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Failure to Identify HIV-Infected Individuals in a Clinical Trial Using a Single HIV Rapid Test for Screening

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Abstract

Background—In the HIV Prevention Trials Network (HPTN) 061 study, 8 (2.3%) of 348 HIV-infected participants identified as HIV uninfected at study enrollment using a single HIV rapid test for screening were found to be HIV infected after additional testing.

Objectives—To evaluate the performance of different HIV assays for detection of HIV infection in HPTN 061 participants with missed infection and individuals with viral suppression.

Methods—Plasma samples from 8 HPTN 061 participants, 17 elite controllers, and 101 individuals on antiretroviral treatment (ART) were tested for HIV with 3 rapid tests, 2 laboratory-

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based immunoassays, and a Western blot assay. The HPTN 061 samples were also tested with 2 HIV RNA assays and an antiretroviral drug assay.

Results—Of the 8 HPTN 061 participants with missed infection, 1 was an elite controller, 1 was taking ART, 2 were missed because of testing or clerical errors, 1 had recent HIV infection (identified using a multi-assay algorithm), and 3 had acute HIV infection. Two (1.7%) of 118 individuals with viral suppression (both taking ART) had at least 1 false-negative test.

Conclusions—In clinical trials, HIV infections can be missed for a variety of reasons. Using more than one assay to screen for HIV infection may reduce the number of missed infections.

Keywords

antiretroviral therapy; elite controller; HIV; rapid test; viral suppression

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HIV rapid tests are commonly used to screen for HIV infection in clinical, community, and research settings. False-negative HIV rapid test results have been observed in some studies during the early stages of HIV infection.[1,2] For example, one study evaluated the performance of 4 US Food and Drug Administration (FDA)–approved HIV rapid tests. In that study, most individuals who had detectable HIV RNA with a negative or indeterminate Western blot had nonreactive rapid test results.[1] False-negative HIV rapid test results have also been observed in individuals with advanced disease[3] and individuals receiving antiretroviral treatment (ART).[4,5] In one study, 3 of 6 FDA-approved HIV rapid tests had at least one false-negative test result when samples from individuals on ART were analyzed.[5] Failure to identify HIV-infected individuals in clinical trials can confound study outcomes and can put those individuals at risk if the study includes an intervention, such as provision of antiretroviral drugs for pre-exposure prophylaxis (PrEP).[6] Although HIV testing is not usually performed for individuals with a prior HIV diagnosis, it may occur in clinical settings or clinical trials to confirm self-reported HIV status or to determine HIV status in individuals who are aware of their HIV infection but choose not to disclose this information.[7–9]

The HIV Prevention Trials Network (HPTN) 061 study (NCT00951249) assessed the feasibility and acceptability of a multicomponent intervention for HIV prevention among Black men who have sex with men (MSM) in the United States.[10,11] The study enrolled 1,553 men, including HIV-uninfected men, HIV-infected men who reported no prior HIV diagnosis, HIV-infected men who reported that they were HIV infected but not in care, and a limited number of HIV-infected men who were in care. Study participants were screened for HIV infection at enrollment at study sites using a single HIV rapid antibody test; testing was repeated 6 and 12 months after enrollment.[10,11] Plasma samples were sent to a centralized laboratory for retrospective quality assurance testing. This retrospective HIV testing identified 8 HIV-infected men who had nonreactive HIV rapid tests among the 1,500 men who had HIV rapid testing performed at study enrollment. In this report, we analyzed samples from those 8 men to understand why their infections were missed. We also evaluated the impact of viral suppression on the performance of HIV screening assays by testing samples from a cohort of elite controllers and from HIV-infected adults on ART.

Methods

Samples Used for Analysis

Plasma samples were obtained from the 8 HPTN 061 participants described above, 17 elite controllers who were virally suppressed in the absence of ART (EC group),[12,13] and 101 individuals who were virally suppressed from ART (ART group).[14] HIV infection was diagnosed a median of 12 years prior to sample collection (interquartile range [IQR], 5–17 years) in the EC group and a median of 8 years prior to sample collection (IQR, 4–13 years) in the ART group. In the ART group, individuals were virally suppressed from ART for a median of 1.6 years prior to sample collection (IQR, 266 days to 6 years). Additional information for the EC and ART groups is provided in Table 1.

Laboratory Testing

Real-time HIV rapid testing was performed at HPTN 061 study sites with venous blood using the OraQuick Advance HIV-1/2 Antibody Test (OraSure Technologies, Bethlehem, PA). Retrospective testing of HPTN 061 samples and testing of samples from the EC and ART groups was performed at a centralized laboratory (HPTN Laboratory Center, Johns Hopkins University, Baltimore, MD) using 3 HIV rapid tests (OraQuick test; Uni-Gold Recombigen HIV Test, Trinity Biotech, Wicklow, Ireland; and INSTI Rapid HIV Test, BioLytical Laboratories, Richmond, BC, Canada). The samples were also analyzed using a third-generation enzyme immunoassay (EIA, VITROS Anti-HIV 1+2 Test, Ortho Clinical Diagnostics, Rochester, NY), a fourth-generation chemiluminescent microparticle immunoassay (CMIA) (ARCHITECT HIV Ag/Ab Combo Assay [List 2P36], Abbott Laboratories, Wiesbaden, Germany), and a Western blot assay (Genetics System HIV-1 Western Blot, Bio-Rad Laboratories, Redmond, WA). Two samples from the ART group did not have sufficient volume to perform all assays. In addition, samples from HPTN 061 participants were tested with 2 HIV RNA assays: (1) either the AMPLICOR HIV-1 Ultrasensitive MONITOR Test, version 1.5 (14 samples) or the COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, v2.0 (8 samples; Roche Molecular Diagnostics, Indianapolis, IN), and (2) the APTIMA HIV-1 RNA Qualitative Assay (Gen-Probe Inc., San Diego, CA). These assays have different window periods for HIV detection.[15] Samples from HPTN 061 participants were also screened for the presence of antiretroviral (ARV) drugs, including 9 protease inhibitors (PIs), 4 nucleoside/nucleotide reverse transcriptase inhibitors, and 2 non-nucleoside reverse transcriptase inhibitors (NNRTIs).[16] Detection of an NNRTI or PI was considered to be indicative of ART. The individuals who performed testing at the centralized laboratory were blinded to the HIV status of the HPTN 061 participants.

Ethical Approval

Written informed consent was obtained for participation in the HPTN 061 study. The study was approved by the institutional review boards at the participating institutions. Individuals in the EC and ART groups provided written informed consent for use of their samples in research studies.

Results

Test results obtained for participants in HPTN 061 are shown in Table 2 (cases 1–8). At enrollment, these participants reported that they were not aware of their HIV status.

Case 1

In this case, the OraQuick test was nonreactive at all 3 study visits (both at study sites and at the centralized laboratory). The UniGold HIV rapid test and the fourth-generation Combo CMIA were also nonreactive at all 3 visits. The INSTI rapid test had a weak reactive result at enrollment, but was nonreactive at both follow-up visits. In contrast, the third-generation Vitros EIA was reactive and the Western blot was positive at all 3 study visits. At all 3 visits, HIV RNA was undetectable using both the ultrasensitive Roche viral load assay and the APTIMA HIV RNA qualitative assay, which is cleared by the FDA for diagnosis of HIV infection. No ARV drugs were detected in the samples, indicating that the participant was most likely an elite controller who may have been unaware of his HIV status.

Case 2

In this case, the OraQuick HIV rapid test was nonreactive at all 3 study visits at the study site, but had weak positive test results when testing was performed at the centralized lab. At all 3 visits, the other 2 HIV rapid tests and both the third-generation EIA and the fourth-generation Combo CMIA were reactive and the Western blot was positive. At all 3 visits, HIV RNA was undetectable using both assays and ARV drugs were detected, indicating that this participant was virally suppressed due to ART.

Cases 3 and 4

In these cases, the only nonreactive test was observed with the OraQuick HIV rapid test at the study site at enrollment. The false-negative test result was likely due to a testing or clerical error. In one case, ARV drugs were detected at all 3 visits, indicating that this participant was on ART; however, HIV RNA was detected at all 3 study visits, indicating inadequate viral suppression.

Case 5

In this case, the OraQuick and UniGold rapid tests were nonreactive at enrollment. The INSTI rapid test and both the third-generation EIA and fourth-generation Combo CMIA were reactive, and the Western blots were positive at enrollment and 6 months. This participant had a very high viral load at enrollment (>750,000 copies/mL) and was determined to be recently infected using a multi-assay algorithm.[17] All of the HIV screening tests, including the HIV rapid test performed at the site, were reactive at the 6-month study visit. No ARV drugs were detected at the first 2 visits. At the 12-month visit, ARV drugs were detected, indicating that the participant was taking ART.

Case 6

In this case, the OraQuick, UniGold, and INSTI rapid tests were nonreactive at enrollment; the third-generation EIA and the fourth-generation Combo CMIA were reactive and the

Western blot was indeterminate. HIV RNA was detectable and no ARV drugs were detected. At the 6-month visit, all of the HIV screening tests were reactive and the Western blot was positive. There was insufficient plasma available from the 12-month visit to complete the planned analyses. These test results were consistent with acute HIV infection at enrollment.

Case 7

In this case, the OraQuick and UniGold rapid tests were nonreactive at enrollment. A weak band was observed with INSTI rapid test. The third-generation EIA and fourth-generation Combo CMIA were reactive and the Western blot was indeterminate. HIV RNA was detectable and no ARV drugs were detected. At the 6- and 12-month visits, all of the HIV screening tests were reactive and the Western blot was positive. These test results were consistent with acute HIV infection at enrollment.

Case 8

In this case, all of the HIV screening assays were nonreactive at enrollment; a Western blot was not performed. HIV RNA was detectable and no ARV drugs were detected. At the 6-month visits, all of the HIV screening tests were reactive and the Western blot was positive. However, at the 12-month visit, ARV drugs were detected, indicating that the participant was on ART. There was insufficient plasma available from the 12-month visit to complete all of the other analyses. These test results were consistent with acute HIV infection at enrollment.

In summary, of the 8 HPTN 061 participants whose HIV infection was missed at the enrollment visit using the OraQuick rapid test, 1 was an elite controller, 1 was on ART, 2 were missed because of testing or clerical errors, 1 had recent HIV infection (identified using a multi-assay algorithm), and 3 had acute HIV infection.

Results from the EC and ART groups are summarized in Table 3. Samples from all 17 individuals in the EC group and samples from 99 (98.0%) of the 101 individuals in the ART group had reactive results using all 5 HIV screening assays. In the ART group, one sample was nonreactive with the OraQuick HIV rapid test and one sample was nonreactive with the UniGold HIV rapid test. In these adults with viral suppression, sensitivity was 99.2% for the OraQuick HIV rapid test and the Uni-Gold HIV rapid test and was 100% for the INSTI rapid HIV test, the third-generation EIA, and the fourth-generation Combo CMIA (Table 3). Overall, 2 (1.7%) of 118 samples from individuals with known viral suppression (EC and ART groups) had a false-negative HIV rapid test with one of the HIV screening tests.

Discussion

This study reports 8 cases where HIV rapid tests failed to detect HIV infection in a clinical trial. These cases were identified by retrospective quality assurance testing, which included screening samples from all of the study participants at the enrollment visit using the fourth-generation HIV Combo test. In 4 cases, the participants had acute or recent HIV infection at study enrollment. In 2 cases, HIV infection was missed due to a testing or clerical error. In these 6 cases, the participants were identified as HIV-infected at the study sites at the 6-

month follow-up visit. These 6 participants were initially classified as new incident cases (ie, cases of HIV seroconversion); based on the results of quality assurance testing, these participants were re-classified as HIV-infected at study enrollment.

This study also demonstrates the importance of quality assurance testing for determining HIV status in clinical trial settings. In the remaining 2 cases, HIV infection was likely missed because of viral suppression. One of these individuals was most likely an elite controller and the other was virally suppressed from ART; nondisclosure of ART was common in this cohort.[8] In these 2 cases, HIV infection was missed at the study site at all 3 study visits (enrollment, 6 months, and 12 months). In one case, false-negative test results were also obtained when samples from the follow-up visits were tested at the centralized laboratory using the same assay; in the other case, weak positive results were obtained at the centralized laboratory. HIV-2 infections may not be detected by some assays; the tests used in this study do not discriminate between HIV-1 and HIV-2.

The results from this report suggest that it may be important to consider use of 2 screening tests for detection of HIV infection in some settings (eg, 2 rapid tests, or a rapid test with an EIA or CMIA). Ideally, one of these would be a fourth-generation HIV test.[18] This testing strategy may be particularly important in clinical trials where ARV drugs are provided to HIV-uninfected individuals for PrEP, because inadvertent use of PrEP in HIV-infected individuals can induce drug resistance.[6] We do note that 5 of the 8 HPTN 061 participants analyzed in this study had HIV infection that was missed by more than one screening test.

To our knowledge, this is the first report evaluating the performance of HIV rapid tests in elite controllers. None of the 17 elite controllers from a well-characterized study cohort had false-negative HIV tests. However, one of the HPTN 061 participants with missed HIV infection was likely to be an elite controller who may have been unaware of his HIV status. In contrast, 2 (2%) of 101 individuals with viral suppression from ART had a false-negative HIV test in this study, and one of the HPTN 061 participants with missed HIV infection was virally suppressed and taking ART. Manufacturers of some HIV rapid tests alert users to the possibility that false-negative test results may be obtained in individuals who are virally suppressed. For example, the package inserts for the OraQuick Advance HIV-1/2 Antibody test and the INSTI Rapid HIV test note that some individuals on ART may have false-negative test results.[19,20] Because HIV-infected individuals with natural or ARV-induced viral suppression have low levels of circulating virus, HIV infection may still be missed using a fourth-generation (antigen/antibody) HIV screening test. In this study, 2 of the 8 HPTN 061 participants with missed HIV infection also had false-negative test results using the fourth-generation Combo test (cases 1 and 8); one of these participants had acute HIV infection with a viral load of 3,031 copies/mL, which is below the estimated antigen sensitivity limit of the assay,[21] and the other was virally suppressed from ART. The samples in this study were obtained from individuals who were likely to be infected with HIV subtype B. Further studies are needed to compare the sensitivity of HIV screening assays in adults with natural and ARV-induced viral suppression. Such studies could include a larger number of elite controllers, as well as virally suppressed individuals in settings outside of the US where nonsubtype B HIV is prevalent.

Conclusion

HIV infection may be missed when a single screening test is used for HIV diagnosis. In research settings, this could have implications for study outcomes and participant safety. Viral suppression (natural or ART-induced) may increase the probability that HIV infection will be missed, even if multiple HIV screening tests are used.

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References

1. Patel P, Bennett B, Sullivan T, Parker MM, Heffelfinger JD, Sullivan PS. Rapid HIV screening: Missed opportunities for HIV diagnosis and prevention. J Clin Virol. 2012; 54(1):42–47. [PubMed: 22381919]
2. Louie B, Wong E, Klausner JD, et al. Assessment of rapid tests for detection of human immunodeficiency virus-specific antibodies in recently infected individuals. J Clin Microbiol. 2008; 46(4):1494–1497. [PubMed: 18234875]
3. Brown P, Merline JR, Levine D, Minces LR. Repeatedly false-negative rapid HIV test results in a patient with undiagnosed advanced AIDS. Ann Intern Med. 2008; 149(1):71–72. [PubMed: 18591648]
4. O'Connell RJ, Merritt TM, Malia JA, 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):2153–2155. [PubMed: 12734265]
5. Delaney KP, Branson BM, Uniyal A, et al. Evaluation of the performance characteristics of 6 rapid HIV antibody tests. Clin Infect Dis. 2011; 52(2):257–263. [PubMed: 21288853]
6. Grant RM, Lama JR, Anderson PL, et al. Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. N Engl J Med. 2010; 363(27):2587–2599. [PubMed: 21091279]
7. Sullivan AK, Savage EJ, Lowndes CM, et al. Non-disclosure of HIV status in UK sexual health clinics–a pilot study to identify non-disclosure within a national unlinked anonymous seroprevalence survey. Sex Transm Infect. 2013; 89(2):120–121. [PubMed: 23408314]
8. Marzinke MA, Clarke W, Wang L, et al. Non-disclosure of HIV status in a clinical trial setting: Antiretroviral drug screening can help distinguish between newly-diagnosed and previously-diagnosed HIV infection. Clin Infect Dis. 2014; 58(1):117–120. [PubMed: 24092804]
9. Fogel JM, Wang L, Parsons TL, et al. Undisclosed antiretroviral drug use in a multi-national clinical trial (HPTN 052). J Infect Dis. 2013; 208(10):1624–1628. [PubMed: 23908493]
10. Koblin BA, Mayer KH, Eshleman SH, et al. Correlates of HIV acquisition in a cohort of black men who have sex with men in the United States: HIV Prevention Trials Network (HPTN) 061. PLoS One. 2013; 8(7):e70413. [PubMed: 23922989]
11. Mayer KH, Wang L, Koblin B, et al. Concomitant socioeconomic, behavioral, and biological factors associated with the disproportionate HIV infection burden among black men who have sex with men in 6 U.S. cities. PLoS One. 2014; 9(1):e87298. [PubMed: 24498067]

12. Han Y, Lai J, Barditch-Crovo P, et al. The role of protective HCP5 and HLA-C associated polymorphisms in the control of HIV-1 replication in a subset of elite suppressors. AIDS. 2008; 22(4):541–544. [PubMed: 18301071]
13. Wendel SK, Mullis CE, Eshleman SH, et al. Effect of natural and ARV-induced viral suppression and viral breakthrough on anti-HIV antibody proportion and avidity in patients with HIV-1 subtype B infection. PLoS One. 2013; 8(2):e55525. [PubMed: 23437058]
14. Moore RD. Understanding the clinical and economic outcomes of HIV therapy: The Johns Hopkins HIV clinical practice cohort. J Acquir Immune Defi c Syndr Hum Retro-virol. 1998; 17(Suppl 1):S38–41.
15. Centers for Disease Control and Prevention. [Accessed January 23, 2014] HIV/AIDS Prevention. Advantages and disadvantages of different types of FDA-approved HIV immunoassays used for screening by generation and platform. http://www.cdc.gov/hiv/pdf/testing_Advantages&Disadvantages.pdf
16. Marzinke MA, Breaud A, Parsons TL, et al. The development and validation of a full scan-high resolution accurate mass spectrometric (HRMS) screening method for the qualitative monitoring of antiretroviral agents in human blood. Manuscript submitted for publication.
17. Laeyendecker O, Brookmeyer R, Cousins MM, et al. HIV incidence determination in the United States: A multiassay approach. J Infect Dis. 2013; 207(2):232–239. [PubMed: 23129760]
18. Centers for Disease Control and Prevention. Detection of acute HIV infection in two evaluations of a new HIV diagnostic testing algorithm - United States, 2011–2013. MMWR Morb Mortal Wkly Rep. 2013; 62(24):489–494. [PubMed: 23784012]
19. US Food and Drug Administration. [Accessed January 18, 2013] INSTI HIV-1 Antibody Test Kit [package insert]. http://www.fda.gov/downloads/BiologicsBloodVaccines/BloodBloodProducts/Approved-Products/PremarketApprovalsPMAs/UCM235249.pdf
20. US Food and Drug Administration. [Accessed January 18, 2013] OraQuick Advance Rapid HIV-1/2 Antibody Test [package insert]. http://www.fda.gov/downloads/BiologicsBloodVaccines/BloodBlood-Products/ApprovedProducts/PremarketApprovalsPMAs/ucm091917.pdf
21. Brennan CA, Yamaguchi J, Vallari A, Swanson P, Hackett J Jr. ARCHITECT HIV Ag/Ab Combo assay: Correlation of HIV-1 p24 antigen sensitivity and RNA viral load using genetically diverse virus isolates. J Clin Virol. 2013; 57(2):169–172. [PubMed: 23485348]

Table 1

Characteristics of virally suppressed study subjects

Group	*n*	Median age at testing (IQR), years	Gender (% male)	Race (% African American)	On ART	Mean CD4 cell count (IQR), cells/mm^3	Mean HIV viral load, copies/mL
EC	17	56 (53–58)	52.9	100.0	No	817 (732–1,141)	<50
ART	101	50 (42–54)	65.3	88.1	Yes	618 (276–2,258)	<50

Note: ART = antiretroviral treatment; ART group = individuals with long-term viral suppression on ART; EC group = elite controllers; IQR = interquartile range.

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Table 2

Test results for eight HIV-infected men in HPTN 061 who had false-negative rapid tests at enrollment

Case no.	Visit	OraQuick (rapid) Real-time[a]	OraQuick (rapid)	UniGold (rapid)	INSTI (rapid)	COMBO (4th gen)	Vitros (3rd gen)	WB	Viral load[b]	Qual RNA	ARV drugs	Comment
1	Enr	**NR**	**NR**	**NR**	R[c]	**NR**	R	P	<50	**NR**	None	Likely elite controller
	6 mo	**NR**	**NR**	**NR**	**NR**	**NR**	R	P	<50	**NR**	None	
	12 mo	**NR**	**NR**	**NR**	**NR**	**NR**	R	P	<50	**NR**	None	
2	Enr	**NR**	R[c]	R	R	R	R	P	<50	**NR**	EFV, FTC, TFV[d]	On ART
	6 mo	**NR**	R[c]	R	R	R	R	P	<50	**NR**	EFV, FTC, TFV[d]	
	12 mo	**NR**	R[c]	R	R	R	R	P	<50	**NR**	EFV, FTC, TFV[d]	
3	Enr	**NR**	R	R	R	R	R	P	135	R	DRV, RTV	Testing/clerical error
	6 mo	R	R	R	R	R	R	P	110	R	DRV	
	12 mo	R	R	R			R	P	4,190	R	DRV, RTV	
4	Enr	**NR**	R	R	R	R	R	P	21,152	R	None	Testing/clerical error
	6 mo	R	R	R	R	R	R	P	18,890	R	None	
	12 mo		R	R	R	R			15,610	R	None	
5	Enr	**NR**	**NR**	**NR**	R	R	R	P	>750,000	R	None	Recently infected[e]
	6 mo	R	R	R	R	R	R	P	1,624	R	None	
	12 mo		R	R	R	R			<20	**NR**	EFV, FTC, TFV[d]	
6	Enr	**NR**	**NR**	**NR**	**NR**	R	R	IND	39,590	R	None	Acutely infected
	6 mo	R	R	R	R	R	R	P	11,962	R	None	
	12 mo									R		
7	Enr	**NR**	**NR**	**NR**	R[c]	R	R	IND	>750,000	R	None	Acutely infected
	6 mo	R	R	R	R	R	R	P	11,762	R	None	
	12 mo		R	R	R	R			75,478	R	None	
8	Enr	**NR**	**NR**	**NR**	**NR**	**NR**	**NR**		3,031[f]	R	None	Acutely infected
	6 mo	R	R	R	R	R	R	P	53,148	R	None	
	12 mo		R	R	R	R			34	R	DRV, RTV, FTC TFV	

Note: The HIV tests used in this study have different window periods for detecting HIV infection.[15] HIV RNA tests can detect HIV infection as early as 1 week after infection. Fourth-generation (4th gen) tests, which detect both HIV antigen and anti-HIV antibodies, have window periods of approximately 2 to 3 weeks. Third-generation (3rd gen) rapid tests and enzyme immunoassays, which detect anti-HIV antibodies only, have window periods of approximately 3 weeks. Western blot tests have window periods of approximately 4 weeks.

Specific information for each of the assays used in this study is provided in the kit inserts. ART = antiretroviral treatment; ARV = antiretroviral; DRV = darunavir; EFV = efavirenz; Enr = enrollment; FTC = emtricitabine; IND = indeterminate; mo = months; NR = nonreactive; P = positive; Qual RNA = APTIMA HIV-1 RNA Qualitative Assay; R = reactive; RTV = ritonavir; SQV = saquinavir; TFV = tenofovir; WB = Western blot.

[a] The OraQuick rapid test was performed in real-time at the HPTN 061 study sites. All other HIV testing, including repeat testing with the OraQuick rapid test, was performed retrospectively.

[b] This testing was performed using either the Roche TaqMan: COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, v2.0 or the Roche Amplicor HIV-1 MONITOR Test, v1.5 (ultra/standard).

[c] A very weak band was observed.

[d] For these samples, efavirenz (EFV) was detected using an alternate method that had a lower limit of detection for EFV.

[e] This case was identified as being recently infected using a multi-assay algorithm.[17]

[f] RNA testing was completed with Abbott RealTime HIV-1 assay (Abbott Molecular Inc., Des Plaines, IL).

Table 3

Sensitivity of assays for detection of HIV infection

	EC group (*n* = 17)		ART group (*n* = 101)		
Assay	**Samples tested**	**False-negative**	**Samples tested**	**False-negative**	**Sensitivity (%)**
OraQuick Advance HIV-1/2 Antibody Test	17	0	101	1	99.2
UniGold Recombigen HIV Test	17	0	101	1	99.2
INSTI Rapid HIV Test	17	0	100	0	100.0
ARCHITECT HIV Ag/Ab Combo	17	0	99	0	100.0
VITROS Anti-HIV 1+2 Test	17	0	99	0	100.0
Genetics System HIV-1 Western Blot	10	0	99	0	100.0

Note: ART group = individuals with long-term viral suppression from ART; EC group = elite controllers.