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Use of Rapid and Conventional Testing Technologies for Human Immunodeficiency Virus Type 1 Serologic Screening in a Rural Kenyan Reference Laboratory

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We report a prospective comparison of human immunodeficiency virus type 1 testing by enzyme immunoassay and Western blot with four rapid tests of 486 subjects performed in rural Kenya. Rapid test sensitivity was 100%. Specificity ranged from 99.1 to 100%. Combined use of two Food and Drug Administration-approved rapid tests yielded a single false-positive result.

Point-of-care diagnosis of human immunodeficiency virus type 1 (HIV-1) infection is critical to increasing serologic awareness and to reduce risk behaviors associated with HIV-1 transmission (7). Rapid HIV-1 tests yield results during a single visit, providing a considerable benefit in settings where return visit adherence is low (6, 22). Rapid HIV-1 tests have been widely used outside the United States (1–4, 8–11, 13, 15, 18, 20, 21, 23). However, studies documenting the direct comparison of reference enzyme immunoassay (EIA) and Western blot HIV-1 testing to rapid HIV-1 testing, in which both modalities were prospectively executed with fresh specimens by the same staff in field laboratories, are few and not recent (19). We report such a study with modern rapid tests in rural Kenya.

Study subjects. Blood samples were obtained from subjects enrolled in the U.S. Military HIV Research Program's HIV and Malaria Cohort Study in Kericho, Kenya. This study of 2,803 adult residents of a Kenyan highland tea plantation is gathering data on HIV incidence and infection risk factors. Volunteers, enrolled in the summer of 2003, return semiannually for physical examination and HIV-1 testing. The study was approved by the appropriate human use review boards.

HIV-1 serologic testing. Serologic point-prevalence HIV-1 testing was performed on-site at the Walter Reed Project laboratory by Kenyan staff. Fresh venous blood specimens from 486 study subjects who were enrolled during July and August 2003 were subjected to initial EIA screening with the Genetic Systems rLAV test (Bio-Rad Laboratories, Redmond, Wash.). If the initial EIA was nonreactive, the sample was interpreted as HIV-1 antibody negative. If the initial EIA result was reactive, the sample progressed to testing with the Vironostika HIV-1 Microelisa system (Organon Teknika, Durham, N.C.), using duplicate serum aliquots. Repeatedly reactive samples (at least two of three reactive EIA reactions) were subjected to confirmatory testing with the Genetic Systems HIV-1 Western blot (Bio-Rad Laboratories). Both EIA and the Western blot tests are approved by the U.S. Food and Drug Administration (FDA).

Rapid serologic testing was performed at the Walter Reed Project laboratory on the same day as phlebotomy. Anticoagulated whole blood (EDTA) was used for the OraQuick HIV-1 (OraSure Technologies, Inc., Bethlehem, Pa.), UniGold Recombigen HIV (Trinity Biotech, Inc., Wicklow, Ireland), and Determine HIV-1/2 (Abbott Laboratories, Inc., Abbott Park, Ill.) tests. Serum was used for the Reveal HIV-1 test (MedMira, Inc., Halifax, Nova Scotia, Canada). The OraQuick HIV-1 and Reveal HIV-1 tests were approved by the FDA for serologic detection of HIV-1 antibody at the time of testing, while the UniGold Recombigen HIV test was approved 23 December 2003.

HIV-1 nucleic acid testing. Frozen plasma samples shipped to Walter Reed Army Institute of Research were subjected to the HIV-1 Monitor test, version 1.5 (Roche Diagnostics, Indianapolis, Ind.), in the standard mode from subjects whose sera tested reactive by rapid test devices but nonreactive by rLAV EIA.

Statistical methodology. Sensitivity, specificity, and predictive values were calculated as described previously (5). Fisher's exact test was performed with StatView, version 5.0.

Initial EIA testing was nonreactive on 424 specimens and reactive on 62 specimens. Subjects whose specimens were nonreactive by initial EIA were defined as HIV-1 seronegative (true negative). All 62 initial EIA reactive specimens were concordantly reactive with both the Vironostika HIV-1 EIA and Genetic Systems HIV-1 Western blot. These subjects were defined as HIV-1 seropositive (true positive), yielding an HIV-1 seroprevalence of 12.8% (95% confidence intervals [CI], 9.8 to 15.7%).

All 62 true-positive specimens were reactive by the OraQuick HIV-1, Determine HIV-1/2, and UniGold Recombigen HIV rapid tests (Table 1). Sixty-one of these specimens were

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TABLE 1. Test results and operating characteristics of four rapid HIV-1 tests

HIV-1 rapid test brand name	Rapid test result[b]				Rapid test operating characteristics[a]			
	Positive[b]		Negative[b]		% Sensitivity (95% CI)	% Specificity (95% CI)	Predictive value	
	No. true	No. false	No. true	No. false			% Positive (95% CI)	% Negative (95% CI)
OraQuick	62	3	421	0	100 (92.7–100)	99.3 (97.8–99.8)	95.4 (86.2–98.8)	100 (98.9–100)
Determine	62	4	420	0	100 (92.7–100)	99.1 (97.4–99.7)	93.9 (84.4–98.0)	100 (98.9–100)
UniGold	62	0	424	0	100 (92.7–100)	100 (98.9–100)	100 (92.7–100)	100 (98.9–100)
Reveal	61	1	423	0	100 (92.6–100)	99.8 (98.4–99.9)	98.4 (90.2–99.9)	100 (98.9–100)

[a] Assuming an overall HIV-1 prevalence rate of 12.8% based upon HIV E1A and Western blot reference testing.
[b] As determined by Western blot testing.

reactive by the Reveal HIV-1 test (remaining sample not tested).

Seven specimens nonreactive by reference serology were reactive in eight rapid test evaluations (false positive) (Table 1). False-reactive results were noted with OraQuick HIV-1 (three specimens), Determine HIV-1/2 (four specimens), and Reveal HIV-1 (one specimen) testing. One specimen was reactive with both the OraQuick HIV-1 and Reveal HIV-1 test. No false-reactive results were observed with the UniGold HIV test. Quantitative HIV-1 RNA testing was below the level of detection on all seven specimens.

Rapid test sensitivity was 100%, with the 95% CI ranging from 92.6 to 100% (Table 1). Specificity ranged from 99.1% (Determine HIV-1/2) to 100% (UniGold HIV), with the 95% CI ranging from 97.4 to 100%. The positive predictive value (PPV) ranged from 93.9 (Determine HIV-1/2) to 100% (UniGold HIV). The lower bounds of the 95% CI for PPV for the Determine HIV-1/2 and UniGold HIV tests were 84.4 and 92.7%, respectively. The negative predictive value for all tests was 100%, with the lower bound of the 95% CI calculated as 98.9%.

Rapid test positive and negative predictive values are given with the observed prevalence (12.8%) and three hypothetical prevalences (Table 2). Singleton rapid test screening predicts the presence of HIV-1 infection with high confidence in populations with an HIV-1 prevalence of 10.0% or greater. This fell sharply in hypothetical populations with a prevalence of 1.0% for the three rapid test devices with specificities less than 100%. Negative predictive values for singleton HIV-1 rapid screening were 100% for all input HIV-1 prevalences.

All four HIV-1 rapid serologic tests were as sensitive as either EIA for the detection of HIV-1 antibody. Only the UniGold HIV test was as specific as the conventional HIV-1 tests. Putative falsely reactive rapid tests were not the result of enhanced serodetection over EIA in primary HIV-1 infection, as shown by the inability to detect HIV-1 RNA in these specimens.

OraQuick HIV-1 specificity was lower in the Kericho cohort (specificity, 99.3%; 95% CI, 97.8 to 99.8%) compared with U.S.-based testing (specificity, 100%; 95% CI, 99.7 to 100%; OraQuick Rapid HIV-1 antibody test, package insert, item 3001-0951, October 2003) ($P < 0.01$ by Fisher's exact test). Reduced specificity with other rapid tests has been previously reported in African settings (12). Antiretrovirus-associated reduction of OraQuick HIV-1 sensitivity (16) was not observed, as these drugs were introduced into Kericho (via the President's Emergency Program for AIDS Relief) 9 months after this study concluded.

Several additional observations merit discussion. First, the use of serial EIA screening tests provided no benefit over a single-screening EIA in this high-prevalence cohort. Second, only paired EIA or EIA and UniGold HIV testing provided the same diagnostic accuracy as conventional serology. Third, the combination of two FDA-approved rapid tests, OraQuick HIV-1 and Reveal HIV-1, would have resulted in a false-positive diagnosis of HIV-1 infection in a single subject. Fourth, parallel screening with two rapid tests would not have increased the sensitivity of detection of HIV-1 antibody in the study population in agreement with recent results by others (14). Last, the PPV of screening HIV-1 rapid tests degraded significantly when applied to hypothetical populations with an HIV-1 prevalence of 1.0%. Such populations define the HIV-1 prevalence seen in sexually transmitted disease clinics in large U.S. urban areas (17), underscoring the critical need for confirmatory HIV-1 testing in these settings.

We demonstrate that robust HIV diagnostic operational research can be executed in regional field laboratories. Rapid HIV-1 tests are critical to the implementation of antiretroviral delivery programs in resource-limited areas. However, newer

TABLE 2. Positive and negative predictive values for four HIV-1 rapid tests used in populations with the observed HIV-1 prevalence rate and three hypothetical rates

HIV-1 rapid test brand name	% Sensitivity	% Specificity	Value for HIV prevalence (%) of:							
			PPV				Negative predictive value			
			12.8	10	5	1	12.8	10	5	1
OraQuick	100	99.3	95.4	94.1	88.3	59.1	100	100	100	100
Determine	100	99.1	93.9	92.5	85.4	52.9	100	100	100	100
UniGold	100	100	100	100	100	100	100	100	100	100
Reveal	100	99.8	98.4	98.2	96.3	83.5	100	100	100	100

rapid tests with high specificity will likely be needed to best realize this goal.

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