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Preclinical and Clinical Performance of the Efoora Test, a Rapid Test for Detection of Human Immunodeficiency Virus-Specific Antibodies

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Barriers to effective diagnostic testing for human immunodeficiency virus type 1 (HIV-1) infection can be reduced with simple, reliable, and rapid detection methods. Our objective was to determine the accuracy, sensitivity, and specificity of a new rapid, lateral-flow immunochromatographic HIV-1 antibody detection device. Preclinical studies were performed using seroconversion, cross-reaction, and interference panels, archived clinical specimens, and fresh whole blood. In a multicenter, prospective clinical trial, a four-sample matrix of capillary (fingerstick) whole-blood specimens and venous whole blood, plasma, and serum was tested for HIV-1 antibodies with the Efoora HIV rapid test (Efoora Inc., Buffalo Grove, IL) and compared with an enzyme immunoassay (EIA) (Abbott Laboratories) licensed by the Food and Drug Administration. Western blot and nucleic acid test supplemental assays were employed to adjudicate discordant samples. Preclinical testing of seroconversion panels showed that antibodies were often detected earlier by the rapid test than by a reference EIA. No significant interference or cross-reactions were observed. Testing of 4,984 archived specimens yielded a sensitivity of 99.2% and a specificity of 99.7%. A prospective multicenter clinical study with 2,954 adult volunteers demonstrated sensitivity and specificity for the Efoora HIV rapid test of 99.8% (95% confidence interval [CI], 99.3 and 99.98%) and 99.0% (95% CI, 98.5 and 99.4%), respectively. Reactive rapid HIV-1 antibody detection was confirmed in 99.6% of those with a known HIV infection ($n = 939$), 5.2% of those in the high-risk group ($n = 1,003$), and 0.1% of those in the low-risk group ($n = 1,012$). For 21 (0.71%) patients, there was discordance between the results of the rapid test and the confirmatory EIA/Western blot tests. We conclude that the Efoora HIV rapid test is a simple, rapid assay for detection of HIV-1 antibodies, with high sensitivity and specificity compared to a standardized HIV-1 EIA.

With the increasing availability of rapid tests for the detection of human immunodeficiency virus type 1 (HIV-1) antibodies, the screening of individuals for HIV infection is moving from laboratories to clinic-based settings (7). The early versions of these tests were neither easy to run nor particularly sensitive or specific (3, 19) Recent advances in the use of lateral-flow immunochromatographic strips and colloidal gold technology have allowed the development of rapid tests with very high sensitivity and specificity that are easy to run and can be used with whole blood (7, 15). Rapid tests for the detection of antibodies to HIV in serum or plasma are commercially available and have been recently reviewed (7, 15).

The potential public health benefits of rapid HIV testing are internationally recognized by the World Health Organization and in the United States by the Centers for Disease Control and Prevention (CDC), the Centers for Medicare and Medicaid Services, and the Food and Drug Administration (FDA) (15, 20). There are at least four reasons to promote rapid HIV testing. First, a change in the paradigm of HIV counseling and testing would enhance a proactive role in HIV testing. According to the CDC client record database, only 25 to 43% of those patients who are ultimately determined to be positive and 33 to 48% of those who are negative return for their results if laboratory-based assays are performed. These rates have improved over the years (18) but are still unacceptably low. However, in clinical settings equipped with rapid HIV testing, patients can be present to observe the rapid testing, be informed of the result in <30 min, and be counseled immediately. Second, rapid HIV tests that can use whole-blood samples are much more efficient than enzyme immunoassay (EIA) testing in resource-limited settings where supplies (sterile needles, blood collection tubes, centrifuges, and electricity, etc.) are scarce and reporting mechanisms cumbersome (9, 21).

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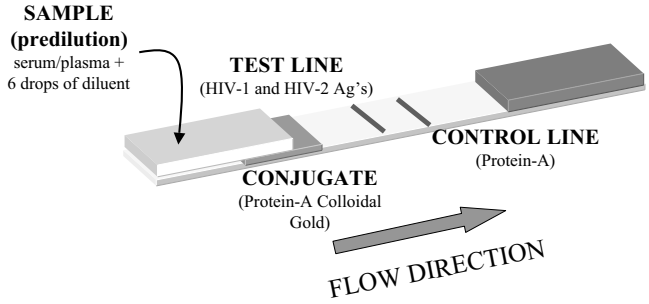

FIG. 1. Diagram of the physical and functional components of the Efoora HIV rapid test. Ag, antigen.

Third, the rapid HIV tests facilitate the initiation and continuation of preemptive antiretroviral therapy for at-risk patients, such as pregnant women (1, 2, 4, 12, 14) and exposed healthcare workers. Lastly, the availability of new technology that is simple, inexpensive, accurate, and comparable to or better than the best laboratory-based EIAs favors the use of these tests. It has been demonstrated that two rapid tests (from different manufacturers) run sequentially or concurrently have sensitivity and specificity equal to those of an EIA and Western blotting (WB) (8, 17), thus lending support to the concept of high accuracy with rapid testing.

We report here the preclinical studies and a multicenter clinical trial that were conducted to determine the accuracy (i.e., sensitivity and specificity) of a new rapid, lateral-flow immunochromatographic HIV-1 antibody detection device compared to traditional tests.

(The preclinical work was presented previously as a poster by Alzona et al. [Pre-clinical evaluation of a rapid, single-use test for detection of HIV antibodies in whole blood, abstr. M28] at the Annual Meeting of the Pan American Society for Clinical Virology, Clearwater Beach, Fla., 27 to 30 April 2003. The clinical trial data were presented previously as a poster by Mundy et al. [Clinical evaluation of an HIV rapid test for detection of HIV antibodies in fingerstick whole blood] at the International AIDS Society Meeting, Paris, France, 13 to 16 July 2003.)

MATERIALS AND METHODS

The Efoora HIV rapid test and test principles. The Efoora HIV rapid test is a unidirectional, lateral-flow immunochromatographic device to which there is binding and concentration of anti-HIV antibodies to HIV antigens located at the test line on the device, followed by detection of bound antibodies with colloidal gold-labeled conjugate (Fig. 1). The plastic housing holds a reagent pad containing an antibody-binding protein conjugated to colloidal gold. The reagent pad is adjoined to a nitrocellulose membrane that has immobilized HIV antigens in a striped test zone. In the reagent pad, the specimen's immunoglobulins bind to an immunoglobulin binding protein. During migration along the nitrocellulose membrane, HIV antibodies, if present, bind to HIV antigens and are immobilized for visualization in the test zone via a deposition of colloidal gold carried by the anti-HIV antibodies. The control zone is reactive in the presence of any immunoglobulin in the test sample. Thus, a positive result has two lines present after the 20-minute development period and a negative test has one line (control line).

Laboratory testing of commercially available panels. These assays were performed between April 2001 and December 2003 at the Efoora laboratory in Buffalo Grove, Ill., or at other contracted laboratories. Seroconversion panels (Table 1) were from Boston Biomedica, Inc. (Boston, MA), Serologicals Corporation (Norcross, GA), and Impath-BioClincial Partners (Franklin, MA). HIV antibodies were spiked by the addition of 10% (vol/vol) HIV-1-positive human plasma into the samples of interference and cross-reactivity panels (Impath-BCP, Boston Biomedica, UCLA [Los Angeles, CA], Focus Laboratories [Cypress, CA], Cambridge Labs [Cambridge, MA], and Teragenix [Ft. Lauderdale, FL]) and assayed with the Efoora HIV rapid test to determine the effects of potentially interfering and cross-reactive substances, such as antibodies to human T-cell leukemia virus (HTLV), Epstein-Barr virus, varicella-zoster virus, cytomegalovirus, hepatitis C virus (HCV), hepatitis B virus (HBV), hepatitis A virus, herpes simplex virus (HSV), *Treponema pallidum*, *Candida* spp., or *Toxoplasma gondii* or containing rheumatoid factor or antinuclear antibody.

Testing of archived and preclinical specimens. Three different kit lots of Efoora HIV rapid tests were used during the course of this testing between March 1999 and February 2001. All lots were tested prior to use and their performance verified in-house with a number of HIV-positive specimens, including low-titer specimens, dilution panels, and negative specimens. Reference tests were performed at the site of the preclinical study or at reference laboratories, utilizing various EIA methods and Western blot analyses (Table 2). Some specimens tested at the various sites were known positives for non-clade B HIV-1, HIV-2 alone, or dual infections of HIV-1 and HIV-2. Specifically, specimens at

TABLE 1. Seroconversion panels: Efoora rapid test versus FDA-licensed enzyme-linked immunosorbent assay tests, p24 antigen tests, and the Roche Amplicor Monitor test[a]

Reference test	Test positivity (in bleed intervals [days]) by panel number (BBI code)						Average
	1 (AD)	2 (AF)	3 (AP)	4 (AS)	5 (AT)	6 (P)	
Abbott							
HIV test	RNP	−1	−1	0	−1	0	−0.6
HIV-1/2 test	+1	0	+1	+2	0	0	+0.67
Genetic Systems							
HIV test	RNP	−2	−3	RNP	RNP	0	−1.67
HIV-1/2 test	0	−1	0	RNP	RNP	0	−0.25
Organon Teknika HIV test	0	−1	−1	RNP	RNP	0	−0.5
Abbott p24 Ag test	+3	0	+2	+3	+3	+1	+2.0
Coulter p24 Ag test	+3	+1	+2	+3	+2	ND	+2.2
Roche Amplicor Monitor test	+3	+1	+3	+5	+4	+2	+3.2

[a] In these BBI seroconversion panels, a bleed interval was usually 3 to 6 days (the mean bleed interval was 4.6 days). Negative numbers indicate that the Efoora rapid test was positive earlier than the reference test. A zero indicates that the Efoora rapid test was positive at the same time as the reference test. Positive numbers indicate that the Efoora rapid test was positive later than the reference test. RNP, reference not positive for any specimen in the panel; ND, not done; Ag, antigen.

TABLE 2. Efoora HIV rapid test preclinical testing locations and information

Site	Date	No. of specimens tested	Reference test(s)[a]	Specimen type(s)
U.S. East Coast	March 1999	1,931	Genetic Systems HIV-1/2, Bio-Rad WB	Serum
	February 2000	291	Genetic Systems HIV-1/2, Genetic Systems rLAV EIA, Bio-Rad WB	Fresh whole blood
	February 2001	528	Genetic Systems HIV-1/2, Bio-Rad WB	Serum, plasma, whole blood
U.S. Midwest	June 1999	1,076	Genetic Systems HIV-1/2, Abbott HIV-1/2, Bio-Rad WB	Serum, plasma
Ivory Coast, West Africa	June 1999	962	Enzygnost Plus, immunocapture HIV	Serum, plasma
South Africa	March 2000	150	Genetic Systems HIV-1/2	Serum, plasma, whole blood

[a] rLAV, recombinant lymphadenopathy-associated virus (HIV-1).

site 1 were from six countries (the United States, Ivory Coast, Philippines, Gabon, Brazil, and Trinidad; non-United States samples, 988) and included 18 specimens that were known to be positive for HIV-2 and HIV-1/2, as well as 10 that were known to be group M, non-clade B specimens; specimens at site 3 (Ivory Coast) were from local patients and thus were most likely subtype CRF_02 A/G, which is the predominant circulating recombinant form in West Africa, or subtype A. Seven specimens were known to be HIV-2 positive and 31 specimens were known to have dual HIV-1/2 infections. Specimens tested at site 5 (South Africa) were from local patients and thus were most likely HIV-1 subtype C; specimens tested at site 6 were mostly United States samples (most likely subtype B), but 10 were known to be dual HIV-1/2 positive, 2 were known to be HIV-2 positive, and 2 were known to be HIV-1 group O positive. Specimens tested at sites 2 and 4 were predominantly, if not exclusively, from patients whose infection originated in the United States and thus were probably subtype B.

Clinical trial study participants and sites. Recruitment, obtaining of consent, and enrollment of study participants occurred at eight United States study sites. Sites comprised various types of healthcare facilities for individuals at different points of care and were designated for the recruitment of three target populations: (i) participants who were infected with HIV and had prior confirmatory HIV tests (i.e., recruitment at HIV clinics), (ii) participants who were at low risk for HIV (i.e., recruitment at general health care facilities with low [<1%] HIV prevalence), and (iii) participants who were at high risk for HIV (i.e., recruitment at sites of high [>3%] HIV prevalence or of individuals who reported HIV risk behaviors as defined by the CDC, such as men who have sex with men, women who have sex with men who have sex with men, injection drug use, or other high-risk sexual activity).

The study eligibility criteria included consenting adults of >18 and <55 years of age. Hosts with non-HIV life-threatening illness or immunosuppression were excluded. Participants were informed of the rapid test results; pretest counseling and posttest counseling were performed for all high- and low-risk study participants. Follow-up confirmatory test results (see below) were provided to the study team at each site. Site-specific data were forwarded to a central data repository. This study underwent institutional review board approval at each site or approval by a third-party institutional review board if none was available locally.

HIV antibody testing. (i)The Efoora HIV rapid test. HIV-1 antibody detection was performed with the Efoora HIV rapid test (Efoora, Inc., Buffalo Grove, IL) with a four-sample matrix of fresh capillary and venous whole blood, plasma (EDTA anticoagulated), and serum. On-site testing by trained personnel was performed on fresh capillary (finger stick) and venous whole-blood specimens. Each site processed venous blood samples and tested serum and plasma by using the HIV rapid test. Each device was for single use, and results were available within 20 min.

(ii) Standard HIV antibody testing. Focus Technologies (Cypress, CA) was the designated central reference lab for the clinical trial. All clinical specimens were sent for HIV antibody EIA assay testing (Abbott HIV-1/2 EIA) with repeat EIA and HIV-1 WB (Bio-Rad) performed on all EIA-reactive specimens.

(iii) Resolution of discordant test results. For specimens with discordance between the Efoora HIV rapid test matrix results and/or the reference EIA/WB results, the central reference laboratory performed triplicate HIV antibody testing (Abbott and Bio-Rad HIV-1/2 EIA), WB (Bio-Rad), and quantification of HIV viral RNA by using the Roche Amplicor Monitor assay (nucleic acid testing [NAT]) on plasma specimens.

For aggregate data analysis of both the Efoora and EIA tests, the multiple test results (i.e., multiple specimen types for Efoora and replicate tests for EIA) were combined into an overall determination of the state of being reactive or nonreactive, based on a majority of the test results for that method. The overall Efoora test result was then compared with the overall EIA test result for concordance, and if disagreement remained, HIV-1 WB and NAT supplemental assays were employed to adjudicate discordant samples. If agreement was found between the overall Efoora test result and NAT result, then the specimen was categorized as concordant in the final analysis; however, if the overall Efoora test result was discordant with the reference, the specimen was categorized as discordant. The final participant status was defined based on NAT results.

(iv) Data analyses. Site-specific data were evaluated at a central data repository. All data were analyzed. Descriptive statistics on all study variables were provided for the entire cohort, for each site, and for each of the target participant populations. Estimates and 95% confidence intervals (CI) for the performance characteristics of the Efoora test on the entire cohort were then calculated. Performance characteristics included the sensitivities, specificities, positive predictive values (PPV), and negative predictive values (NPV) of the Efoora HIV rapid test, both for the overall rapid test result and for each of the four individual matrices of the Efoora device. Performance characteristics were also estimated for the Efoora HIV rapid test at each site and for each target participant population (both overall and for each matrix). All statistical tests were performed with StatXact version 5.

Clinical testing locations in the Northeast United States were as follows: Bellevue Hospital, New York, N.Y.; Erie County Medical Center, Buffalo, N.Y.; and Tapestry Health System, Inc. Northampton, Massachusetts. Clinical testing locations in the Midwest United States were as follows: Chicago Recovery Alliance, Chicago, Ill.; Howard Brown Health Center, Chicago, Ill.; and Washington University Medical Center, St. Louis, Mo. Clinical testing locations in the Western United States were as follows: Focus Technologies, Inc., Cypress, Calif., and UCLA, Los Angeles, Calif.

RESULTS

Preclinical studies. Six HIV seroconversion panels were used to compare the sensitivity of the Efoora HIV rapid test to that of FDA-licensed EIA assays (Abbott HIV EIA) (Table 1). The p24 antigen tests (Abbott and Coulter) and the Roche Amplicor Monitor test for the detection of HIV RNA were

TABLE 3. Assigned HIV risk group categorization of 2,954 study participants compared with HIV status by confirmatory HIV-1 EIA/WB testing

Patient group (no. of participants)	No. (%) of indicated specimens	
	HIV EIA reactive[a]	HIV EIA nonreactive
HIV infection (939)	935 (99.6)	4 (0.4)
High-risk HIV (1,003)	52 (5.2)	951 (94.8)
Low-risk HIV (1,012)	1 (0.10)	1,011 (99.9)
Total	988	1,966

[a] HIV EIA, Abbott.

TABLE 4. Comparison of Efoora HIV rapid test and standard HIV EIA/WB

Overall test result	No. of participants with or without infection		
	HIV infection	No HIV infection	Total
Efoora test reactive	986	19	1,005
Efoora test nonreactive	2	1,947	1,949
Total	988	1,966	2,954

used for comparison, since both of these assays were known to detect their respective analytes prior to the appearance of antibody in recently infected patients (5). Seroconversion panels had six to nine members (i.e., different bleed times). The mean bleed interval was 4.8 days (range, 2 to 15 days; standard deviation, 3.1 days). Across all six panels, the Efoora test was positive sooner than four of the five reference EIAs (negative average numbers in the right column of Table 1). In contrast, the Abbott HIV-1/2 EIA detected HIV antibodies in the six panels 0.67 bleed intervals sooner than the Efoora rapid test. The Abbott HIV-1/2 and Efoora tests detected antibody at the same time in three of the panels; the Abbott test detected antibody sooner by one bleed interval in two of the panels (no. 1 and 3) and by two bleed intervals in one of the panels (no. 4). As expected, the antigen assays and the HIV RNA assay were positive prior to any of the antibody tests (Table 1). The Efoora rapid test detected HIV antibodies 2.0 bleed intervals after the Abbott p24 antigen assay, 2.2 bleed intervals after the Coulter p24 antigen assay and 3.2 bleed intervals after the Roche Amplicor Monitor assay.

Eleven HIV seroconversion panels were used to compare the sensitivity of the Efoora HIV rapid test to those of FDA-licensed EIA assays and Western blot methods (Abbott HIV EIA and Bio-Rad WB). Seroconversion panels had 5 to 14 members. For 10 of the 11 panels tested, the mean bleed interval was 4.9 days (range, 2 to 28 days; standard deviation, 3.8 days). Across all 11 panels, the Efoora test was reactive at the same bleed as or sooner than two of the three reference EIA methods. Efoora and Abbott EIA detection bleeds were equivalent for six panels, while Abbott EIA detected antibody one bleed earlier in 4 of the 11 panels tested. Efoora detection was observed two bleeds earlier than Abbott EIA detection for one panel. Efoora results closely correlated with positive results confirmed in all Western blotting methods.

Collections of samples containing substances that could potentially interfere with the proper development of the test or control lines were tested. These samples were first tested neat with both Efoora and the Abbott HIV-1/2 reference test and found to be HIV negative. Then, they were spiked with one-tenth of a volume of a human plasma sample known to contain antibodies to HIV-1 (i.e., the known positive plasma sample was diluted 1:10 into the potentially interfering sample so that the resulting mixture was 10% known-positive plasma and 90% sample with potentially interfering substances). The spiked samples were mixed and tested again with the Efoora assay. Panels of samples that were icteric ($n = 30$), lipemic ($n = 30$), and hemolyzed ($n = 30$) and with high hematocrits ($n = 14$) all tested negative with Abbott and Efoora kits before the spike, and all tested positive with Efoora after the addition of HIV antibodies (data not shown). A panel of samples with low hematocrits ($n = 15$) all tested negative with Abbott and Efoora tests before the spike, and one tested negative (no visible band in the test area) with Efoora after the spike (one false negative; data not shown). A panel of whole-blood samples ($n = 30$) tested negative with the Abbott test before the spike, and 29 of 30 tested negative with the Efoora test before the spike (i.e., one false positive). The same panel of whole-blood samples tested with Efoora after the spike yielded 29 of 30 positives (one false negative; data not shown).

A viral coinfection panel (catalog no. PCA201; Boston Biomedica Inc.) was used to test for possible interference with the detection of HIV antibodies due to the presence of coinfections with HBV, HCV, and/or HTLV. Serum was tested from patients who were coinfected with HIV-1 and also HBV ($n = 7$); HBV and HCV ($n = 4$); HCV and HTLV ($n = 3$); and HBV, HCV, and HTLV ($n = 2$). As negative controls, sera were tested from patients who were infected with HBV, HCV, and HTLV ($n = 5$); HBV and HTLV ($n = 2$); or none of the viruses mention above ($n = 2$). The Efoora results for all 25 specimens were concordant with the reference HIV EIA/WB results (data not shown).

A cross-reactivity performance panel constructed of serum or plasma samples from patients infected with various viruses or infectious agents or having conditions that potentially engender antibody production that may interfere with the HIV assay was tested. The specimens were positive for antibodies against HTLV ($n = 20$), Epstein-Barr virus viral capsid antigens ($n = 10$), varicella-zoster virus ($n = 10$), cytomegalovirus ($n = 10$), HSV type 1 and HSV type 2 ($n = 10$), HCV ($n = 10$), HBV ($n = 10$), hepatitis A virus ($n = 10$), rheumatoid factor ($n = 9$), *T. pallidum* ($n = 6$), toxoplasmosis ($n = 10$), *Candida* spp. ($n = 10$), and chlamydia ($n = 10$) and samples from multiparous females ($n = 10$), patients who have received multiple transfusions ($n = 9$) and from patients with antinuclear antibodies ($n = 10$). In this set of 164 specimens, all of which were

TABLE 5. Sensitivity and specificity, by the Efoora HIV rapid test, overall and by specimen type, for the entire cohort ($n = 2,954$)[a]

Matrix	Sensitivity estimate (%) (no. detected/no. HIV+)	Sensitivity 95% CI (%)	Specificity estimate (%) (no. undetected/no. HIV−)	Specificity 95% CI (%)
Overall	99.8 (986/988)	99.3, 99.98	99.0 (1,947/1,966)	98.5, 99.4
Fingerstick	99.8 (986/988)	99.3, 99.98	99.2 (1,951/1,966)	98.7, 99.6
Whole blood	99.8 (986/988)	99.3, 99.98	99.2 (1,950/1,966)	98.7, 99.5
Plasma	99.8 (986/988)	99.3, 99.98	99.1 (1,949/1,966)	98.6, 99.5
Serum	99.8 (986/988)	99.3, 99.98	99.2 (1,950/1,966)	98.7, 99.5

[a] +, positive; −, negative.

TABLE 6. Positive and negative predictive values by the Efoora HIV rapid test, overall and by specimen type, for the entire cohort (n = 2,954)[a]

Matrix	PPV estimate (%) (no. HIV+/no. detected)	PPV 95% CI (%)	NPV estimate (%) (no. HIV−/no. undetected)	NPV 95% CI (%)
Overall	98.1 (986/1,005)	97.1, 98.9	99.9 (1,947/1,949)	99.6, 99.99
Fingerstick	98.5 (986/1,001)	97.5, 99.2	99.9 (1,951/1,953)	99.6, 99.99
Whole blood	98.4 (986/1,002)	97.4, 99.1	99.9 (1,950/1,952)	99.6, 99.99
Plasma	98.3 (986/1,003)	97.3, 99.0	99.9 (1,949/1,951)	99.6, 99.99
Serum	98.4 (986/1,002)	97.4, 99.1	99.9 (1,950/1,952)	99.6, 99.99

[a] +, positive; −, negative.

from patients not infected with HIV, there was one false-positive result with the Efoora HIV rapid test (data not shown).

For the final step in the preclinical evaluation of the Efoora HIV rapid test, specimens were tested at several international sites to assess the sensitivity and specificity of the assay as performed on a variety of archived or fresh specimens in a laboratory setting. Table 2 summarizes the locations, test initiation dates, number of specimens tested, and reference tests that were used for comparison. Specimens differed in type and presence of HIV-1 and HIV-2. The testing yielded 1,417 positive concordant results, 3,544 negative concordant results, 11 Efoora rapid test false-positive results, and 12 Efoora false-negative results. The overall sensitivity and specificity of the 4,984 specimens using the Efoora rapid HIV test were 99.2% and 99.7%, respectively.

Clinical studies. From December 2001 through February 2003, 2,954 adult volunteers were screened for HIV infection with the Efoora rapid HIV test and standard HIV-1 antibody EIA detection methods. By recruitment status, there were 939 (32%) participants who had HIV infection, 1,003 (34%) who were at high risk for HIV infection, and 1,012 (34%) who were at low risk for HIV infection. Confirmatory HIV-1 EIA/WB testing revealed that 99.5% of the specimens from those with a known HIV infection were reactive, 5.2% of the specimens from high-risk participants were reactive, and 0.1% of the specimens from low-risk participants were reactive (Table 3). For the overall rapid test analysis, sensitivity was based on 988 (33%) confirmed HIV-positive cases and specificity was based on 1,966 (67%) confirmed HIV-negative cases. Additionally, there were 1,005 (34%) Efoora test-positive cases and 1,949 (66%) Efoora test-negative cases who served as the bases for the overall estimates of predictive values. The predictive values determined by using the aggregate data were skewed due to the inclusion in our aggregate population of 939 patients who were recruited as known positives. Predictive values for the low-risk (6 and 1,006 test-positive and test-negative cases, respectively) and high-risk (64 and 939 test-positive and test-negative cases, respectively) populations in this study were therefore also calculated separately.

Performance characteristics overall. The Efoora HIV rapid test was reactive for the specimens from 986 of 988 participants with confirmed HIV infections, for a sensitivity estimate of 99.8% (95% CI, 99.3 to 99.98%) (Table 4, Table 5, and Table 6). The Efoora HIV rapid test was nonreactive for 1,947 of 1,966 participants who were confirmed not to have HIV infection, for a specificity estimate of 99.0% (95% CI, 98.5 to 99.4%). Per the study definitions, if the majority of the four-sample matrix test results were discordant with the reference HIV EIA/WB results, the overall EIA test result, and the NAT result, the specimen was considered discordant in the final analysis. The final participant status was determined by the NAT results. Notably, for 21 (0.71%) participants, there was discordance between the matrix of rapid HIV tests and the confirmatory HIV EIA/WB tests. Thirteen high-risk and five low-risk (n = 18) participants had false-positive (reactive) rapid tests that gave discordant nonreactive HIV EIA/WB results; there was one known HIV-positive participant whose rapid test result was reactive but the HIV EIA/WB test was nonreactive (i.e., a false-positive rapid test), thus accounting for a total of 19 false positives by the Efoora rapid test (Table 4). Two participants with confirmed HIV by EIA/WB had false-negative (nonreactive) rapid tests (Table 4).

There were four other participants with nonreactive Efoora HIV rapid tests, reactive HIV EIA/WB tests, and HIV RNA at <50 copies/ml (via NAT); the rapid test results were concordant with the NAT results. Since the NAT result was the final arbiter, these were recorded as true rapid test negatives and were categorized among the total of 1,947 true negatives (Table 4). Of note, rapid HIV tests may be negative for hosts on aggressive antiretroviral therapy (13), and these four patients may have had results that were confounded by this (data not available). This Efoora HIV rapid test is intended as a screening test and will not routinely be used for the testing of hosts with known HIV infections and who are on antiretroviral ther-

TABLE 7. Sensitivity and specificity, by the Efoora HIV rapid test, overall and by specimen type, for the low-risk population only (n = 1,012)[a]

Matrix	Sensitivity estimate (%) (no. detected/no. HIV+)	Sensitivity 95% CI (%)	Specificity estimate (%) (no. undetected/no. HIV−)	Specificity 95% CI (%)
Overall	100.0 (1/1)	2.5, 100.0	99.5 (1,006/1,011)	98.9, 99.8
Fingerstick	100.0 (1/1)	2.5, 100.0	99.6 (1,007/1,011)	99.0, 99.9
Whole blood	100.0 (1/1)	2.5, 100.0	99.5 (1,006/1,011)	98.9, 99.8
Plasma	100.0 (1/1)	2.5, 100.0	99.7 (1,008/1,011)	99.1, 99.9
Serum	100.0 (1/1)	2.5, 100.0	99.7 (1,008/1,011)	99.1, 99.9

[a] +, positive; −, negative.

TABLE 8. Positive and negative predictive values, by the Efoora HIV rapid test, overall and by specimen type, for the low-risk population only (n = 1,012)[a]

Matrix	PPV estimate (%) (no. HIV+/no. detected)	PPV 95% CI (%)	NPV estimate (%) (no. HIV−/no. undetected)	NPV 95% CI (%)
Overall	16.7 (1/6)	0.4, 64.1	100.0 (1,006/1,006)	99.6, 100.0
Fingerstick	20.0 (1/5)	0.5, 71.6	100.0 (1,007/1,007)	99.6, 100.0
Whole blood	16.7 (1/6)	0.4, 64.1	100.0 (1,006/1,006)	99.6, 100.0
Plasma	25.0 (1/4)	0.6, 80.6	100.0 (1,008/1,008)	99.6, 100.0
Serum	25.0 (1/4)	0.6, 80.6	100.0 (1,008/1,008)	99.6, 100.0

[a] +, positive; −, negative.

apy. The positive predictive value and negative predictive value for the aggregate population were estimated to be 98.1% (95% CI, 97.1 to 98.9%) and 99.9% (95% CI, 99.6 to 99.99%), respectively. Formal statistical testing for homogeneity of effects across sites and across populations was not possible, as some centers enrolled only one specific patient population or the test characteristics had no variability (e.g., test performance of 100%). Informal assessments of the estimates of test performance across sites and across populations, however, suggest strong consistency and support for pooling data for an overall analysis.

The overall performance characteristics for all 2,954 participants with results of the accuracy for each specimen type are summarized in Table 5 and Table 6. All specimen types correctly detected 986 of the 988 specimens determined to be true positives, for a sensitivity estimate of 99.8% for each specimen type. When fingerstick whole blood was used in the rapid HIV test, 1,951 of the 1,966 truly negative participants were correctly identified, for an overall specificity for that specimen type of 99.2%. For either whole-blood or serum specimen type, 1,950 negative participants were correctly identified (specificity, 99.2%), and when plasma was the specimen type, 1,949 negative participants were correctly identified (specificity, 99.1%).

Performance characteristics in low- and high-risk populations. There were 1,012 low-risk and 1,003 high-risk study participants. One recruited low-risk participant was determined to be HIV positive by both the rapid test and the reference tests. All four of the test specimens correctly and independently recorded this result, as shown in the sensitivity estimate column (Table 7). Of the 1,011 true-negative low-risk participants, the fingerstick specimens correctly identified 1,007 cases, whole blood identified 1,006 cases, and plasma and serum each identified 1,008 cases (Table 7, specificity estimate column), for specificities of 99.5% (whole blood) to 99.7% (serum and plasma). The estimates of positive and negative predictive values are shown in Table 8 for the low-risk population, and the wide confidence intervals limit the interpretation of this finding.

Among 1,003 high-risk participants, 52 were HIV positive by the reference tests, 51 of whom were Efoora rapid test positive by all four specimen types, for a sensitivity in this population of 98.1% (95% CI, 89.7 to 99.95%) (Table 9). There were 11 false positives with the fingerstick and whole blood specimens, 12 false positives with the serum specimens, and 13 false positives with plasma specimens, which resulted in a specificity range from 98.6% to 98.8% (95% CI, 97.9 to 99.4%) for fingerstick and whole blood (Table 9). The estimates of positive predictive values were 79.7% for plasma and 82.3% for fingerstick and whole blood (Table 10). The estimates of negative predictive values were 99.9% for all four of the sample types.

DISCUSSION

There is rising international interest in the capability for rapid, on-site HIV testing with reliable and accurate same-day results (3, 4). The findings from the studies reported here suggest that the Efoora HIV rapid test can meet this need in several health service delivery systems. In this series of studies, this rapid HIV antibody detection assay performed well with capillary whole blood, venous whole blood, plasma, and serum and demonstrated high sensitivity and specificity compared to a standardized HIV EIA. In addition, the specimens used in the preclinical and clinical phases of testing represented a broad spectrum of geographic locations, patient populations, and HIV types and subtypes, including HIV-2. The test performed well with all four test matrices and on specimens from diverse populations from around the world.

The evaluation of this rapid test was conducted at a variety of testing sites and healthcare facilities. Although the device is intended only for professional use, the implementation of testing is simple and applicable to resource-limited settings. One of the three currently FDA-licensed tests, the OraQuick test, has been given the rating of "low complexity" by Clinical Lab-

TABLE 9. Sensitivity and specificity, by the Efoora HIV rapid test, overall and by specimen type, for the high-risk population only (n = 1,003)[a]

Matrix	Sensitivity estimate (%) (no. detected/no. HIV+)	Sensitivity 95% CI (%)	Specificity estimate (%) (no. undetected/no. HIV−)	Specificity 95% CI (%)
Overall	98.1 (51/52)	89.7, 99.95	98.6 (938/951)	97.7, 99.3
Fingerstick	98.1 (51/52)	89.7, 99.95	98.8 (940/951)	97.9, 99.4
Whole blood	98.1 (51/52)	89.7, 99.95	98.8 (940/951)	97.9, 99.4
Plasma	98.1 (51/52)	89.7, 99.95	98.6 (938/951)	97.7, 99.3
Serum	98.1 (51/52)	89.7, 99.95	98.7 (939/951)	97.8, 99.4

[a] +, positive; −, negative.

TABLE 10. Positive and negative predictive values, by the Efoora HIV rapid test, overall and by specimen type, for the high-risk population only (n = 1,003)[a]

Matrix	PPV estimate (%) (no. HIV+/no. detected)	PPV 95% CI (%)	NPV estimate (%) (no. HIV−/no. undetected)	NPV 95% CI (%)
Overall	79.7 (51/64)	67.8, 88.7	99.9 (938/939)	99.4, 100.0
Fingerstick	82.3 (51/62)	70.5, 90.8	99.9 (940/941)	99.4, 100.0
Whole blood	82.3 (51/62)	70.5, 90.8	99.9 (940/941)	99.4, 100.0
Plasma	79.7 (51/64)	67.8, 88.7	99.9 (938/939)	99.4, 100.0
Serum	81.0 (51/63)	69.1, 89.8	99.9 (939/940)	99.4, 100.0

[a] +, positive; −, negative.

oratories Improvement Act of 1988 and thus has recently received "waived status." Waived status allows the test to be performed by non-laboratory health care professionals and provides the option for true point-of-care testing in a clinic, doctor's office, or emergency department. The Efoora test is stored at room temperature, requires no special equipment, and should be considered for use as a screening device in clinical and outreach settings. In addition, the Efoora test is a closed system: the specimen and test diluent are added directly to the test cartridge and then are completely contained within the cartridge. Other rapid tests require dilution of the blood outside the assay or addition of multiple reagents to the assay. It is anticipated that the Efoora HIV rapid test will soon join the short list of FDA-licensed rapid HIV tests.

There are at least 25 rapid HIV tests currently available on the worldwide commercial market, thus affirming international interest in rapid testing formats (3). HIV testing facilities have recognized the advantages of rapid testing, the CDC has actively promoted the use of rapid testing, and the manufacturers are answering the call for more and better rapid HIV tests (20).

Furthermore, the widespread use of rapid tests for the detection of HIV antibodies is shifting the paradigm for the screening of at-risk patients for HIV infection (11, 16). In developing countries, these rapid tests are the standard assay for the detection of HIV antibodies. The most common algorithm employs a rapid test for the initial screen and, if the specimen is nonreactive, the patient is considered uninfected, counseled as such, and released. If reactive, the specimen is tested with a rapid test from a different manufacturer than that of the first test. If the specimen is reactive in the second test, the patient is considered a confirmed positive and counseled as such. If the second test is nonreactive, the patient is considered uninfected and such specimens may be sent to a lab for adjudication of the discordant rapid results by further testing (the CDC has recently recommended that in the United States, these specimens be further tested with a Western blot or immunofluorescent antibody assay) (6). The optimal situation would be to use the most sensitive rapid assay of the two as the first or screening test and the most specific rapid assay as the second or supplemental test, thus assuring that the greatest possible number of potentially reactive specimens is harvested with the first test and the fewest possible false reactive specimens are recorded as reactive on the second test.

In developed countries, the algorithm for use of rapid HIV tests will be different, at least temporarily. Most of our screening tests will continue to be lab-based, and supplemental testing will be all lab-based for the foreseeable future. However, the rapid tests will find usefulness, particularly with regard to needlestick and surgical accident cases and in labor and delivery clinics (10). In these situations, in which the patient is most likely captive, the actual testing may be performed either point-of-care or in the laboratory, if transport to the lab is expedited. However, in other hospital or clinic-based cases, the turnaround time (TAT) is very important, since, in one study, more than half of the patients did not stay for their results when the mean TAT was 107 min (10). Thus, in situations in which the patients are not captive, it is important that the test be performed on-site so as to minimize the TAT and optimize reporting directly to the patient. The needlestick and labor and delivery situations require rapid testing for important medical decisions, such as whether to treat or not treat the recipient of the needlestick or to treat or not treat the mother in order to reduce the risk of transmission of HIV to her infant. Other situations involving rapid HIV testing are less critical in terms of medical decision making but are nonetheless important in identifying infected persons and informing them of their infection so that they can be counseled immediately to modify their behaviors and lower the risk of transmission to others.

As in all studies, there were recognized limitations in both the study design and the interpretation of findings. For a select few participants, linking the tests results with the medical chart review would have guided the test interpretation, but data were deidentified at study entry. Notably, the risk-group-specific predictive values are more meaningful than those for the overall population.

The performance characteristics of the Efoora HIV rapid test were based on a comparison to an FDA-licensed EIA for the detection of human anti-HIV antibodies to HIV-1. Further studies are needed to document the performance characteristics of this rapid HIV test for HIV-2, but the indications from the preclinical testing done in Africa are that this test will detect antibodies to the non-B subtypes of HIV-1 and also to HIV-2.

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