HIV antigens provides a simple and reliable test that is capable of distinguishing HIV infections on the basis of viral type.

Human immunodeficiency virus (HIV) strains are divided into two distinct types, HIV type 1 (HIV-1) and HIV-2. Genetic analysis of HIV-1 isolates has revealed that they are separated into two groups: M (major) and O (outlier). HIV-1 group M isolates can be further subdivided into 10 different subtypes (subtypes A to J), while HIV-2 is classified into five subtypes (subtypes A to E) (21). Although numerous isolates of HIV-1 group O have been characterized, classification of group O viruses into subtypes has not been established. HIV-1 group M infections predominate worldwide, while HIV-2 is found primarily in West Africa. Although HIV-1 group O infection is endemic in west central Africa (Cameroon, Gabon, and Equatorial Guinea) (12, 14), patients infected with group O isolates have been identified in Belgium (7), France (6, 16), Germany (13), Spain (18), and the United States (25).

HIV serology is characterized in large part by the immune response to viral proteins (antigens), particularly those comprising the *gag* and *env* regions. For the majority of commercial diagnostic tests, the main serological target for the detection of HIV infections is based on antibody reactivity to the envelope transmembrane protein: gp41 for HIV-1 and gp36 for HIV-2. The transmembrane protein is highly immunogenic and elicits a strong and sustained antibody response in individuals infected with HIV. Antibodies to this protein are among the first to appear at seroconversion, and the antibody response remains persistent throughout the course of the disease (1, 22, 28). The majority of the antibody response to gp41 or gp36 is directed toward the immunodominant region (9–11). Comparisons of the *env* genes of gp41 for HIV-1 group M, gp41 for HIV-1 group O, and gp36 for HIV-2 show up to 50% divergence in amino acid sequences among the genes. As a consequence of this divergence there is limited serological cross-reactivity between these *env* glycoproteins. This may in part explain why serological assays with HIV-1 group M subtype B reagents are unable to detect antibodies from some individuals infected with HIV-1 group O or HIV-2 (27). However, differences in the serological responses to *env* proteins would allow one to discriminate between HIV-1 group M, HIV-1 group O, and HIV-2.

The conventional enzyme immunoassays (EIAs) available for the detection of antibodies to HIV require instrumentation (i.e., incubators and mechanical washing and optical reading devices) and generally take 2 to 4 h to produce a result. The need for simpler, faster, less expensive, and easier-to-perform tests has become more acute as the HIV pandemic has expanded; thus, a variety of rapid test formats continue to be evaluated worldwide (20, 26, 30, 31, 33). Rapid tests for the detection of HIV (HIV rapid tests) which provide results concurrent with the patient's visit were preferred and resulted in significant improvement in the delivery of counseling without increasing the cost or decreasing the effectiveness of testing (15). In addition, simple, rapid, economical tests for the diag-

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nosis of HIV infections could improve the safety of the blood supply worldwide.

In response to this need, a rapid self-performing immunochromatographic assay for the detection of antibodies to HIV-1 and HIV-2 was developed. This instrument-free assay is performed at room temperature and produces results in 5 min. The unique feature of this rapid assay is its ability to determine whether an individual is infected with HIV-1 group M, HIV-1 group O, or HIV-2. Discrimination between the viral types would prove to be useful in epidemiological studies, when choosing antiviral therapy, or when counseling a patient on disease progression.

MATERIALS AND METHODS

HIV antigens. Three recombinant proteins derived from the *env* regions of HIV-1 group M, HIV-1 group O, or HIV-2 were expressed in *Escherichia coli* as fusion proteins with CTP:CMP–3-deoxy-D-*manno*-octulosonate cytidylyltransferase (CKS) (24). These antigens were extracted and purified by standard protocols for the production of recombinant proteins in *E. coli* (29). The HIV-1 group M *env* construct was derived from subtype B isolate HXB2R (21) and comprises the carboxy-terminal 42 amino acids of gp120 (residues 477 to 518) and the amino-terminal 185 amino acids of gp41 (residues 1 to 155 and 194 to 223) fused to CKS. The HIV-1 group O *env* construct was derived from the group O isolate HAM112 and comprises the carboxy-terminal 45 amino acids of gp120 (residues 476 to 520) and the amino-terminal 199 amino acids of gp41 (residues 1 to 169 and 196 to 225) (11a). The HIV-2 *env* construct was derived from subtype A isolate D194 (21) and comprises the carboxy-terminal 60 amino acids of gp105 (residues 432 to 491) and the amino-terminal 159 amino acids of gp36 (residues 1 to 159).

Colloidal selenium-antibody conjugates. A selenium colloid suspension (Abbott Laboratories, Abbott Park, Ill.) was concentrated to an absorbance of 25 optical density units (wavelength scan, 400 to 800 nm) in distilled water. MOPS [3-(*N*-morpholino)propanesulfonic acid] was added to the selenium colloid suspension at a final concentration of 10 mM (pH 7.4). Affinity-purified goat anti-human immunoglobulin G (IgG; Fc specific) antibodies (Jackson Immuno Research Laboratories, Inc., West Grove, Pa.) were diluted in 50 mM phosphate buffer (pH 7.4) to a concentration of 0.75 mg/ml. The diluted antibody was added to the selenium colloid suspension to achieve a final antibody concentration of 75 μg/ml. The mixture was stirred for 40 min at room temperature. Bovine serum albumin (11% [wt/vol] in 10 mM MOPS [pH 7.4]) was added to a final concentration of 1% (vol/vol), and the selenium colloid-antibody conjugate solution was stirred for an additional 15 min, followed by centrifugation at 5,000 × *g* for 90 min. After centrifugation, 90% of the supernatant was removed and the pellet was resuspended with the remaining supernatant.

The conjugate pad is a resin-bonded glass fiber material (Lydal, Inc., Rochester, N.H.) that is immersion coated in selenium colloid–anti-IgG conjugate and then dried with hot air. A glass fiber material that is not coated with the selenium colloid–anti-IgG conjugate is used as the sample application pad.

Preparation of material for chromatography. All antigens were applied to a nitrocellulose membrane (Schleicher & Schuell, Keene, N.H.) by charge and deflect reagent jetting (Abbott Laboratories). HIV recombinant proteins were diluted to a concentration of 0.5 to 1 mg/ml in jetting diluent (100 mM Tris [pH 7.6] with 1% [wt/vol] sucrose, 0.9% [wt/vol] NaCl, and 5 μg of fluorescein per ml) and jetted at a band width of 0.5 mm onto the nitrocellulose membrane strip as three discrete zones (lines perpendicular to the direction of the fluid flow). The jetted nitrocellulose, blotter pad, conjugate pad, and sample application pad components are manually assembled and held permanently in place with a Mylar film laminating material. The laminate is then cut into individual 7.5-mm strips and placed in plastic housing sticks for final assembly (Fig. 1).

Assay procedure. Serum or plasma (1 μl) and five drops (100 μl) of sample elution buffer are applied to the sample well of the HIV rapid test stick. For testing of urine, 50 μl of sample and 50 μl of sample elution buffer are used. The sample and buffer are allowed to diffuse through the application pad and migrate past the result window. As the anti-IgG-coated selenium–anti-HIV complexes flow, they pass over discrete zones, each of which contains a recombinant antigen. Complexes containing HIV-specific antibodies bind to recombinant antigens immobilized on the nitrocellulose strip, resulting in a red color at the capture bar. When the end-of-test window turns red (about 5 min), the assay is completed.

Serum, plasma, and urine specimens. The serum and plasma samples (n = 470) included in the study were obtained from Cameroon, Uganda, Brazil, Thailand, Côte d'Ivoire, Equatorial Guinea, France, Spain, the United States, and Germany. HIV-1 seroconversion panels were purchased from Boston Biomedica, Inc. (West Bridgewater, Mass.) and NABI (Boca Raton, Fla.). Matched plasma and urine samples (n = 103) were purchased from Research Sample Bank (Pompano Beach, Fla.) or were from healthy donors (Abbott Laboratories). The samples were tested for antibodies to HIV-1 or HIV-2 by commercially available assays; either an HIV-1 lysate EIA (3A11; Abbott Laboratories) or an HIV-1 and HIV-2 recombinant antigen sandwich EIA (3A77; Abbott Laboratories).

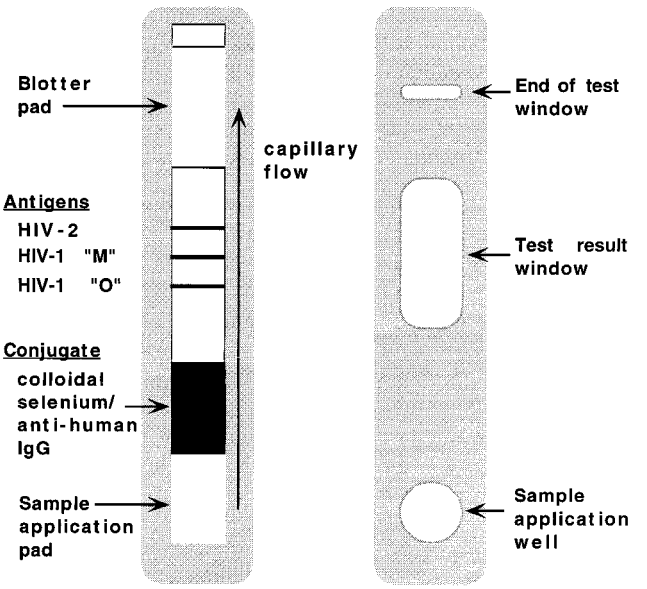

FIG. 1. HIV rapid assay format. Three recombinant envelope antigens are jetted separately onto distinct areas of a nitrocellulose strip. The assembly also includes a glass fiber conjugate pad containing colloidal selenium coated with goat anti-human IgG. The entire assembly including the conjugate sample application pad and blotter pad are held together by clear laminating Mylar film.

Positive controls consisted of two HIV-1 group M-, two HIV-1 group O-, and two HIV-2-infected serum or plasma samples. Two uninfected serum samples were included as part of the control panel. This control panel was used to test all batches of strips prepared as part of an internal quality control. HIV-1-positive samples were confirmed by Western blotting (Cambridge Biotech, Worcester, Mass.) or by sequence analysis after PCR amplification. HIV isolates were subtyped on the basis of phylogenetic analysis of PCR-amplified gp41 sequences (2, 4). Samples positive for HIV-2 were confirmed to be positive by a Western blot assay for HIV-2 (Sanofi-Pasteur) and/or sequence analysis after PCR amplification (3).

Assignment of virus type. Results of the rapid assay were recorded when the sample chromatographed past the end-of-test window (approximately 5 min). Unequivocal results were obtained in most cases when a single line of reactivity developed at the site of the target antigen. For samples showing more than one line of reactivity, the capture bar showing the highest intensity was used as the basis for assignment of the viral type.

RESULTS

A control panel of serum or plasma samples known to be positive for HIV-1 group M subtype B, HIV-1 group O, and HIV-2 was used to demonstrate the ability of the HIV rapid assay to detect and discriminate antibodies to HIV, as shown in Fig. 2. Positive test results were usually interpretable within 2 min. However, final test results were not recorded until 5 min had passed to allow the colloidal selenium particles to completely migrate past the immobilized HIV antigens in the test result window. This additional time was necessary for the detection of weakly reactive samples by allowing excess selenium conjugate to fully migrate to the end-of-test window. Samples nonreactive at 5 min were interpreted as negative. In most cases only one antigen was reactive; thus, the test result was definitive in determining whether an individual was infected with either HIV-1 group M, HIV-1 group O, or HIV-2. However, some samples showed various degrees of serological cross-reactivity with the *env* antigens. When such cross-reactivities were observed, they occurred between the HIV-1 group M and HIV-1 group O antigens. No cross-reactions between HIV-1 group M and HIV-2 were observed. Despite some cross-reactivity, the type of virus present in the infected individual could still be determined on the basis of preferential

FIG. 2. Results of HIV rapid assay for control serum panel. Lane 1, negative control; lane 2, HIV-1 group O-positive control; lane 3, HIV-1 group M-positive control; lane 4, HIV-2-positive control.

immunoreactivity to the respective *env* antigen; i.e., the relative intensity of the signal was the basis for determining the viral type.

A total of 470 plasma samples from HIV-infected individuals were tested by the HIV rapid assay. These samples were obtained from various geographical regions of the world including Cameroon (n = 111), Equatorial Guinea (n = 8), Uganda (n = 65), Brazil (n = 50), Thailand (n = 107), Côte d'Ivoire (n = 117), France (n = 5), Spain (n = 4), the United States (n = 2), and Germany (n = 1). All 470 samples were seropositive by one or more commercial HIV screening assays. The results for all HIV-positive samples were subsequently confirmed by either Western blotting or PCR amplification. Subtype determinations were based on phylogenetic analysis of PCR-amplified sequences from gp41 (HIV-1) or gp36 (HIV-2). The HIV rapid assay detected and discriminated the viral types in all HIV-1-seropositive specimens (Table 1), including 319 specimens positive for HIV-1 group M isolates representing subtypes A to G (Table 2) and 29 specimens positive for HIV-1 group O isolates. The rapid HIV assay detected and discriminated as HIV-2 infections 117 of 118 HIV-2-antibody positive specimens with subtypes A and B represented (Table 1 and Table 2). For two samples the intensities were equivalent for both the HIV-1 group M and HIV-2 antigens, and the samples were interpreted as having dual infections. Only HIV-1 nucleic acid sequences were detected by PCR amplification. These two samples were subsequently tested for the presence of antibodies to HIV-2 by an HIV-2-specific Western blotting assay and a synthetic peptide EIA that discriminates HIV-1 from HIV-2. Both samples were reactive by Western blotting and with the type-specific immunodominant region peptide from gp36, indicating infection with HIV-2 (data not shown). One sample confirmed to be positive for HIV-2 was negative by the HIV rapid assay. This HIV-2-positive sample was weakly reactive in a commercial recombinant HIV-1 and HIV-2 sandwich assay.

TABLE 1. Detection and differentiation of HIV in HIV antibody-positive plasma samples[a] from various geographical locations by the HIV rapid assay

Country	No. of samples tested	No. of samples positive for the following:		
		HIV-1 group M	HIV-1 group O	HIV-2
Cameroon	111	101	9	1
Uganda	65	65		
Brazil	50	50		
Thailand	107	107		
Côte d'Ivoire	117			116
Equatorial Guinea	8		8	
France	5		5	
Spain	4		4	
United States	2		2	
Germany	1		1	
Total	470	323	29	117

[a] Samples were previously determined to be EIA positive and were subsequently confirmed to be positive by Western blotting and/or PCR amplification and analysis.

A collection of plasma samples from Equatorial Guinea and Cameroon (n = 409) seronegative for HIV by EIA was obtained for testing. All of the samples were also seronegative by the rapid assay. The specificity of the rapid assay, based on the total number of negative samples tested, was 100%.

To determine the sensitivity of the HIV rapid assay during seroconversion, HIV-1 seroconversion panels were tested. The results are presented in Table 3. The rapid assay was as sensitive as a third-generation HIV-1 and HIV-2 recombinant antigen sandwich EIA for 9 of the 12 panels tested. For the three remaining panels, the rapid assay was positive one bleed later than the bleed with which the sandwich EIA was positive. No seroconversion panels positive for HIV-1 group O or HIV-2 were tested.

Testing was performed with 103 matched plasma and urine specimens collected in the United States. Ninety-three of the plasma samples were EIA positive and were confirmed to be positive by Western blotting. The remaining 10 samples were EIA negative. The rapid assay detected 100% (93 of 93) of the plasma specimens positive by EIA. The 10 negative plasma specimens were also negative by the rapid assay. When the matched urine samples from the EIA-positive individuals were tested, 91 of 93 were found to be positive. All 10 EIA-negative samples were also negative by the rapid assay. Thus, the overall sensitivity of the rapid assay was 97.89% (91 of 93) for urine samples. All urine samples were typed as HIV group M by the rapid assay.

DISCUSSION

We have developed a highly specific and sensitive rapid assay which detects and discriminates between HIV-1 group M, HIV-1 group O, and HIV-2. This assay can be used to evaluate quickly and inexpensively large numbers of samples even in the most difficult of testing environments. Discrimination between the virus types is important for epidemiological studies to track prevalence and/or changes in the epidemic. In addition, discrimination of HIV-1- from HIV-2-infected individuals is important due to the biological and pathological differences between these viruses; HIV-2 has lower rates of viral transmission and disease progression than HIV-1 (17). The identification of HIV-1 group O from HIV-1 group M may

TABLE 2. Genotyping of HIV in HIV-infected individuals by the HIV rapid assay

Country	Total	No. of samples with the following rapid assay result:									
		HIV-1 group M (n = 319)							HIV-2 (n = 13)		HIV-1 group O N = 29
		A[a]	B	C	D	E	F	G	A	B	
Cameroon	101	86	1		1		3	10			9
Uganda	63	34		3	26						
Brazil	48		38	2			8				
Thailand	107		5		102						
Côte d'Ivoire	13								7	6	
Equatorial Guinea	8										8
France	5										5
Spain	4										4
United States	2										2
Germany	1										1
Total	322	120	44	5	27	102	11	10	7	6	29

[a] Letters represent HIV subtypes. HIV subtypes determinations were based on phylogenetic analysis of PCR-amplified sequences of gp41 for HIV-1 and gp36 of HIV-2.

affect the choice of drug therapy due to the natural resistance of HIV-1 group O to nonnucleoside reverse transcriptase inhibitors (8).

Identification of all HIV-infected individuals continues to be the major emphasis in serological detection. Due to the extensive genetic variation and antigenic diversity of HIV isolates (21), immunoassays for HIV must have the capability to detect a wide spectrum of divergent strains. With this in mind, the HIV rapid assay was used to screen a wide variety of genotyped HIV strains in specimens from various geographical regions (2–4). The rapid test showed broad specificity in accurately detecting HIV-1 group M seropositive specimens regardless of subtype, in addition to detecting HIV-1 group O and HIV-2 infections. Although the overall sensitivity was high (469 of 470; 99.79%), one HIV-2-positive sample was negative by the HIV rapid assay. This sample may have been from a patient in the process of seroconversion or may have had a low antibody titer, as shown by a low signal-to-cutoff ratio when tested by a commercial HIV EIA (data not shown).

TABLE 3. Seroconversion sensitivity of the HIV rapid assay

Seroconversion panel (no. of bleeds)	No. of positive samples/total no. of samples	
	Rapid assay	HIV-1 and HIV-2 EIA[a]
Boston Biomedica		
PRB908 (6)	1/6	1/6
PRB916 (6)	2/6	2/6
PBR917 (5)	2/5	2/5
PBR923 (13)	4/13	5/13[b]
PRB924 (8)	3/8	3/8
PRB926 (6)	2/6	2/6
PBR929 (7)	1/7	2/7[c]
PRB932 (9)	5/9	5/9
NABI		
SVO-241 (7)	4/7	4/7
SVO-331 (6)	0/6	1/6[d]
4888 (7)	6/7	6/7
37748 (6)	5/6	5/6
Total	35/86	38/86

[a] Abbott Laboratories recombinant HIV-1/2 EIA (3A77).
[b] The rapid assay was positive one bleed (37 days) later than the bleed with which the EIA was positive.
[c] The rapid assay was positive one bleed (3 days) later than the bleed with which the EIA was positive.
[d] The last bleed (20 days past the first bleed) of the seroconversion panel was detected by EIA but not by the rapid assay.

Twelve HIV-1 seroconversion panels were tested to examine the sensitivity of the rapid test for the detection of HIV infections during seroconversion. A third-generation EIA for HIV-1 and HIV-2 was used as the reference test. The rapid assay detected HIV antibody on the same day that the reference test detected HIV antibody for 9 of 12 panels tested. Of the remaining three panels, the rapid test was positive one bleed later than the third-generation EIA. This sandwich EIA can simultaneously detect IgG and IgM antibodies to HIV-1 and HIV-2 in samples from infected individuals, whereas the rapid assay uses only an anti-IgG conjugate. The lack of IgM detection may account, at least in part, for the difference in sensitivity between the two tests. In addition, the seroconversion panel members missed by the rapid assay were predominantly reactive with p24 when they were examined by Western blotting (data not shown). The third-generation EIA used in the present study has the capability of detecting anti-p24 antibodies, whereas the rapid assay does not. Thus, the lack of anti-p24 detection by the rapid assay may result in a lower sensitivity for HIV detection during seroconversion in some cases. The sensitivity of the rapid assay for the detection of HIV during seroconversion is close but not equivalent to that of the third-generation EIA.

The utility of the rapid assay for the detection of HIV antibodies from specimens that can be obtained by noninvasive means was also examined. The overall rate of detection with urine samples was 97.85%, in contrast to a 100% rate of detection for the matched plasma samples. The observed differences in sensitivity are likely due to the relatively lower concentrations of anti-HIV antibodies in urine in comparison to the levels in serum or plasma (5). Further studies with additional samples are needed for a more accurate determination of the sensitivity of the rapid assay with this type of specimen.

On-site rapid testing for HIV provides an inexpensive and effective method of determining the HIV serological status of an individual. As such, rapid screening tests can be a valuable alternative to testing by EIA especially in areas with a high prevalence of individuals infected with HIV. These areas are often in resource-poor and/or rural settings, where access to HIV testing is minimal. In these settings, the use of a rapid assay for the detection of HIV would provide immediate test

results and would facilitate result-specific counseling on the day of the initial visit. To date, most individuals infected with either HIV-1 group O or HIV-2 have originated from or were connected to west central Africa. However, immigration and travel have resulted in the spread of these viruses to new geographical regions. The ability of the rapid assay to discriminate the viral type provides an excellent tool for epidemiological studies designed to monitor the spread of HIV-1 group O or HIV-2.

Rapid tests that detect antibodies to HIV have not traditionally been used to screen specimens in clinical settings. Simple and rapid test methods may provide an acceptable alternative if their sensitivities and specificities are comparable to those of the standard EIAs. Most commercial EIAs detect but do not discriminate between HIV-1 group M, HIV-1 group O, and HIV-2. Competitive immunoblotting assays (32), peptide immunoassays (23), and peptide-blocking EIAs (19) are among a few tests used to differentiate HIV isolates serologically, but these type of assays require significant amounts of equipment, time, and expertise to achieve a result.

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