Post-marketing surveillance of OraQuick whole blood and oral fluid rapid HIV testing

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Objective: Post-marketing surveillance was conducted to monitor the performance of the OraQuick Advance rapid HIV-1/2 antibody test (OraQuick) on whole blood and oral fluid.

Design: Surveillance of routinely collected data on clients tested with OraQuick in 368 testing sites affiliated with 17 state and city health departments between 11 August 2004 and 30 June 2005.

Methods: For whole blood and oral fluid, we report the median (range) health department OraQuick specificity and positive predictive value (PPV), and the number of clients with discordant results (e.g. who had a reactive rapid test not confirmed positive by Western blot or indirect immunofluorescence). At one site with lower than expected oral-fluid specificity, we evaluated whether device expiration, manufacturing lot, operator practices, or device-storage or testing-area temperatures were associated with false-positive tests.

Results: During the surveillance period, 135 724 whole blood and 26 066 oral fluid rapid tests were conducted. The median health department whole blood OraQuick specificity was 99.98% (range: 99.73–100%) and PPV was 99.24% (range: 66.67–100%); the median oral fluid specificity was 99.89% (range: 99.44–100%) and PPV was 90.00% (range: 50.00–100%). A total of 124 discordant results were reported from 68 (0.05%) whole blood and 56 (0.22%) oral fluid rapid tests. The oral fluid specificity at the site with excess oral fluid false-positive tests was 98.7% (95% confidence interval: 98.18–99.11%). The increase in false-positive tests at that site was not associated with any specific device characteristic, operator procedure or temperature condition.

Conclusion: The specificity of OraQuick performed on whole blood and oral fluid during post-marketing surveillance was compatible with the manufacturer's claim within the package insert. However, one site experienced lower than expected oral fluid specificity. Sites that observe that the specificity of OraQuick is lower than the range indicated in the package insert should notify the manufacturer and evaluate quality assurance procedures.

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* See Appendix.

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Introduction

Approximately 24–27% of the 1 039 000–1 185 000 persons in the United States living with HIV do not know their serostatus [1]. The Advancing HIV Prevention initiative, introduced by the Centers for Disease Control and Prevention (CDC) in 2003, aims to reduce the number of HIV-infected persons who are unaware of their status [2]. As part of this initiative, CDC purchased and distributed OraQuick Advance rapid HIV-1/2 antibody tests (OraQuick) to health departments and community-based organizations, and conducted post-marketing surveillance to monitor the product's performance. OraQuick was the first rapid HIV test waived under the Clinical Laboratory Improvement Amendments of 1988 for use at point of care [3]. It can be used by trained operators in non-clinical settings on whole blood and oral fluid. The manufacturer's specificity claim for whole blood is 100% (95% confidence interval (CI): 99.7–100%) and 99.8% for oral fluid (95% CI: 99.6–99.9%) [4]. In this paper, we describe the whole blood and oral fluid specificity and positive predictive value of OraQuick at health departments that conducted surveillance, and the outcomes of an investigation at one site that reported excess oral fluid false-positive tests.

Methods

Design

Health departments in three cities (Chicago, New York City and San Francisco) and 14 states (Arizona, Delaware, Florida, Indiana, Louisiana, Massachusetts, Michigan, Montana, Nebraska, New Jersey, New York, North Carolina, Utah and Wisconsin) selected rapid test sites in their jurisdictions to participate in post-marketing surveillance. Whole blood and oral fluid OraQuick tests conducted at 368 sites between 11 August 2004 and 30 June 2005 were included in surveillance. Specimens from clients with reactive rapid tests were submitted to local laboratories for Western blot (WB) or indirect immunofluorescence (IFA) confirmation. Clients with discordant results (e.g. those who had a reactive rapid test not confirmed positive by WB or IFA) were asked to return for follow-up testing 4 weeks after their initial test [5]. Demographic data and HIV test results were entered into local health department data systems. Electronic data were submitted to CDC in accordance with variable maps designed to create one standard dataset.

Definitions

Non-reactive OraQuick tests were assumed to be true negatives. A true positive was defined as a reactive OraQuick test followed by a positive WB or IFA. Initially discordant results were reclassified as: (1) true positive if the follow-up WB or IFA was positive or the client had a detectable viral load; (2) false positive if the last follow-up confirmatory tests was negative or if no record of follow-up testing was obtained; and (3) indeterminate if the last WB or IFA was indeterminate. Specificity was defined as the number of negative tests divided by the sum of negative and false-positive tests. Positive predictive value (PPV) was defined as the number of true-positive tests divided by the sum of true-positive plus false-positive tests.

Analyses

For each health department, we report the number of participating sites and the following outcomes by specimen type: the number of negative and true-positive tests, percent seropositivity, number of false-positive tests, specificity and PPV. Exact confidence intervals were calculated for specificity using the Clopper–Pearson method [6]. The median (range) seropositivity, specificity and PPV for the 17 health departments were also calculated. Specificity was compared with the manufacturer's claim for whole blood and oral fluid [4]. Statistical comparisons were made using the chi-square test. All analyses were conducted using SAS (Statistical Application Software v9.1, SAS Institute Inc., Cary, North Carolina, USA).

Investigation of excess false-positive oral fluid tests

One site (site X) in San Francisco that conducted oral fluid tests from March 2005 to November 2005 reported an excess of false-positive tests starting in June 2005. Staff from the San Francisco Department of Public Health, California State Office of AIDS, CDC, and OraSure Technologies conducted an investigation to assess whether device expiration, manufacturing lot, operator practices, device-storage or testing-area temperatures might have been associated with these false-positive tests. As part of our investigation of excess false-positive tests, we report the seropositivity, specificity and PPV for site X by month. We also compare the specificity of oral fluid OraQuick at site X with 11 other rapid test sites in San Francisco from March to November 2005.

Human subjects

Surveillance activities and investigations were conducted as part of post-marketing surveillance determined at CDC to be non-research.

Results

Surveillance population characteristics

A total of 162 317 OraQuick tests were conducted at the 368 sites. Of these, 29.5% were conducted at counseling

and testing sites, 29.0% at sexually transmitted disease (STD) clinics, and 17.7% at correctional facilities; 63.8% were conducted on males, 50.0% on clients $\leq$29 years of age, and 41.7% on clients who were black, 39.8% white, 17.4% Hispanic, 2.1% Asian, 1.3% American Indian/Alaskan Natives, and 0.3% Native Hawaiian or other Pacific Islander. Of the OraQuick tests, 135 724 (83.6%) were conducted on whole blood, 26 066 (16.1%) on oral fluid, and 527 (0.3%) on an unrecorded specimen type.

Discordant results

A total of 124 initially discordant results were reported; 68 (0.05%) of 135 724 whole blood rapid tests, and 56 (0.22%) of 26 066 oral fluid rapid tests. Of the 68 discordant whole blood results, 13 (19.1%) were classified as true positive, 15 (22.1%) as indeterminate and 40 (58.8%) as false positive. Of the 56 discordant oral fluid results, 4 (7.1%) were classified as true positive, 5 (8.9%) as indeterminate and 47 (83.9%) as false positive. Of discordant results that were classified as false positive, a similar proportion of false-positive whole blood versus false-positive oral fluid results were based on $\geq$1 follow-up tests (52.5 versus 38.3%, $P = 0.18$).

Seropositivity, specificity, positive predictive value

The median HIV seropositivity was 0.83% (range among 17 health departments: 0.08–2.60%) among tests conducted using whole blood and 1.00% (range: 0–4.02%) among tests conducted using oral fluid. The median specificity was 99.98% (range: 99.73–100%) for whole blood and 99.89% (range: 99.44–100%) for oral fluid. The median PPV was 99.24% (range: 66.67–100%) for whole blood and 90.00% (range: 50.00–100%) for oral fluid. Point estimates for whole blood specificity at all health departments exceeded 99.7%, the lower bound of the 95% confidence interval of the manufacturer's specificity claim [4]. Point estimates for oral fluid specificity at all health departments except one (Arizona) exceeded 99.6% (Table 1). At this health department, the upper bound of the 95% CI for oral-fluid specificity overlaps the lower bound of the 95% CI of the manufacturer's claim (Table 1).

Investigation of excess oral fluid false-positive tests

Specificity and positive predictive value

At site X in San Francisco, of 2585 oral fluid OraQuick tests conducted, one was invalid and 33 (1.3%) were classified as false positive (11 of the 33 did not have follow-up testing). The oral fluid specificity at this site fell below the manufacturer's claim and was significantly lower than 11 other sites in San Francisco (Table 2). The number of false-positive oral fluid tests increased from July through October (Fig. 1). Because of staff concerns, the site discontinued use of oral fluid OraQuick on 16 November 2005.

Device characteristics

The 33 false-positive OraQuick tests at site X were from unexpired devices from four lots: 17 (51.5%) from one lot, 13 (39.4%) from a second, 2 (6.1%) from a third and 1 (3.0%) from a fourth. Each of these lots was used at other sites in San Francisco. Of the 33 devices with false-positive results, 29 (87.9%) had test lines (lines that denote a preliminary positive result) that were qualitatively described as 'gray', 'extremely faint' or 'shadowy'.

Operator practices

Seven primary operators at site X performed and interpreted the 33 false-positive OraQuick tests within 20–40 min. One operator read 14 (42.4%) devices, one read eight (24.2%), one read seven (21.2%) and four read one each (12.0%). In all 33 cases, the test line was read by more than one operator before the preliminary positive result was given to the client. Operator practices were observed to be in accordance with the package insert, with the exception of oral fluid specimen collection (some operators recommended that clients swab the upper and lower gum lines more than once with the OraQuick device). Operators were retrained on oral fluid specimen collection practices in October. Thirteen (39.4%) of the 33 false-positive results occurred after this retraining.

External controls and temperatures

From March through November 2005, staff performed 163 (6.3% of tests conducted) OraQuick external control runs at site X. Of these, two invalid results were observed; the remaining results were concordant with the negative or positive control. All temperatures recorded in storage and test logs during this period were within the manufacturer's specifications.

Discussion

From August 2004 through June 2005, surveillance of the OraQuick Advance rapid HIV-1/2 antibody test on over 150 000 clients in 17 health departments demonstrated that the device performed in accordance with manufacturer's specificity claim for use with both whole blood and oral fluid. Our findings support those from independent studies that found high OraQuick specificity for whole blood and oral fluid [7–9]. However, in accordance with the manufacturer's claim, we found that OraQuick specificity on oral fluid is slightly lower than that on whole blood [4]. Based on our observed median specificities, 11 false-positive results can be expected for every 10 000 oral fluid OraQuick tests performed on non-infected persons, and 2 false-positive results can be expected for every 10 000 whole blood OraQuick tests on non-infected persons.

Table 1. Specificity and positive predictive value (PPV) of the OraQuick Advance rapid HIV-1/2 antibody test, by specimen type; 17 health departments, 11 August 2004 to 30 June 2005.

Health department	Test sites (*n*)	Test negative (*n*)	True positive[a] [*n* (%)[b]]	False positive (*n*)	Specificity[c] [% (95%CI)]	PPV[d] (%)
Arizona	7					
Whole blood		3549	83 (2.3)	4	99.89 (99.71–99.97)	95.4
Oral fluid		532	12 (2.2)	3	99.44 (98.37–99.88)	80.0
Chicago	22					
Whole blood		3687	55 (1.5)	1	99.97 (99.85–100)	98.2
Oral fluid		2018	15 (0.7)	2	99.90 (99.64–99.99)	88.2
Delaware	5					
Whole blood		7364	50 (0.7)	0	100 (99.95–100)	100
Oral fluid		n/a	n/a	n/a	n/a	n/a
Florida	29					
Whole blood		24 635	651 (2.6)	5	99.98 (99.95–99.99)	99.2
Oral fluid		988	13 (1.3)	1	99.90 (99.44–100)	92.9
Indiana	6					
Whole blood		4868	37 (0.8)	1	99.98 (99.89–100)	97.4
Oral fluid		593	1 (0.2)	1	99.83 (99.50–100)	50.0
Louisiana	10					
Whole blood		11 784	179 (1.5)	0	100 (99.97–100)	100
Oral fluid		1595	43 (2.6)	3	99.81 (99.45–99.96)	93.5
Massachusetts	9					
Whole blood		3238	16 (0.5)	0	100 (99.89–100)	100
Oral fluid		n/a	n/a	n/a	n/a	n/a
Michigan	6					
Whole blood		9100	76 (0.8)	0	100 (99.96–100)	100
Oral fluid		72	1 (1.4)	0	100 (95.01–100)	100
Montana	10					
Whole blood		1274	1 (0.1)	0	100 (99.71–100)	100
Oral fluid		n/a	n/a	n/a	n/a	n/a
Nebraska	18					
Whole blood		702	12 (1.7)	0	100 (99.48–100)	100
Oral fluid		219	1 (0.5)	0	100 (98.33–100)	100
New Jersey	64					
Whole blood		20 707	392 (1.9)	12	99.94 (99.90–99.97)	97.0
Oral fluid		166	7 (4.0)	0	100 (97.80–100)	100
New York City	4					
Whole blood		13 673	199 (1.4)	7	99.95 (99.90–99.98)	96.6
Oral fluid		8248	92 (1.1)	11	99.87 (99.76–99.93)	89.3
New York State	130					
Whole blood		16 199	72 (0.4)	5	99.97 (99.93–99.99)	93.5
Oral fluid		5778	17 (0.3)	14	99.76 (99.60–99.87)	54.8
North Carolina	6					
Whole blood		363	2 (0.6)	1	99.73 (98.48–99.99)	66.7
Oral fluid		299	0 (0.0)	0	100 (98.77–100)	n/a
San Francisco	12					
Whole blood		3474	93 (2.6)	0	100 (99.89–100)	100
Oral fluid		2928	72 (2.4)	8	99.73 (99.46–99.88)	90.0
Utah	14					
Whole blood		2547	21 (0.8)	0	100 (99.86–100)	100
Oral fluid		1558	14 (0.9)	4	99.74 (99.35–99.93)	77.8
Wisconsin	16					
Whole blood		6334	52 (0.8)	4	99.94 (99.84–99.98)	92.9
Oral fluid		691	6 (0.9)	0	100 (99.47–100)	100

[a]Confirmed by indirect immunofluorescence or Western blot.
[b]% calculated as confirmed positives/rapid test negative plus rapid test positive (whether or not confirmed).
[c]Calculated as test negatives/test negatives plus false positives.
[d]Calculated as true positives/true positives plus false positives.

Given the low HIV seropositivity observed in some health department sites, we were not surprised to find some low positive predictive values. The lower PPV for oral fluid than whole blood tests at some health departments resulted primarily from fewer HIV-infected persons who tested with oral fluid rather than from lower specificity of the oral fluid test. These findings underscore the importance that all persons who have a reactive rapid HIV test need to be informed that the positive result is preliminary and that confirmatory testing is necessary [10]. When the confirmatory test following a preliminary positive rapid test is not positive, current guidelines for follow-up testing should be heeded [5,11].

Post-marketing surveillance was subject to several limitations. First, health departments and rapid test sites were not randomly selected and some sites may have been

Table 2. Comparison of specificity of the OraQuick Advance rapid HIV-1/2 antibody test used on oral fluid between site X and 11 other rapid HIV test sites, San Francisco, March 2005–November, 2005.

Test site	Test sites (*n*)	Test negative (*n*)	True positive[a] [*n* (%)[b]]	False positive (*n*)	Specificity[c] [% (95%CI)]	PPV[d] (%)
Site X	1	2512	42 (1.6)	33	98.70 (98.18–99.11)	56.0
All other sites	11	4272	118 (2.2)	14	99.67 (99.45–99.82)*	89.4

[a]Confirmed by indirect immunofluorescence or Western blot.
[b]% calculated as confirmed positives/rapid test negative plus rapid test positive (whether or not confirmed).
[c]Calculated as test negatives/test negatives plus false positives.
[d]PPV, positive predictive value; calculated as true positives/true positives plus false positives.
*Compared with site X, $P < 0.001$.

selected to participate because of their prior experience with rapid testing or higher quality test performance. If true, our specificity estimates might be overestimates. It is unlikely, however, that OraQuick performance at test sites included in surveillance was very different than at sites that were not included. Approximately half of the 162 317 rapid tests were conducted by five health departments that included all of their rapid test sites in post-marketing surveillance. Also, the specificity at these five health departments was comparable with all other health departments. Second, false-positive designations were based on the Western blot and IFA, which are older assays and which might be less sensitive in identifying persons with recent infection compared with newer generation rapid tests [12]. However, additional diagnostic tests performed on the 87 clients with false-positive results indicate that HIV was not present. Remnant sera from clients with false-positive results were tested at CDC from initial confirmatory specimens ($n = 55$) and from second confirmatory specimens ($n = 22$) and all tests were non-reactive by third-generation enzyme immunoassay. Additionally, all 32 nucleic acid amplification tests conducted on specimens from the 87 clients with false-positive results had undetectable viral loads. These results were concordant with Western blot and IFA results on these clients.

We observed lower oral-fluid OraQuick specificity at site X compared with other sites in San Francisco. We did not identify a clear cause for the lower specificity at this one site. Manufacturing error seems an unlikely cause since the lots used at this site were used at other

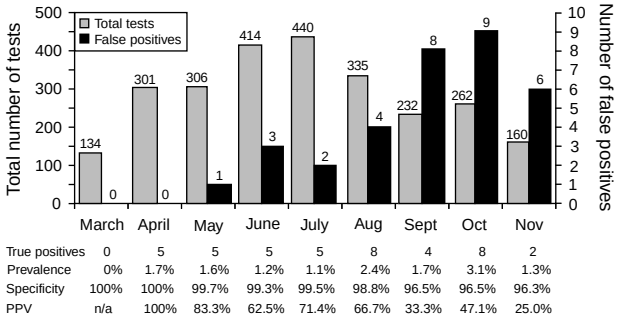

Fig. 1. OraQuick Advance rapid HIV-1/2 antibody oral fluid test summary, by month, site X, San Francisco, 16 March 2005 to 16 November 2005. PPV, positive predictive value.

sites in San Francisco where an increase in whole blood false-positive tests did not occur. Although it is possible that the observed lower specificity at site X was a random event due to chance alone, the increase in false positives is probably attributed to one or more unmeasured site-specific operator or host factors. These factors may be sufficiently rare that they are difficult to identify.

One or more operators may have routinely overcollected oral fluid specimens by instructing clients to swab around the gums more than once, rather than once as recommended [4]. Although unknown, it is plausible that overcollection of oral fluid might result in higher concentrations of antibodies that either cross-react or bind non-specifically with antigen in the test strip. Also, increased saliva might affect dilution or flow through the test device. However, over one-third of the false-positive tests at site X occurred after operators were retrained on oral fluid collection, and operators generally performed the tests correctly when observed. The reported increase in false-positive tests at site X might also be attributed to differences in interpreting test results. In other rapid test sites, gray lines may be unrecognized by staff or classified as non-reactive or invalid. The manufacturer should consider modifying the package insert to clarify how gray lines should be interpreted [4].

During post-marketing surveillance, whole blood and oral fluid OraQuick performed in the specificity range documented in the package insert. However, in one site, OraQuick specificity fell below this range during a specific time period. Operators who observe preliminary positive test results on whole blood or oral fluid specimens should continue to follow existing counseling and confirmatory testing guidelines [13]. If test providers observe an increase in false-positive OraQuick rapid HIV tests, they should notify the manufacturer, and assess adherence to package insert instructions and CDC quality assurance guidelines [4,11].

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Appendix: Post-marketing Surveillance Team

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