Performance of an oral fluid rapid HIV-1/2 test: experience from four CDC studies

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Objective: To evaluate the performance of a rapid HIV antibody test used with whole blood and oral fluid in settings where the test is likely to be used.

Design: In four separate studies, we compared the accuracy of the rapid test performed on whole blood and oral fluid specimens with the results of conventional HIV tests.

Methods: Oral fluid and whole blood from persons of unknown HIV status recruited from clinics, labor and delivery units, and outreach venues were tested with the OraQuick Advance rapid HIV-1/2 antibody test. Sensitivity and specificity were compared with results of the enzyme immunoassay (EIA) and Western blot algorithm used by the study sites.

Results: OraQuick sensitivity was 99.7% with whole blood and 99.1% with oral fluid from 327 persons who were HIV antibody positive by the conventional algorithm. OraQuick specificity was 99.9% with whole blood and 99.6% with oral fluid from 12 010 HIV-negative persons; EIA specificity was 99.7%. A cluster of 16 false-positive oral fluid tests occurred in one study, in which specificity was lower (99.0%) than in the other three studies (99.6–99.8%).

Conclusions: In diverse settings in four studies, the OraQuick test showed high sensitivity and specificity for HIV antibody in whole blood and oral fluid specimens. Slightly more false-positive and false-negative results occurred with oral fluid than with whole blood, but performance with both specimen types was similar to, or better than, that of conventional EIAs.

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Introduction

In March 2004, the US Food and Drug Administration (FDA) approved the OraQuick rapid HIV-1 antibody test for detection of antibodies to HIV-1 in oral fluid [1]. (At the time of the studies reported here the device was called OraQuick HIV-1. On 22 June 2004 the FDA approved an additional indication for detection of HIV-2 in oral fluid,

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whole blood and plasma specimens and change of the name to OraQuick Advance. Website: http://www.fda.gov/cber/pma/p01004716.htm) Waived under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) [2], this test is intended as a point-of-care screening test for HIV antibodies when used with oral fluid, fingerstick and venous whole blood specimens. The test can be performed with 5 μl of whole blood or on an oral fluid specimen collected by swabbing the flat pad of the test device once around the outer surface of the upper and lower gums [3]. Results are read in 20–40 min. The manufacturer indicates a specificity of 99.8% [95% confidence interval (CI): 99.6–99.9] with oral fluid and 100% (95% CI: 99.7–100) with whole blood [3].

Previously, only one system for HIV testing of oral fluid had received FDA approval for use in the USA [4]. Conventional oral fluid testing follows the algorithm for testing blood [5]: specimens are screened by enzyme immunoassay (EIA); those that are repeatedly reactive are confirmed by Western blot. Results for EIA-reactive, Western blot-negative specimens are reported as HIV negative. The process of laboratory EIA/Western blot testing may take several days to weeks.

Oral fluid collection for HIV testing offers significant advantages in outreach settings: it is non-invasive, can be conducted almost anywhere, eliminates costs of phlebotomy training and equipment, and reduces biohazardous risks and waste. In 2000, at testing sites in the USA funded by the Centers for Disease Control and Prevention (CDC), nearly 30% of the 1.53 million tests were performed on oral fluid, most often in field outreach or other non-clinical settings [6]. Persons tested at these sites were more likely to test positive than persons at serum testing sites. However, approximately half of those tested failed to return for their test results [6]. Thus, an accurate oral fluid rapid HIV test could help persons at higher risk to both access testing and learn their test results on the same day.

Since 2000, CDC has sponsored four studies in which OraQuick whole blood and oral fluid testing was performed on the same individual in settings where the test is likely to be used. In this paper, we compare the accuracy of OraQuick testing with results of the conventional EIA and Western blot algorithm in these four studies.

Methods

Study settings and participants

One of these four studies (A), conducted between July 2003 and December 2004, included pregnant women undergoing HIV screening at 18 hospitals in six US cities as part of the Mother–Infant Rapid Intervention At Delivery (MIRIAD) Study [7,8]. The other three studies enrolled high-risk persons at (B) 41 community outreach sites in Minneapolis, Minnesota (MN) between July 2002 and August 2004, and three HIV testing sites and two sexually transmitted disease (STD) clinics in (C) Los Angeles, California (LA) between April 2000 and January 2005, and (D) Phoenix, Arizona between April 2001 and February 2003. In each of the four studies, all participants provided oral fluid and fingerstick or anticoagulated whole blood specimens for testing with OraQuick (on both oral fluid and whole blood.) At hospital sites, OraQuick was either performed in the laboratory by trained technicians or in labor and delivery by trained nurses, midwives and/or physicians [7]. At community outreach sites, OraQuick was performed by trained HIV counselors, most of whom did not have previous laboratory training. In Los Angeles and Phoenix, OraQuick was performed by trained technicians in onsite laboratories.

Gold standard comparison tests

The specimen type and EIAs and Western blots used as gold standard comparison tests varied by study. The LA, Phoenix and MN studies used the Vironostika HIV-1 Microelisa (bioMerieux Inc., Durham, North Carolina, USA), the EIA most commonly used in state public health labs in the USA [9]. Serum specimens repeatedly reactive by EIA and those from persons with reactive rapid tests were tested with Genetic Systems HIV-1 Western blot (Bio-Rad Laboratories, Inc., Hercules, California, USA) (LA and MN) or the Cambridge Biotech Western blot (Calypte Biomedical, Rockville, Maryland, USA) (Phoenix). In MIRIAD, FDA-approved EIA and Western blot tests were performed on blood specimens according to protocols already in place at participating hospitals; none used the Vironostika Microelisa [7]. In MN, the specimen collected for comparison testing was determined by the result of the fingerstick whole blood OraQuick test. For participants with negative fingerstick OraQuick results, oral fluid specimens were collected for testing with the Vironostika Microelisa approved for use on oral fluid, and if repeatedly reactive, by the Orasure Western blot (Orasure Technologies, Bethlehem, Pennsylvania, USA). For participants with reactive fingerstick OraQuick results, serum specimens were collected and tested with comparison tests as described above.

Quality assurance and quality control measurements

Staff in all four studies were trained to perform the test according to the instructions in the manufacturer's package insert [3]. Initial quality assurance guidance for these studies included the following: both a positive and negative external control was run each day testing was performed, and staff were asked to record the test lot number, daily temperatures in device storage and testing environments, and the times when the test was started and

read. The MN study continued to run positive and negative external quality control tests daily for the duration of the study. The other three studies ran external quality controls in accordance with the OraQuick package insert [3] and CDC quality assurance guidelines [10] when these were released in 2003.

Analyses

Only participants with valid results for both rapid and conventional tests were included in the analysis. Participants with indeterminate Western blot results were excluded from calculations of rapid test performance because true infection status could not be determined in this group. Sensitivity, specificity, and positive and negative predictive values were calculated based on the results of the EIAs and Western blots from each site using standard formulas. Exact 95% confidence intervals for these proportions were calculated [11]. For calculation of EIA specificity, persons with a non-reactive EIA, a reactive screening EIA that was non-reactive on repeat testing, or a negative Western blot result after a repeatedly reactive EIA were classified as uninfected. Differences in performance of tests on the same individuals were evaluated using McNemar's test. Differences in performance on the same specimen type across studies were evaluated using Fisher's exact test. All analyses were conducted using SAS system version 8.2 for Windows (SAS Institute Inc., Cary, North Carolina, USA).

Human subjects

All four studies were conducted under protocols approved and monitored by institutional review boards at CDC and at each participating site.

Results

Sensitivity

Of 12 343 participants, 6 with indeterminate Western blots whose infection status was unresolved were excluded from further analysis. EIA and Western blot confirmed 327 (2.7%) of the remaining 12 337 as HIV positive (Table 1). Of these, 326 tested positive by OraQuick with whole blood (sensitivity 99.7%) and 324 with oral fluid (sensitivity 99.1%, $P = 0.63$). Two of the three persons with false-negative oral fluid tests had reactive whole blood OraQuick tests. No Western blot positive person had a reactive oral fluid and non-reactive whole blood OraQuick test. Sensitivity of OraQuick on each specimen type varied slightly but not significantly across the four studies (Table 1).

Specificity

Overall

Of the 12 010 persons who tested negative by the reference algorithm, 11 975 were EIA negative and 35 were initially reactive by EIA but negative by the EIA/Western blot algorithm (EIA specificity 99.7%). For all studies combined, OraQuick specificity was 99.9% with whole blood and 99.6% with oral fluid ($P < 0.0001$) (Table 1). Nine (0.07%) participants were OraQuick false positive with both blood and oral fluid; 3 (0.02%) were false positive with only whole blood, and 45 (0.37%) were false positive with only oral fluid.

Minnesota study

Although specificity was slightly lower with oral fluid than with whole blood in all four studies, in the Minnesota study, oral fluid specificity (99.0%) was significantly lower ($P < 0.05$) than in any of the other three studies. In the first 2 years of the study, among 2017 HIV-negative persons, oral fluid test performance in the Minnesota study was similar to that observed in the other studies (specificity 99.7%, 95% CI: 99.3–99.8).

The decrease in observed specificity was attributed to 16 false-positive results, of which only 1 was also OraQuick false positive with whole blood, occurring in 388 HIV-negative persons tested (specificity 95.9%, 95% CI: 93.4–97.6) between April 2004 and the end of the study in August 2004.

Outreach workers performing the test recalled that, during this period, oral fluid tests with very faintly reactive results occurred which appeared qualitatively different from the usual weakly reactive OraQuick result, including some test lines that they described as gray or without color. However, the intensity and color of the test lines was not recorded during the study. Daily temperatures were recorded in both test and external control storage logs from 15 April 2004 through 31 August 2004, and in individual test logs completed at testing sites from 7 June 2004 through 31 August 2004. All temperatures were within the manufacturer's specifications for OraQuick at that time (2–27°C for storage and 15–27°C for testing.) All test devices run as part of external quality control gave the expected results. The false positives occurred with devices from six different test lots. The manufacturer reported that all implicated test lots were manufactured and shipped according to standard procedures and that all components met quality control specifications.

Positive and negative predictive value

OraQuick specificity with whole blood was 99.9% in all four prospective studies, but the observed positive predictive value of a reactive whole blood OraQuick test ranged from 81.8% among Minnesota participants (HIV prevalence 0.3%) to 98.6% among Los Angeles participants (HIV prevalence 5.1%) (Table 1). In all studies, the positive predictive value of OraQuick with oral fluid was lower than that observed with OraQuick screening of whole blood. Although estimates of the positive predictive value of oral fluid OraQuick and conventional EIA test results varied (Table 1) these

Table 1. Performance characteristics of the OraQuick Advance rapid HIV 1/2 test and enzyme immunoassay (EIA) in persons of unknown status, four US studies.

Study	Test and specimen	Reference positive	False negative	Reference negative	False positive	Sensitivity (95% CI)	Specificity (95% CI)	Prevalence (HIV infections/ 1000 tested)	Predictive value negative	Predictive value positive
(A) MIRIAD: pregnant women	Rapid test whole blood	16	0	2278	2	100.0% (79.4–100.0)	99.9% (99.7–100.0)	7/1000	100.0%	88.88%
	Rapid test oral fluid	16	0	2278	5	100.0% (79.4–100.0)	99.8% (99.5–99.9)		100.0%	76.19%
	Serum EIA			2278	7		99.7% (99.4–99.9)			69.57%
(B) Minnesota outreach	Rapid test whole blood	9	0	2405	2	100.0% (66.4–100.0)	99.9% (99.6–100.0)	3/1000	100.0%	81.82%
	Rapid test oral fluid	9	1	2405	23	88.9% (51.8–99.7)	99.0% (98.6–99.4)		99.96%	28.13%
	EIA[a]			2405	5		99.8% (99.5–99.9)			64.29%
(C) Los Angeles County rapid testing study	Rapid test whole blood	289	1	5327	4	99.7% (98.1–100.0)	99.9% (99.8–100.0)	51/1000	99.98%	98.63%
	Rapid test oral fluid	289	2	5327	21	99.3% (97.5–99.9)	99.6% (99.4–99.8)		99.96%	93.18%
	Serum EIA			5327	23		99.6% (99.4–99.7)			92.62%
(D) Arizona STD and HIV testing clinics	Rapid test whole blood	13	0	2000	3	100.0% (75.3–100.0)	99.9% (99.6–100)	6/1000	100.0%	81.25%
	Rapid test oral fluid	13	0	2000	5	100.0% (75.3–100.0)	99.8% (99.4–99.9)		100.0%	72.22%
	Serum EIA			2000	0		100% (99.9–100.0)			100.0%
Total	Rapid test whole blood	327	1	12 010	12	99.7% (98.3–100.0)	99.9% (99.8–100.0)	27/1000	99.99%	96.45%
	Rapid test oral fluid	327	3	12 010	54	99.1% (97.3–99.8)	99.6% (99.4–99.7)		99.97%	85.71%
	EIA[a]			12 010	35		99.7% (99.6–99.8)			90.33%

[a]The two persons who tested false positive on whole-blood OraQuick in the Minnesota study were tested with a serum specimen on the EIA; the remaining 2403 of 2405 reference negative EIA results were performed on an oral fluid specimen, including all EIA false positives. MIRIAD, Mother–Infant Rapid Intervention At Delivery Study; STD, sexually transmitted disease; CI, confidence interval.

differences were not significant (data not shown). The predictive value of a negative rapid test was >99.9% with whole blood and oral fluid in all four studies.

Discussion

In four separate studies, the OraQuick test demonstrated high sensitivity and specificity for HIV antibody with both whole blood and oral fluid specimens. Our findings are consistent with the clinical trial data reported by the manufacturer to the FDA [3] and other evaluations [12,13]. We also found that OraQuick sensitivity and specificity were lower with oral fluid than with whole blood. The negative predictive value was high in all four studies, and thus, counselors and clients can have confidence that a negative OraQuick test result, in the absence of a recent exposure to HIV, is conclusive.

The lower specificity with oral fluid is certain to have practical implications, especially in populations with low HIV prevalence. The 95% confidence intervals for specificity for the combined studies suggest that false-positive OraQuick tests can be expected to occur at a rate 2–6 times higher with oral fluid than with whole blood. In low-prevalence settings, this will reduce the positive predictive value considerably. Although we found that the positive predictive values for OraQuick with both oral fluid and whole blood were comparable to, or sometimes better than, that of conventional EIAs, unlike the EIA, clients receive the rapid test result at the point of care, before confirmation. Thus, counselors and clients must be aware of the limitations of reactive rapid HIV screening tests and the need for confirmation in accordance with current guidelines [14].

The consistent performance of OraQuick in real-world settings represented by these four studies is reassuring. Similar accuracy was achieved in different populations (pregnant women, high-risk persons with both high and low HIV prevalence) and by both laboratory technicians and persons with little or no prior experience with laboratory testing (hospital labor and delivery staff and trained HIV counselors).

However, we observed a cluster of false-positive oral fluid OraQuick tests in the Minnesota study. Although the causes for this and other reported clusters are currently unknown [15,16], possible operator errors such as over-collection of samples, which we did not assess in any of the four studies reported here, or differences in the interpretation of very faint or gray lines, which were interpreted as preliminary positive in Minnesota and not reported in any of the other three studies, may have played a part in these clusters [16]. Furthermore, in the Minnesota study, information on medical conditions, e.g. Epstein–Barr virus, hepatitis A or B infection, rheumatoid factor or multiparity, which may be associated with false-positive results [3], was not collected. Efforts to recontact clients to obtain this information after the study had ended were unsuccessful. Importantly, all clients with false-positive preliminary rapid HIV test results will be correctly classified as uninfected if CDC guidelines for confirmation are followed. Rapid test providers must also implement appropriate quality-assurance procedures to monitor operators and test performance, and to ensure that operators follow the manufacturer's instructions consistently [3,10]. Higher than expected numbers of false-positive OraQuick test results should be reported to the manufacturer, which is obligated to investigate and report such complaints to the FDA [15].

In these four studies, most serum and oral fluid specimens were screened with the Vironostika HIV-1 Microelisa, which uses a whole viral lysate substrate, and confirmed with Western blot. Seroconversion studies [3,17] and post-marketing surveillance [14] suggest that this EIA and the Western blot are less sensitive to early infection than some newer EIAs and rapid tests. Thus, it is possible that some clients classified as negative by the EIA used as the gold standard were actually infected with HIV. If such clients were missed by both the Vironostika and OraQuick tests, sensitivity estimates reported here would be artificially inflated. Conversely, misclassifying reactive OraQuick tests of truly infected persons as false positive would have biased the reported specificity downwards. Theoretically the latter form of misclassification could partially explain the cluster observed in the Minnesota study. However, none of the 327 Western blot positive persons identified in these studies had a reactive oral fluid and non-reactive whole blood OraQuick test, as was observed in 15 of the 16 false-positive oral fluid tests in the reported cluster. The sensitivity of the whole blood test suggests it is unlikely that false-negative whole blood OraQuick and Vironostika tests could explain the decrease in specificity observed in Minnesota, given the prevalence of HIV infection observed in that study. The problems of imperfect gold standards exist in all evaluations of antibody tests. In practice, discrepancies can only be resolved through follow-up testing. A limitation of these four studies is the lack of follow-up testing for persons with discordant (false-positive or false-negative) OraQuick results relative to these imperfect gold standards; for such clients their gold standard result was considered definitive.

A recently published randomized controlled trial which assessed client HIV testing preferences in two outreach settings [18] found that, compared with conventional tests, more clients accepted testing with oral fluid tests and rapid tests, and more clients tested with either of these alternatives received their test results. Because oral fluid OraQuick tests produce results almost immediately, most clients learn their HIV test result. With conventional oral fluid collection, nearly half of those who test

HIV-positive never receive their results [6]. Thus, using the OraQuick test with oral fluid in outreach settings where obtaining blood specimens is not feasible will help to identify more HIV-positive persons, even though its sensitivity is slightly lower with oral fluid than with whole blood [19]. In settings where blood is routinely available, the difference in specificity should be considered when deciding which specimen to use for OraQuick testing.

With adequate quality assurance, the CLIA-waived OraQuick Advance test produced accurate results with both oral fluid and whole blood. Such CLIA-waived, versatile tests offer the ability to provide HIV testing that is acceptable and useful in outreach settings for persons at high risk for acquiring HIV infection who may not learn their HIV status any other way. The convenience, safety and acceptability of non-invasive oral fluid collection, combined with nearly immediate rapid test results, suggest there will be a continuing demand for oral fluid rapid tests, even if they are slightly less sensitive and specific than whole blood or serum tests. Use of the OraQuick test, currently the only rapid test approved for oral fluid, will help identify more HIV-infected persons and link them to vital care and treatment services.

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