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Performance of the OraQuick Rapid Antibody Test for Diagnosis of Human Immunodeficiency Virus Type 1 Infection in Patients with Various Levels of Exposure to Highly Active Antiretroviral Therapy

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With oral mucosal transudate and serum samples from 101 human immunodeficiency virus type 1 (HIV-1)-infected subjects and 100 HIV-1-negative volunteers, the OraQuick HIV-1 test demonstrated 100% specificity and 96% sensitivity. Four false-negative subjects, who were characterized by early initiation of effective antiretroviral therapy, demonstrated waning serum anti-gp41 titers and Western blot band intensities.

Point-of-care human immunodeficiency virus (HIV) diagnosis has advantages over conventional testing in time-critical settings (1–3, 5, 6, 8, 11, 12). The OraQuick HIV type 1 (HIV-1) rapid antibody test (OraQuick; OraSure Technologies, Bethlehem, Pa.), is designed as a rapid test that uses oral mucosal transudate (OMT), whole blood, serum, or plasma. It was approved by the U.S. Food and Drug Administration in November 2002 for HIV-1 diagnosis by using finger-stick whole blood. OraQuick detects gp41 immunodominant domain antibody. Early trials of OraQuick on OMT, blood, and serum demonstrated 100% sensitivity and 99.8% specificity (B. Branson, M. Uniyal, C. Fridlund, T. Granade, and P. Kerndt, 9th Conf. Retrovir. Opportunistic Infect., abstr. 599-T, 2002; S. Nookai, C. Sinthuwattannawibool, P. Phanuphak, and R. George, 8th Conf. Retrovir. Opportunistic Infect., abstr. 232, 2001).

To evaluate OraQuick with serum and OMT, we studied a cohort of patients whose HIV infection status was known and then expanded the evaluation to characterize the clinical features and antibody response characteristics associated with lower-than-expected OraQuick sensitivity. One hundred volunteers at low risk for HIV infection and 101 HIV-1-infected patients were recruited from an ongoing HIV natural history study. The voluntary, fully informed consent of the subjects used in this research was obtained as required by Air Force Regulation 169-9.

OraQuick testing was performed according to the directions on the package insert. OraQuick test results were read by the study technician and an observer unaware of the patient's HIV status. Serum specimens were subjected to enzyme immunoassay (EIA) (Genetic Systems rLAV; Bio-Rad Laboratories, Redmond, Wash.). Repeatedly reactive specimens were tested by Western blotting (Cambridge Biotech HIV-1 Western blot; Calypte Biomedical Corp., Rockville, Md.). Band reactivity was assigned a value from 0 (no reactivity) to 3 (strongly reactive). Viral load (VL) testing was performed with the Amplicor HIV-1 Monitor test (Roche Molecular Systems, Inc., Branchburg, N.J.) in ultrasensitive mode. Highly active antiretroviral therapy (HAART) was defined as a regimen containing at least three medications, including nucleoside reverse transcriptase inhibitors, nonnucleoside reverse transcriptase inhibitors, and protease inhibitors, in which at least one agent was abacavir, any protease inhibitor, or any nonnucleoside reverse transcriptase inhibitor.

After four HIV-infected subjects tested negative with OraQuick (case subjects), 20 subjects were randomly selected from the 97 HIV-1-infected subjects who tested positive with OraQuick (control subjects). Serum had been collected semiannually from all HIV-infected participants in the natural history study and stored at −20°C. The earliest available archived serum was tested with OraQuick. A gp41 peptide EIA (10) was performed on all specimens.

Statistical analysis was performed with the Statistical Package for the Social Sciences, version 10.0.7 (SPSS Inc., Chicago, Ill.). Continuous variables were analyzed by an independent-sample *t* test or a Mann-Whitney U test; categorical variables were analyzed by a chi-square test, Fisher's exact test, or Kruskal-Wallis one-way analysis of variance. Interobserver agreement was examined with Cohen's kappa test. Nonnumeric VL values reported as <50 were assigned values of 25, and those reported as >750,000 were assigned values of 750,001. Control subjects were selected by using random numbers generated by Microsoft Excel 97.

OraQuick was reactive with OMT and sera from 97 of 101 HIV-infected subjects and 0 of 100 subjects who were uninfected (sensitivity and specificity, 96 and 100%, respectively). Concordance between serum and OMT testing and interobserver concordance (kappa = 1.0, $P < 0.001$) were 100%. The mean time since HIV diagnosis for the 101 HIV-infected subjects was 7.0 years, 91 (90%) subjects had ever received

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TABLE 1. Anti-gp41 titers in archived and study specimens from OraQuick-reactive and OraQuick-negative HIV-infected patients

Specimen type	True positive (n = 20)		False negative (n = 4)	
	gp41 titer[a]	Fraction detectable[b]	gp41 titer[a]	Fraction detectable[b]
Archived[c]	3,976 ± 2,862	19/20 (95)	1,600 ± 1,286	3/4 (75)
Study[d]	17,148 ± 12,633	20/20 (100)	<100	0/4 (0)

[a] Geometric mean ± standard error of the mean.
[b] Number detected/total number (percent).
[c] By use of Mann-Whitney test (two-tailed) to compare titers and numbers detectable, P is 0.26 and 0.31, respectively.
[d] By use of Mann-Whitney test (two-tailed) to compare titers and numbers detectable, P is 0.002 and 0.00047, respectively.

HAART, and 80 (79%) subjects were taking HAART on the OraQuick study date.

OraQuick was reactive with archival sera from all four subjects who were HIV negative by OraQuick and from all 20 control subjects. Anti-gp41 was detectable in three of four archival specimens from the OraQuick false-negative subjects but in none of their study date specimens (Table 1). gp41 antibody was detectable in 19 of 20 control group archival specimens and all 20 study specimens. For the archived specimens, there was no statistically significant difference in anti-gp41 reactivity between the four OraQuick false-negative subjects and the 20 OraQuick-reactive control subjects (P = 0.31). For the study specimens, however, a significantly higher proportion of OraQuick false-negative specimens had undetectable anti-gp41 titers than did the 20 OraQuick-reactive control specimens (P = 0.00047). Both archived and study specimens from all 24 subjects in the case-control study were reactive by HIV-1 EIA and Western blotting.

The 20 control subjects and the 77 subjects whose specimens were OraQuick reactive and who were not included in the case-control study did not differ significantly by age, HIV-1 infection time, proportion on HAART, HAART duration, or CD4 count (data not shown). These characteristics were also similar for case and control subjects (Table 2). However, VL was undetectable (<50 copies/ml) at the time of the study in all four OraQuick false-negative subjects compared with 7 of 20 control subjects (P = 0.034). The OraQuick false-negative subjects had initiated HAART sooner after HIV diagnosis than had the 17 HAART-experienced OraQuick-reactive subjects (0.31 versus 3.52 years, P = 0.025). The OraQuick-negative subjects also had significantly higher HAART ratios (HAART duration divided by known duration of HIV infection) than did the OraQuick-reactive control subjects (0.92 versus 0.45, P = 0.002).

Western blot band intensity was significantly lower with study sera from the four OraQuick-negative subjects than with those of the 20 OraQuick-reactive control subjects. Furthermore, Western blot band intensity was inversely proportional to effective HAART exposure when the 101 HIV-infected subjects were stratified into four groups with ascending HAART exposure defined as follows: no HAART therapy ("never HAART"), HAART with detectable HIV VL ("HAART$^+$ detectable"), HAART with undetectable HIV VL ("HAART$^+$ undetectable"), and the four OraQuick-negative subjects (OraQuick false negative) (Table 3). Five of the 51 HAART$^+$ undetectable subjects had HAART ratios that equaled or exceeded the mean HAART ratio for the four OraQuick false-negative subjects.

OraQuick contains a synthetic gp41 peptide for HIV antibody detection. While OraQuick performed equally well with

TABLE 2. Characteristics of HIV-infected case subjects whose specimens were OraQuick false negative and control subjects whose specimens were OraQuick reactive[a]

Characteristic	OraQuick false negative (n = 4)	OraQuick reactive (n = 20)	P[b]
Age (yr)	31.7 ± 2.2	38.9 ± 9.8	0.16
Gender (% male)	75	85	0.54
Race (% white)	75	55	0.40
Seroconverters (%)[c]	75	60	1.000
Time since HIV Dx (yr)	3.08 ± 1.7	6.85 ± 3.99	0.081
CD4 count (cells/mm^3)	540 ± 171	450 ± 275	0.54
VL (copies/ml)	<50	623.9[d]	0.034
HAART			
Ever treated (%)	100	85	1.00
Duration (yr) (n = 17)	2.8 ± 1.3	1.9 ± 1.5	0.69
Duration/time positive (n = 17)	0.92 ± 0.084	0.45 ± 0.25	0.002
Time elapsed from Dx to HAART (yr) (n = 17)	0.31 ± 0.48	3.52 ± 3.30	0.025

[a] For age, time since HIV diagnosis (Dx), CD4 count, HAART duration, HAART duration/time positive, and time from diagnosis to HAART, values are shown as means ± standard deviations. For VL, values are shown as geometric means.
[b] The Fisher exact test (two-tailed) was used for treatment with HAART, gender, and seroconverters; the t test (two-tailed) was used for age, time since HIV diagnosis, CD4 count, HAART duration, and HAART duration/time positive; and the Mann-Whitney test (two-tailed) was used for race, VL, and time elapsed from diagnosis to HAART.
[c] Seroconverters were defined as patients with known prior negative HIV-1 serology.
[d] Median value, 199; interquartile range, 9,136.

TABLE 3. Comparison of Western blot band intensities for specimens of HIV-infected subjects, by OraQuick reactivity and exposure to effective HAART

Band	OraQuick reactivity			HAART exposure				
	Negative ($n = 4$)	Reactive ($n = 20$)	P^a	Never HAART ($n = 10$)	HAART$^+$ detectable ($n = 36$)	HAART$^+$ undetectable ($n = 51$)	OraQuick false negative ($n = 4$)	P^b
p17	0.38 ± 0.48	1.33 ± 0.82	0.033	1.65 ± 1.00	1.35 ± 0.75	1.22 ± 0.80	0.38 ± 0.48	0.056
p24	1.38 ± 1.11	2.50 ± 0.51	0.034	2.70 ± 0.48	2.47 ± 0.70	2.29 ± 0.76	1.38 ± 1.11	0.0632
gp41	0.63 ± 0.48	2.08 ± 0.77	0.0049	2.20 ± 0.63	2.12 ± 0.67	1.71 ± 0.68	0.63 ± 0.48	0.0003
gp120	1.25 ± 0.87	2.20 ± 0.70	0.044	1.80 ± 0.63	2.14 ± 0.64	1.86 ± 0.57	1.25 ± 0.87	0.0389
gp160	2.50 ± 1.00	3.00 ± 0.00	0.025	3.00 ± 0.00	2.94 ± 0.23	2.94 ± 0.31	2.50 ± 1.00	0.2328

[a] Mann-Whitney test (two-tailed).
[b] Kruskal-Wallis one-way analysis of variance.

OMT and serum, sensitivity was reduced with specimens from some HIV-infected subjects with heavy HAART exposure. Sera and OMT collected for the study from four HIV-infected subjects were not reactive by OraQuick, but archived sera were reactive. All four subjects had undetectable anti-gp41 levels on the date of the nonreactive OraQuick test and viral loads of <50 copies/ml. These data suggest that these subjects experienced anti-gp41 seroreversion as a consequence of early and prolonged suppression of viremia by HAART. Reduction in antibodies to HIV gp120, gp41, and p24 following HAART has been recognized elsewhere (4, 7, 9). Morris and colleagues suggested that reduction in HIV-1-specific antibody levels with HAART is due to a reduction of B-cell hyperresponsiveness (9). Kim and colleagues showed specific reductions in HIV-1-specific antibody responses to the gp41 immunodominant domain in chronically infected patients treated with HAART (7). Antibody levels promptly increase if viral suppression is not maintained (4, 7, 9). The data presented here suggest that diagnostic devices do not always perform comparably in HAART-naïve and HAART-treated individuals.

HAART-treated patients should be included in the evaluation of HIV-1 diagnostic tests. Such patients may present for testing being unable or unwilling to disclose their infection status. Clinical testing in support of Food and Drug Administration submissions for HIV diagnostic device approval must be performed on known HIV-seropositive and HIV-seronegative individuals with a significant proportion of seropositive subjects in resource-rich countries receiving HAART. Present data preclude an estimate of the number of HIV-1-seropositive individuals on HAART who present for testing without revealing their serostatus. HIV diagnostics based solely on gp41 reactivity may fail to detect HIV infection in HAART-treated patients treated early and effectively following diagnosis. OraQuick might optimally be used in parallel with an HIV diagnostic test based on different or multiple epitopes in which discordant test pairs are submitted to confirmatory testing when clinical suspicion of HIV infection is high.

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