Analysis of False Negative HIV Tests Based on Oral Fluid in Three Clinical Trials

Marcel E. Curlin[1,2], Michael Martin[1,2], Wanna Leelawiwat[1], Roman Gvetadze[2], Charles E. Rose[2], Sarika Pattanasin[1], Richard W. Niska[2], Timothy H. Holtz[1,2], Kachit Choopanya[3], Janet M. McNicholl[2]

[1]Thailand MOPH – U.S. CDC Collaboration, Nonthaburi, Thailand; [2]Centers for Disease Control and Prevention, Atlanta, GA, USA; [3]Bangkok Tenofovir Study Group, Bangkok, Thailand

Background

- HIV continues to be a major global public health concern. There are more than 39 million infected lives so far[1]
- 95% of the HIV infected people worldwide remain unaware of their infection status[1]
- Only 5-10% of people in many countries have ever had an HIV test[1]
- Low rate of HIV testing drives the rapid growing of HIV epidemic
- Promoting HIV diagnosis improves access to HIV care and prevention services
- A rapid point-of-care HIV test offers several advantages:
  - Simplicity, ease of implementation
  - Minimal invasiveness
  - Low equipment requirments
  - Test results typically available within 30 minutes
- OraQuick Advance Rapid HIV1/2 Ab[2] test:
  - A non-invasive rapid HIV test capable of detecting HIV-Ab in blood and oral fluid (OF)
  - U.S. FDA approved rapid HIV test for clinic and home used
  - High test performance is reported in the package insert
    - Sensitivity: 99.3% (98.4%-99.9%)
    - Specificity: 99.8% (9.6%- 99.9%)
- However, test performance may be affected by several factors:
  - HIV prevalence in the population
  - stage of illness
  - use of antiretroviral agents
  - test operators
  - other factors
- In this study, we examined oral fluid OraQuick (OF OQ) performance among all seroconvertors participating in three longitudinal clinical studies

Methods

Study population

- TDF2[3]
  - A randomized double-blinded placebo-controlled clinical trial of tenofovir-emtricitabine (TDF-FTC) PrEP to reduce HIV incidence
  - 1219 HIV-negative heterosexual men and women in Francistown and Gaborone, Botswana were enrolled between Feb 2007-Oct 2009
  - 36 participants HIV seroconverted during follow up (median follow up for all participants was 1.1 years, max 3.7 years)
  - Of 36, three cases were determined to have been infected at enrollment
- Bangkok Tenofovir Study[4] (BTS)
  - A randomized double-blinded placebo-controlled clinical trial of daily oral TDF PrEP to reduce HIV incidence
  - 2413 men and women HIV-negative PWID in Bangkok, Thailand were enrolled between Jun 2005-Jul 2010
  - 53 participants HIV seroconverted during follow up (mean follow up for all participants was 4.0 years, max 6.9 years)
  - Of 53, two cases were determined to have been infected at enrollment, and one case was lost to followup before confirmation
- The Bangkok MSM Cohort Study[5] (BMCS)
  - An ongoing cohort study in MSM to investigate the epidemiology of HIV and other sexually transmitted diseases, and HIV prevention methods among Thai MSM
  - 1371 HIV-negative MSM in Bangkok, Thailand were enrolled between Apr 2006-Nov 2010
  - As of Jan 2012, 221 participants HIV seroconverted during follow up (mean follow up for all participants was 2.9 years, max 5.4 years)
  - Of 221 seroconverted, 206 cases were determined by OF OQ (15 were identified by NAAT) and 201 had available samples for this study

Testing scheme of parental study

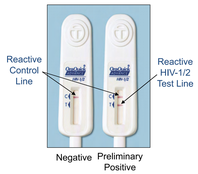
- TDF2
  - At enrollment
    - Screening for HIV infection using a combination of either OraQuick ADVANCE® Rapid HIV-1/2 Antibody Test (OraSure Technologies, Bethlehem, PA, USA) or Uni-Gold™ Recombigen® HIV Test (Trinity Biotech PLC Bray, Co, Wicklow, Ireland), and Determine® HIV-1/2 Test (Alere, Chiba, Japan)
  - At follow up (monthly visits)
    - HIV screening in oral fluid using OraQuick ADVANCE® Rapid HIV-1/2 Antibody Test
    - Blood collected and stored every four months and at exit
    - At exit, 5% of participants with negative OF OQ randomly tested for HIV status using EIA
  - At seroconversion
    - If OF OQ reactive, confirmed with EIA (Biorad, Genetic Systems, Redmond, WA, USA) and viral load (VL) on blood (Cobas Amplicor Monitor v1.5, Roche Molecular Systems, Branchburg, NJ, USA)
    - Retrospective testing with EIA and VL to determine the first HIV positive date
- BTS
  - At enrollment and follow up (monthly visits)
    - HIV screening in oral fluid using OraQuick Rapid HIV-1/2 Antibody Test (Test identical to OraQuick ADVANCE but manufactured for distribution outside the United States)
    - Blood for plasma samples collected and stored quarterly and at exit
    - At exit, all participants with negative OF OQ tested for HIV infection using EIA and NAAT (Aptima, Genprobe, San Diego, CA, US)
  - At seroconversion
    - If OF OQ reactive, confirmed with EIA and Western blot (Biorad, Genetic Systems, Redmond, WA, USA) and VL tests on blood (Amplicor Monitor v1.5, Roche Molecular Systems, Branchburg, NJ)
    - Samples retrospectively tested by EIA and VL (Roche Cobas TaqMan) to determine the first HIV positive date
- BMCS
  - At enrollment and follow up (every 4 months)
    - Oral fluid (OF) screening for HIV infection using OraQuick Rapid HIV-1/2 Antibody Test (Test is identical to OraQuick ADVANCE but is manufactured for distribution outside the United States)
    - Blood for plasma samples were collected and stored annually
    - From Feb 2010 onward, all participants with negative OF OQ were tested for acute HIV infection using 4[th] generation EIA (Asxym, Abbott, Wiesbaden, Germany) and NAAT (Aptima, Genprobe, San Diego, CA, US)
  - At seroconversion
    - If OF rapid test reactive, confirmed with blood sample using three rapid tests; either Determine, DoubleCheck (Orgenics, Israel) or SD Bioline (Standard Diagnostics, Korea) and Capillus (Trinity Biotech, USA) or HIV Core (Core Diagnostic, UK
    - VL determined on blood samples (Roche Cobas Taqman)
    - Samples retrospectively tested using EIA and VL to determine the first HIV positive date

Oral fluid OraQuick (OF OQ) test

- Detects anti-HIV (gp41 and gp36) IgG antibodies in oral fluid
- Simple two step procedure
- Results available within 20-40 minutes
- OF OQ tests in all three studies were performed by well-trained staff at research sites. Annual competency tests were done to maintain staff competence

1. Swab upper and lower gums once each with flat pad of test device

2. Insert device in developer vial. Read result between 20 and 40 minutes



Definition of variables

- True Negative (TN) or False Negative (FN): Negative OF OQ results with reference to a gold standard of EIA/NAAT results
- True Positive (TP) or False Positive (FP): Positive OF OQ results with reference to a gold standard of EIA/NAAT results
- Estimated date of infection: Midpoint between last negative and first positive identified by EIA/NAAT
- OF OQ Delay time: Time between estimated date of infection and estimated date of OF OQ conversion

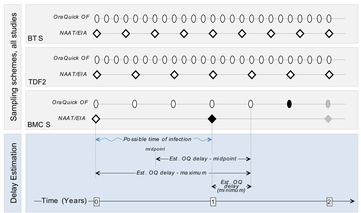


Figure 1. Testing schemes in BTS, TDF2 and BMCS studies (upper panels), and schematic showing calculation of the estimated OF OQ conversion delay time (bottom panel) in a hypothetical BMCS seroconverter. OF OQ tests are represented by ovals, EIA/NAAT tests are represented by diamonds. Open and filled symbols represent nonreactive and reactive test results, respectively. In this example, a hypothetical BMCS seroconverter was noted to be HIV-infected by OF OQ at 20 months (black oval), and look-back testing in blood revealed the estimated time of infection to be 6 months by the midpoint method (midpoint between open and solid diamonds).

Statistical analysis

- The relationships between primary outcome (proportion of FN) and potential predictors were analyzed using generalized estimating equations (GEE)
- Repeated HIV viral load measures were analyzed using a linear mixed-effects regression model and accounting for lower and upper detection limits
- Statistical hypotheses tests were interpreted at the 0.05 level of significance

OF OQ-related descriptive characteristics

Variables	TDF2 FN test		BTS FN test		BMCS FN test		Combined FN test	
	Yes	No	Yes	No	Yes	No	Yes	No
Subjects (%)	12 (34%)	23 (66%)	32 (60%)	21 (40%)	37 (18%)	184 (82%)	81 (28%)	208 (72%)
Median age (range)	24 (22-36)	26 (21-29)	30 (20-50)	27 (20-53)	24 (19-48)	24 (18-42)	28 (20-51)	26 (18-57)
Treatment received TDF Placebo	4 (33%) 8 (67%)	4 (17%) 19 (83%)	11 (34%) 21 (66%)	6 (29%) 15 (71%)	NA	NA	15 (34%) 29 (66%)	10 (23%) 34 (77%)
HIV subtype B/BE CRF01_AE C Nontypeable	0 (0%) 0 (0%) 12 (34%) 0 (0%)	0 (0%) 0 (0%) 23 (66%) 0 (0%)	4 (12.5%) 24 (75%) 0 (0%) 4 (12.5%)	2 (9.5%) 19 (90.5%) 0 (0%) 0 (0%)	6 (16%) 24 (65%) 0 (0%) 7 (19%)	26 (16%) 122 (74%) 0 (0%) 16 (10%)	10 (12%) 48 (60%) 12 (15%) 11 (14%)	28 (13.5%) 141 (68%) 23 (11%) 16 (8%)
# Tests found	34 (12%)	258 (88%)	123 (16%)	635 (84%)	53 (5%)	966 (95%)	210 (10%)	1859 (90%)
# Sites	1 (50%)	1 (50%)	13 (87%)	2 (13%)	NA	NA	14 (82%)	3 (18%)
# Operators	5 (38.5%)	8 (61.5%)	23 (62%)	14 (38%)	2 (15%)	11 (85%)	30 (48%)	33 (52%)
Median delay time, day (range)	93 (35-307)	NA	125 (14-547)	NA	48 (32-302)	NA	98 (14-547)	NA
# Test lot found	5 (21%)	19 (79%)	48 (73%)	18 (27%)	4 (7%)	53 (93%)	57 (39%)	90 (61%)
Mean viral load, cpm (95% CI)	24,801 (9,808-62,710)	19,337 (9,754-38,334)	16,657 (6,629-41,657)	33,102 (13,933-78,642)	46,770 (17,828-122,698)	52,009 (36,808-73,488)	24,409 (14,064-42,361)	42,978 (31,642-58,374)

Results

- TDF2
  - The median OF OQ delay time was 93 days (range 35-307 days)
  - The occurrence of FN tests were significantly associated with
    - Test site, site 001 (prevalence ratio = 23.68, 95%CI; 3.93-142.63)
    - Not proficient operator (prevalence ratio = 4.41, 95%CI; 1.75-11.13)
    - Test kit in excess of 100 days prior to expiration date (prevalence ratio = 3.41, 95%CI 1.65-7.04)
  - Gender, treatment arm and operator workload were not significant factors
- BTS
  - The median OF OQ delay time was 125 days (range 14-547 days)
  - The occurrence of FN tests were significantly associated with
    - Received TDF treatment (prevalence ratio = 2.29, 95%CI; 1.1-4.75)
    - Test kit in excess of 100 days prior to expiration date (prevalence ratio = 2.36, 95%CI 1.29-4.3)
  - Gender, age, operator workload and HIV subtype were not significant factors
- BMCS
  - The median OF OQ delay time was 66 days (range 32-302 days)
  - The occurrence of FN tests were significantly associated with
    - Operator age < 35 years (prevalence ratio = 0.37, 95%CI; 0.18-0.74)
    - Test kit in excess of 100 days prior to expiration date (prevalence ratio = 4.34, 95%CI 2.11-8.92)
  - Gender, participant age, operator workload and HIV subtype were not significant factors
- Analyses of other factors in correlation with OF OQ FN
  - The occurrence of OF OQ FN was not associated with the absence of anti-gp41 in blood
  - There was a trend towards lower viral load in the group with longer delay time (> 180 days)
  - Test kit lot was not associated with FN test
  - The sensitivity of the OF OQ on a per-person basis in these three research settings was 72%

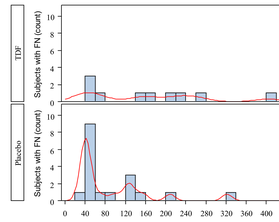


Figure 2. Distribution of estimated OF OQ conversion delay times in the BTS study. TDF recipients (top panel) and Placebo recipients (bottom panel) are shown. Estimated delay time calculated using the midpoint method.

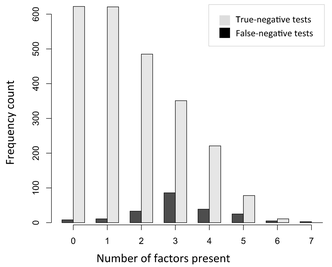


Figure 3. Frequency of factors significantly associated with a FN OF OQ tests. Plot showing count (Y-axis) of all OF OQ tests performed in 290 seroconverters, grouped by the number of contributing factors present (X-axis), from among 6 factors significantly associated with a FN OF OQ test. Factors: test operator, clinic, ARV exposure, time to kit expiration, time within 90 days of infection, test prior to 1st gp41(+), test performed before 1st EIA(+). FN tests and TN tests shown as black and grey bars, respectively. Some FN tests have no associated factors; conversely, some true-negative tests have 5-6 factors, indicating that factors identified in this series are contributory but not deterministic of a FN result.

Discussion

- In all, 81 of 290 HIV-infected participants had one or more false-negative OF OQ tests.
- False-negative OF OQ tests were frequently observed in this cohort during longitudinal follow-up.
- Some OF OQ conversion delays were very long, exceeding 500 days
- Most FN test results occurred after the appearance of HIV-specific antibodies (EIA) or gp41-specific antibodies (Western blot) in blood.
- Factors significantly associated with false-negative tests in one or more studies included:
  - test location
  - test operator
  - use of test kits in excess of 100 days prior to kit expiration date (paradoxical)
  - randomization to oral TDF prophylaxis
  - low plasma viral load
  - operator age significant in TDF2 and BTS, but in opposite directions
- Factors not associated with FN tests included:
  - participant age
  - participant gender
  - operator workload
- FN test results may be due to lower antibody titers or delayed appearance of antibodies in gingival crevicular fluid in comparison with blood[6-9]
- Other studies show that negative tests are more likely during late infection or during long-term suppressive therapy where HIV specific antibody titers wane[10-11]
- Delayed Oraquick conversion is multifactorial. Few FN tests had no factors. However, many true-negative tests had one or more factors.
- Caution must be exercised when interpreting a negative OF OQ test, particularly under circumstance where
  - acute infection is likely
  - PrEP has been used for HIV prevention
  - ARV has been widely used for viral suppression
- Use of HIV rapid test by non-laboratory personnel in the clinical trial should be implemented with caution, appropriate measures should be taken to ensure proper training and ongoing quality assurance

References

1. UNAIDS/WHO: HIV/AIDS update 2014
2. OraSure Technologies Inc. OraQuick Advance Rapid HIV-1/2 Antibody Test [package insert]. In. Bethlehem, Pennsylvania; 2004
3. Thigpen MC, Kebaabetswe PM, Paxton LA, Smith DK, Rose CE, Segolodi TM, et al. Antiretroviral preexposure prophylaxis for heterosexual HIV transmission in Botswana. The New England journal of medicine. 2012;367(5):423-34. Epub 2012/07/13.
4. Choopanya K, Martin M, Suntharasamai P, Sangkum U, Mock PA, Leethochawalit M, et al. Antiretroviral prophylaxis for HIV infection in injecting drug users in Bangkok, Thailand (the Bangkok Tenofovir Study): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet. 2013;381(9883):2083-90. Epub 2013/06/19.
5. van Griensven F, Thienkrua W, McNicholl J, Wimonsate W, Chaikummao S, Chonwattana W, et al. Evidence of an explosive epidemic of HIV infection in a cohort of men who have sex with men in Thailand. AIDS (London, England). 2013;27(5):825-32. Epub 2012/11/22.
6. Scott LE, Noble LD, Langeveldt M, Jentsch U, Francois Venter WD, Stevens W. Can oral fluid testing be used to replace blood-based HIV rapid testing to improve access to diagnosis in South Africa? Journal of acquired immune deficiency syndromes (1999). 2009;51(5):646-8; author reply 8-9. Epub 2009/07/25.
7. Luo W, Masciotra S, Delaney KP, Chanurat M, Croxton T, Constantine N, et al. Comparison of HIV oral fluid and plasma antibody results during early infection in a longitudinal Nigerian cohort. J Clin Virol. 2013;58 Suppl 1:e113-8. Epub 2013/12/18.
8. Granade TC, Phillips SK, Parekh B, Gomez P, Kitson-Piggott W, Oleander H, et al. Detection of antibodies to human immunodeficiency virus type 1 in oral fluids: a large-scale evaluation of immunoassay performance. Clinical and diagnostic laboratory immunology. 1998;5(2):171-5. Epub 1998/04/01.
9. Soto-Ramirez LE, Hernandez-Gomez L, Sifuentes-Osornio J, Barriga-Angulo G, Duarte de Lima D, Lopez-Portillo M, et al. Detection of specific antibodies in gingival crevicular transudate by enzyme-linked immunosorbent assay for diagnosis of human immunodeficiency virus type 1 infection. J Clin Microbiol. 1992;30(11):2780-3. Epub 1992/11/01
10. O'Connell RJ, Merritt TM, Malia JA, VanCott TC, Dolan MJ, Zahwa H, et al. 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. J Clin Microbiol. 2003;41(5):2153-5. Epub 2003/05/08
11. Merchant M, Wright M, Kabat W, Yogev R. Long-term highly suppressed HIV-infected children and adolescents with negative rapid HIV tests due to significant antibody loss. J Clin Virol. 2014;59(3):172-6. Epub 2014/01/21

Acknowledgements

- Additional contributors included: Dr. Michele Owen, Dr. Taraz Samandari, Pairote Tararut, Jaray Tongtoyai, Wannee Chonwattana and Dr. Yen Duong
- We gratefully acknowledge the many volunteers who participated in the TDF2, BTS and BMCS studies
- We thank the study staff, clinicians and investigators responsible for the conduct of these three studies
- We are grateful for assistance from the Bangkok Metropolitan Administration, the Thai Ministry of Public Health and the Botswana Public Health Authoritye among all seroconvertors participating in three longitudinal clinical studies