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Relative Accuracy of Serum, Whole Blood, and Oral Fluid HIV Tests among Seattle Men Who Have Sex with Men

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Abstract

Background—Point-of-care (POC) rapid HIV tests have sensitivity during the "window period" comparable only to earliest generation EIAs. To date, it is unclear whether any POC test performs significantly better than others.

Objective—Compare abilities of POC tests to detect early infection in real time.

Study Design—Men who have sex with men (MSM) were recruited into a prospective, cross-sectional study at two HIV testing sites and a research clinic. Procedures compared four POC tests: one performed on oral fluids and three on fingerstick whole blood specimens. Specimens from participants with negative POC results were tested by EIA and pooled nucleic acid amplification testing (NAAT). McNemar's exact tests compared numbers of HIV-infected participants detected.

Results—Between February 2010 and May 2013, 104 men tested HIV-positive during 2479 visits. Eighty-two participants had concordant reactive POC results, 3 participants had concordant non-reactive POC tests but reactive EIAs, and 8 participants had acute infection. Of 12 participants with discordant POC results, OraQuick ADVANCE Rapid HIV-1/2 Antibody Test performed on oral fluids identified fewer infections than OraQuick performed on fingerstick (p=.005), Uni-Gold Recombigen HIV Test (p=.01), and Determine HIV-1/2 Ag/Ab Combo (p=.005).

Conclusions—These data confirm that oral fluid POC testing detects fewer infections than other methods and is best reserved for circumstances precluding fingerstick or venipuncture. Regardless of specimen type, POC tests failed to identify many HIV-infected MSM in Seattle. In populations

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with high HIV incidence, the currently approved POC antibody tests are inadequate unless supplemented with p24 antigen tests or NAAT.

Keywords

HIV testing; rapid HIV test; oral fluids

Introduction

In 2002, the OraQuick ADVANCE Rapid HIV-1/2 Antibody Test (OraQuick, OraSure Technologies Inc.) became the first CLIA-waived rapid HIV test approved by the Food and Drug Administration (FDA). The following year, Public Health – Seattle & King County (PHSKC) began to offer rapid testing to men who have sex with men (MSM) to provide point-of-care (POC) results and pooled nucleic acid amplification testing (NAAT) [1] to identify highly infectious antibody-negative individuals with acute HIV infection [2, 3]. Since 2003, OraQuick has identified approximately 80% of HIV-infected MSM testing through PHSKC [4]. When serum specimens from MSM with negative POC tests were tested by an enzyme immunoassay (EIA) prior to pooling, about one-third of HIV-infected men had detectable antibodies [4], including some detected by a 1st generation EIA [5] with a “window period” of six weeks or more [6].

One advantage attributed to POC tests over laboratory-based testing is that more persons receive test results [7, 8], although this may or may not translate into greater likelihood of linkage to HIV care [9, 10]. One disadvantage of currently FDA-approved POC tests is that they have sensitivity during the “window period” comparable to the earliest generation EIAs [11-14]. To date, it has been unclear whether any FDA-approved POC test performs substantially better than other POC tests; retrospective testing of frozen specimens has produced conflicting results [4, 14-16]. However, evidence from various settings and populations suggests that POC testing on oral fluid specimens has lower sensitivity [17-19] and specificity [20] compared to fingerstick whole blood specimens.

In 2010, we began a prospective, cross-sectional study to compare the ability of different HIV tests, all performed on fresh specimens from the same individuals, to detect acute and early infection in real-time. We previously reported that Seattle MSM, a population with high incidence and frequent HIV testing, prefer less invasive specimen collection methods but have greater trust in results of tests performed on fingerstick and venipuncture specimens [21]. Here, we report HIV test results from this study.

Methods

Population

Men and transgender persons reporting sex with men in the prior year were recruited when seeking HIV testing at the PHSKC STD Clinic, Gay City Health Project (a community-based organization), or University of Washington Primary Infection Clinic (PIC, a research clinic). At the STD Clinic, a full-time research staff member attempted to test all MSM seeking only HIV testing or those referred by clinicians for rapid testing. At Gay City, all counselors participated, and the study was offered primarily to men considered to be at higher risk for HIV acquisition; this included men with symptoms of acute infection, who reported sex with an HIV-infected partner, or who had a condom break or had no recollection of events during or after a sexual exposure. Finally, to enrich the analysis with persons with early infection, study enrollment was offered to persons referred to the PIC [22] for suspected or confirmed diagnosis of acute HIV infection.

The University of Washington Institutional Review Board approved this study, and all participants gave verbal consent. Participants received $20 in compensation and could participate quarterly.

HIV Testing

Study procedures included one POC test performed on oral fluids (OraQuick) and three POC tests performed on fingerstick whole blood specimens: OraQuick (5μL), Uni-Gold Recombigen HIV Test (Uni-Gold, Trinity Biotech, 50μL), and Determine HIV-1/2 Ag/Ab Combo (Determine Combo, Alere Inc., 50μL). (Determine Combo was not FDA-approved or available for sale in the United States at the time of this study; the manufacturer provided devices for investigational use ten months after study enrollment began, with occasional interruptions in supply.) We selected these devices because OraQuick is widely used for testing on oral fluids, Uni-Gold has been suggested to be more sensitive during early infection [15, 23], and Determine Combo is designed to detect HIV-1/HIV-2 antibody and HIV-1 p24 antigen. Each test was performed on a separate fingerstick specimen. Participants with any reactive fingerstick test result were linked to HIV care.

At the STD Clinic and Gay City, participants with negative POC results had serum specimens sent for EIA and pooled NAAT as previously described [24]. Specimens from PIC participants were tested using the GS HIV-1/HIV-2 Plus O antibody EIA (Bio-Rad) until May 2011 and the ARCHITECT HIV Ag/Ab Combo assay (ARCHITECT, Abbott Diagnostics Division) thereafter; HIV RNA testing was performed on all individual plasma specimens using the Abbott RealTime HIV-1 RNA assay (Abbott Molecular Inc).

Data Analysis

Methods for maintaining and retrieving study results varied for each site. At the STD Clinic, HIV test results were entered into electronic medical records and retrieved via clinic database. At Gay City, a tally was kept of participants with reactive POC test results. Chart reviews were conducted for participants with discordant test results at these sites. At the PIC, results for this project were regularly abstracted from research charts, combined with data obtained via the PIC research database, de-identified, and maintained in a spreadsheet.

Participants with a reactive EIA and positive Western blot or detectable HIV RNA were considered to have confirmed infection. Estimates of sensitivity and specificity were generated for STD Clinic participants, as these participants were more likely to be representative of HIV-negative MSM seeking HIV testing. McNemar's exact tests were used to compare the numbers of participants detected by the different tests at all sites. Analyses were performed using Stata12 software (StataCorp LP, College Station, TX).

Results

Between February 22, 2010 and May 1, 2013, there were 2479 visits by 2456 men, 17 transgender women, and six transgender men. Minority representation paralleled the race/ethnicity of clients at these sites [25].

One hundred and four participants were newly diagnosed with HIV-1 infection (Table 1). The overall proportion testing positive was similar at the STD Clinic and Gay City (3.3% versus 3.4%, p=.9). Eighty-two HIV-infected participants had concordantly reactive POC test results. Three participants had non-reactive results on all POC tests (including Determine Combo for one participant) but a reactive EIA. Eight participants were acutely infected (antibody-negative/NAAT-positive); one of these participants, tested after Determine Combo was incorporated into the study, had a reactive p24 antigen result. Eleven other HIV-infected participants had at least one reactive and one non-reactive POC test

result (Table 2). OraQuick performed on oral fluid detected significantly fewer HIV-infected persons compared to OraQuick performed on fingerstick whole blood (p=.005), Uni-Gold (p=.01), and Determine Combo (p=.005). There were no significant differences identified between OraQuick performed on fingerstick whole blood compared to Uni-Gold (p=1.0) or to Determine Combo (p=.5) or between Uni-Gold and Determine Combo (p=.5).

Among 53 persons newly diagnosed with HIV at the STD Clinic, EIA identified 46 of 52 (88.5%, 95% CI 76.6-95.6%), OraQuick performed on oral fluids identified 42 (79.2%, 95% CI 65.9-89.1%), OraQuick performed on fingerstick specimens identified 44 (83.0%, 95% CI 70.2-91.9%), Uni-Gold identified 43 (81.1%, 95% CI 68.0-90.6%), and Determine Combo identified 22 of 25 tested (88.0%, 95% CI 68.8-97.4%). Seven (0.4%) of 1562 HIV-negative participants screened at the STD Clinic had false-positive test results on a single test; no participant had false-positive results on more than one test. False-positive results were obtained for two participants tested by OraQuick performed on oral fluids (specificity 99.9%), two participants on the Determine Combo antigen and one on the antibody (specificity 99.7%), and two by EIA (specificity 99.9%). There were no false positive tests with OraQuick performed on fingerstick specimens or Uni-Gold.

Discussion

These data reinforce and further illuminate our previously published data showing that POC tests fail to diagnose many HIV-infected MSM with early infection and confirm that testing performed on oral fluids is less accurate than testing on fingerstick whole blood. MSM familiar with HIV testing prefer oral fluid as a specimen collection method, but they have less trust that tests performed on oral fluid provide accurate results [21]. Together, our data support the 2008 observation from the Centers for Disease Control and Prevention that “testing with blood or serum specimens is more accurate than testing with oral fluid and is preferred when feasible, especially in settings where blood specimens already are obtained routinely.” [20] To date we have not yet identified any POC test performed on fingerstick whole blood that is significantly better than others in detecting early infection.

Our finding that HIV tests performed on oral fluids are less accurate than tests performed on fingerstick whole blood specimens is consistent with the OraQuick package insert and previously published studies [17-19]. However, the magnitude of difference seen in our study was greater because HIV incidence is high in Seattle MSM and because the comparator was NAAT. This difference in ability to detect early infection has potential public health consequences. For example, in a deterministic model where HIV-negative MSM in Seattle replaced clinic-based HIV NAAT with home, self-testing using oral fluids, HIV prevalence was predicted to increase from 19% to 28% because of transmissions that could have been averted by earlier detection [26]. This model was extremely sensitive to changes in the “window period” duration because of the high infectiousness of persons with undetected recent infection [3]. It is therefore critical for programs that utilize oral fluid tests, whether at home or in a clinical setting, to ensure that such testing does not substitute for regular, blood-based testing.

Our study had several limitations. Point estimates for less-sensitive POC tests are likely overestimated because tests were not performed independently, and faint bands were read as reactive in the context of other reactive tests. Although study participants had higher rates of HIV infection than previously reported at the STD Clinic [24], our sensitivity results are likely generalizable to populations with high HIV incidence and frequent testing, factors producing a high likelihood of testing during early infection. However, our findings may not be generalizable to populations with lower incidence or less frequent testing. Finally, although we have not identified a POC test that is more accurate than others, this study will

have greater power in the future as it continues with more participants and a different array of tests.

In conclusion, our findings suggest that programs that offer POC HIV rapid tests for high-risk populations should use fingerstick specimens. Our recommendation to avoid HIV tests on oral fluids must be balanced against concerns that some people might not otherwise accept testing, as persons who have never tested often express preferences for oral fluid specimens [27]. Ideally, programs that perform POC tests should offer back-up testing, e.g., either pooled NAAT or antigen-antibody combination assays [4, 28, 29], to reconcile the potentially conflicting goals of satisfying client preferences for same-day results while facilitating the earliest detection of HIV infections. With the focus on “test-and-treat” as HIV prevention and findings that the 20% of persons unaware of their infection transmit half of new HIV infections [30], we cannot fail to diagnose 20% of the HIV-infections present in persons at the time they seek testing, our care, and expertise.

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Table 1

Distribution of point-of-care and laboratory HIV test results among participants, Seattle, 2010-2013

	STD Clinic n=1615	Gay City n = 842	PIC n=22	Total n=2479
HIV-negative	1562[1]	813	0	2375
Total HIV Positive	53 (3.3%)	29 (3.4%)	22	104
Concordant Positive POC Tests	43 (81%)	26 (90%)	13	82
Discordant POC Antibody Tests	3 (6%)	0	8	11
All POC Tests Negative/EIA-Positive	1 (2%)	2 (7%)	0	3
Acute (EIA-Negative / NAAT-Positive)	6[2] (11%)	1 (3%)	1[3]	8

POC: point-of-care; EIA: enzyme immunoassay; NAAT: nucleic acid amplification test

[1] Includes one participant with reactive EIA, indeterminate Western blot, and negative NAAT

[2] Includes one participant with positive p24 antigen of two participants screened by Determine Combo

[3] This participant had a negative Determine Combo, reactive ARCHITECT, negative Multispot HIV-1/HIV-2 Rapid Test and Western blot, and HIV RNA of 33,000 copies/mL.

Table 2

Results of HIV-infected Participants with Discordant Point-of-Care HIV Test Results, Seattle, 2010-2013

	OraQuick OF	OraQuick FS	Uni-Gold	Determine Ag Ab		3rd or 4th gen EIA[1]		Western blot band pattern	HIV RNA (copies/mL)
1	–	–	–	+	–	3rd	–	negative	5.7 million
2	+	+	–	ND		3rd	+	24, 31, 40, 55, 120	141,000
3	–	+	+	ND		3rd	+	24, 31, 40, 55, 160	128,000
4	–	+	+	ND		3rd	+	18, 24, 31, 40, 51, 55, 120, 160	25,000
5	–	–	+	ND		3rd	+	24, 51, 55, 160	12.8 million
6	–	–	+	–	+	3rd	+	24, 40, 55, 160	21,000
7	–	+	–	–	+	4th	Ab+	24, 51, 55	719,000
8	–	+	+	–	+	4th	Ab+	24, 31, 55, 160	436,000
9	–	+	+	–	+	4th	Ab+	24, 55, 160	33,000
10	–	+	+	–	+	4th	Ab+	24, 55, 160	9000
11	–	+	+	–	+	4th	Ab+	18, 24, 55, 160	32,000
12	–	+	+	–	+	4th	Ab+	24, 160	94,000
		p=0.005[2]	p=0.01[2]	p=0.005[2]					

OF: oral fluids; FS: fingerstick; Ag: antigen; Ab: antibody; EIA: enzyme immunoassay; ND: Not done

[1]The ARCHITECT assay was considered to be reactive for antibody if the Multispot HIV-1/HIV-2 Rapid Test, performed for confirmatory testing, was reactive.

[2]Compared to OraQuick performed on oral fluid