HHS Public Access

Rapid HIV screening: Missed opportunities for HIV diagnosis and prevention

Pragna Patel[a,*], **Berry Bennett**[b], **Timothy Sullivan**[c], **Monica M. Parker**[c], **James D. Heffelfinger**[a], and **Patrick S. Sullivan**[d] **for the CDC AHI Study Group**[e]

[a]Division of HIV/AIDS Prevention, Centers for Disease Control and Prevention, Atlanta, GA, United States

[b]Retrovirology Section, Florida Bureau of Laboratories, Jacksonville, FL, United States

[c]Wadsworth Center, New York State Department of Health, Albany, NY, United States

[d]Department of Epidemiology, Emory University, Rollins School of Public Health, Atlanta, GA, United States

Abstract

Background—Although rapid HIV tests increase the number of persons who are aware of their HIV status, they may fail to detect early HIV infection.

Objectives—To evaluate the sensitivity for early HIV infection of several rapid tests and third- and fourth-generation assays compared with nucleic acid amplification testing (NAAT).

Study design—Sensitivity for early HIV infection was evaluated using 62 NAAT-positive/WB-negative or indeterminate specimens from the CDC Acute HIV Infection study. Specimens underwent third-generation testing with Genetic Systems 1/2 + O® and rapid testing with Multispot HIV-1/HIV-2. A subset was also tested with four FDA-approved rapid tests and Determine HIV-1 Antigen/Antibody Rapid Test® and Architect HIV Antigen/Antibody Combo®, both fourth-generation tests.

Results—Of 99,111 specimens screened from April 2006 to March 2008, 62 met the definition for early HIV infection (60 NAAT-positive/seronegative and 2 NAAT-positive/Western blot indeterminate). Third-generation testing correctly detected antibody in 34 specimens (55%; 95%

confidence interval (CI): 42–67); Multispot detected antibody in 16 (26%; 95% CI: 16–38). Of the 62 specimens, 33 (53%) had sufficient quantity for further testing. Rapid test sensitivities for early HIV infection ranged from 22–33% compared with 55–57% for the third-generation assay and 76–88% for the fourth-generation tests.

Conclusions—Many rapid HIV tests failed to detect half of the early HIV infection cases in whom antibody was present. Programs that screen high-incidence populations with rapid tests should consider supplemental testing with NAAT or other antigen-based tests. These data support the need for more sensitive antigen-based point-of-care screening tests for early HIV infection.

Keywords

HIV; Rapid tests; Acute HIV infection; Early HIV infection; Fourth-generation

1. Background

The Centers for Disease Control and Prevention has encouraged expanded HIV testing[1] to identify the estimated 21% of persons living with HIV who remain undiagnosed.[2] Rapid HIV tests have been widely adopted as an essential component of HIV prevention programs because they are ideal tools to increase the number of persons who are aware of their HIV status. Rapid HIV tests offer several advantages because they are simple and easy to use; can be used in outreach, point-of-care, and nonclinical settings; return results faster than conventional tests (usually in 30 min); and minimize the extent to which clients fail to return for test results.[3] For these reasons, rapid HIV tests are commonly used in venues that reach persons at high risk for acquiring HIV.

Although rapid HIV tests increase the number of persons who are aware of their HIV status, the current Food and Drug Administration (FDA)-approved rapid tests may have limited sensitivity for the diagnosis of early HIV infection,[4,5] resulting in missed opportunities for HIV prevention. Early HIV infection is the stage of infection prior to HIV seroconversion or Western blot positivity (Fig. 1).[6] Many rapid tests seem to detect HIV only a few days before the Western blot.[4,5] The earliest phase of early HIV infection is acute HIV infection (AHI) which represents the stage of infection in which HIV RNA and p24 antigen can be detected but HIV antibodies are not yet detectable by earlier generation immunoassays (IAs), such as third-generation IAs which detect IgM and IgG HIV antibodies using an antigen–antibody–antigen sandwich technique, second-generation IAs which detect IgG antibodies to HIV recombinant proteins, and first-generation IAs which detect IgG antibodies to viral lysate.[4,6] Only more-sensitive IAs, such as fourth-generation IAs which detect p24 antigen in addition to IgM and IgG HIV antibodies using the third-generation technique, and nucleic acid amplification tests (NAAT) can detect persons with AHI.[7,8] Because NAAT is difficult to perform in outreach settings, we evaluated the sensitivity of rapid tests, including fourth-generation rapid tests, for early HIV infection compared with NAAT and third- and fourth-generation IAs. We believe these results are vital to inform HIV screening practices in high-risk outreach settings.

2. Objectives

The aim of this study was to examine the sensitivity of rapid and fourth-generation tests to detect early HIV infection.

3. Study design

3.1. CDC AHI study

The CDC AHI study was an observational study to evaluate several strategies for detecting AHI in Los Angeles (LA), New York City (NYC) and Florida. From April 24, 2006 to March 28, 2008, all persons who consented for HIV testing, including NAAT, at 14 county sexually transmitted disease (STD) clinics and one gay community clinic in LA, three health department STD clinics in NYC, and approximately 80 public health clinics in four Florida counties participated in this study. The primary objective was to evaluate the yield of pooled NAAT after first-, second-, and third-generation HIV antibody screening. However, one secondary objective was to examine the sensitivity of rapid and fourth-generation tests to detect early HIV infection. The study design and methods have been previously described.[8]

3.2. Testing protocol

Plasma specimens were collected from all consenting patients at the study sites. All persons were initially screened for HIV antibody; however, each project area used a different test for initial screening: Florida used a third-generation assay (Genetic Systems HIV 1/2 + O®) on serum specimens and in a few study sites, a second-generation rapid test on fingerstick specimens (Oraquick®), NYC used a second-generation rapid test (Oraquick Advance®) on oral fluid specimens, and LA used a first-generation assay (Vironostika HIV-1 Microelisa System®) on serum specimens. Specimens that were negative or indeterminate by serologic testing underwent NAAT (APTIMA® HIV-1 RNA® Qualitative assay) and viral load quantification (Siemens Versant® HIV-1 RNA 3.0 assay).

To evaluate sensitivity for early HIV infection using NAAT as a gold standard, all NAAT-positive specimens underwent third-generation testing with Genetic Systems 1/2 + O® IA and rapid testing with Multispot HIV-1/HIV-2®. Initial and follow-up specimens that remained antibody (Ab)-negative by screening IAs from 55 persons with AHI were included in this panel of NAAT-positive specimens; therefore all specimens were collected during early HIV infection. A subset of plasma specimens with sufficient quantity was stored at −70 °C and later tested with four other FDA-approved rapid HIV tests (Clearview Complete®, Unigold Recombigen®, Clearview HIV 1/2 Statpak®, and Oraquick Advance Rapid HIV-1/2®) and underwent fourth-generation testing with Determine HIV-1 Antigen/Antibody Rapid Test® and Architect HIV Antigen/Antibody Combo®, a lab-based immunoassay (Fig. 2). All rapid test devices were read by two trained laboratorians at each site and consensus was obtained regarding the test results. Characteristics of the lab-based assays and rapid test devices used in this evaluation are provided in Table 1. Test performance characteristics with confidence intervals (e.g., sensitivity) were calculated using OpenEpi version 2.2.1 (Emory Rollins School of Public Health, Atlanta, Georgia).

To evaluate fourth-generation screening for AHI detection, an unlinked, anonymous, blinded panel of 40 AHI, 44 HIV Ab-positive, and 30 HIV-negative specimens was tested with Determine HIV-1 Antigen/Antibody Rapid Test® and Architect HIV Antigen/Antibody Combo® IA. The 40 AHI specimens were from persons with documented subsequent seroconversion and included initial and follow-up specimens from the same individual. Because these assays were not approved by the FDA at the time, this evaluation was conducted retrospectively and results were not reported to participants; however it was expected that the fourth-generation test results would not alter the interpretation of participants' HIV test results.

4. Results

Of 99,111 specimens screened from April 2006 to March 2008, 60 specimens were NAAT-positive/Ab-negative by one of three different screening IAs and 2 were NAAT-positive/WB-indeterminate. Of these 62 specimens, half had HIV viral loads >500,000 copies/mL (range: <75–6,334,400 copies/mL). Genetic Systems $1/2 + O$® correctly detected antibody in 34 of 62 specimens (55%; 95% confidence interval (CI): 42–67); Multispot detected antibody in 16 of 62 specimens (26%; 95% CI: 16–38).

Of the 62 specimens, 33 (53%) had sufficient quantity for further testing. Because testing was done sequentially, the amount of specimen available for testing was exhausted in a few instances. Fourth-generation testing with Architect was positive in 29 of 33 (88%) specimens; Determine identified antibody or antigen (Ag) in 25 of 33 (76%) specimens (Table 2): 8 specimens tested Ab-positive/Ag-negative, 13 specimens tested Ab-negative/Ag-positive, 4 specimens tested Ab-positive/Ag-positive. Genetic Systems $1/2 + O$® correctly detected antibody in 19 of 33 (58%) specimens; Multispot in 11 of 33 (33%); Clearview Complete in 8 of 27 (30%); Unigold Recombigen HIV in 8 of 33 (24%); Clearview HIV 1/2 Statpak in 7 of 31 (23%); and Oraquick Advance Rapid HIV-1/2 in 7 of 32 (22%; Table 2).

Fourth-generation testing of the blinded specimen panel resulted in 0 false-positive and 10 false-negative results by Determine and 2 false-positive, 5 false-negative, and 2 error results by Architect. Therefore, the sensitivity of Determine compared with NAAT was 0.88 (95% CL: 0.79–0.94) and specificity was 1.00 (95% CL: 0.91–1.00). The sensitivity of Architect compared with NAAT was 0.94 (95% CL: 0.87–0.98) and specificity was 0.93 (95% CL: 0.79–0.99).

5. Discussion

Our findings suggest that, as expected, many FDA-approved rapid HIV tests failed to detect early HIV infection in half of the cases in whom antibody was present. Rapid test sensitivities for early HIV infection in our study ranged from 22–33% compared with 55–57% for the third-generation assay and 76–88% for the fourth-generation assays. Although the sensitivities of rapid HIV tests for established HIV infection are high,[9] rapid tests have varying ability to detect HIV infection during the early seroconversion period[4,5,10] and their ability to do so is substantially lower than third- and fourth-generation lab-based assays as well as rapid tests that also detect HIV antigens. Therefore, widespread use of rapid HIV

testing, particularly among high-risk/high-incidence populations, may have insufficient public health benefits by failing to detect persons with early HIV infection and missing opportunities to interrupt onward HIV transmission. Persons with early HIV infection are more likely to transmit HIV infection than persons with established infection,[11] contributing significantly to overall HIV transmission.[12] Studies have shown that NAAT can detect a high number of persons with early HIV infection who screened false-negative by a rapid HIV test.[8,13] Thus, programs that screen high-risk/high-incidence populations with rapid tests should consider supplemental testing with NAAT or other antigen-based tests.

Although NAAT is presently the gold standard for the diagnosis of AHI, its use is limited by cost,[14] the longer turnaround time of test results, and the need for venipuncture and a sophisticated laboratory. Fourth-generation combination assays that detect both HIV antibodies and p24 antigen may be a practical substitute for NAAT.[8] These more-sensitive assays are used worldwide, have a high sensitivity for HIV infection overall[15] and detected up to 88% of early HIV infection specimens in our study. Although fourth-generation testing will miss a few cases that may be detected by NAAT, frequent testing of persons who have recently participated in high risk activity should be encouraged to maximize HIV detection rates.[16] Even NAAT will miss cases of HIV particularly during the eclipse period when HIV RNA may be present in very small quantities but is undetectable.[6] and among a small number of persons who maintain viral suppression without antiretroviral therapy.

In outreach settings where rapid tests are traditionally used, the fourth-generation rapid test may be a good screening assay. In our evaluation, the fourth-generation rapid test was able to detect early HIV infection in 76% of specimens, at least twice as many as the other rapid tests and more than the third-generation lab-based assay. Furthermore, although the fourth-generation rapid test had a lower sensitivity than the lab-based fourth-generation assay, its specificity was higher which is important when delivering positive HIV results to high-risk individuals at the point of care.

Our study had a few limitations. We conducted this evaluation on a small sample size; however specimens collected during early HIV infection are in general rare. We conduct testing on the initial sample submitted by the participant for HIV testing so specimens were often exhausted or of limited quantity after routine HIV testing for screening and confirmation was conducted. We did not conduct a real-time evaluation of the fourth-generation tests because neither was FDA-approved at the time that the CDC AHI study was conducted. Therefore, specimens were frozen and stored for future testing. Freeze-thaw cycles were limited (2) but test results may have been affected by freezing and thawing.

These data support the need for FDA-approval of more sensitive antigen-based point-of-care screening tests for early HIV infection. Fourth-generation rapid tests are presently used worldwide and the approval of their use in the United States would allow for more effective screening of early HIV infection in hard-to-reach, high-risk populations to increase the number of persons who are aware of their HIV infection. Furthermore, point-of-care HIV RNA tests, which are currently being tested in clinical trials, may also be a useful tool for detection of early HIV infection in outreach settings.

References

1. Centers for Disease Control and Prevention. Advancing HIV prevention: new strategies for a changing epidemic – United States, 2003. MMWR Morb Mortal Wkly Rep. 2003; 52:329–32. [PubMed: 12733863]

2. Campsmith ML, Rhodes PH, Hall HI, Green TA. Undiagnosed HIV prevalence among adults and adolescents in the United States at the end of 2006. J Acq Immune Def Synd. 2010; 53:619–24.

3. Branson BM. State of the art for diagnosis of HIV infection. Clin Infect Dis. 2007; 45:S221–5. [PubMed: 18190290]

4. Owen SM, Yang C, Spira T, Ou CY, Pau CP, Parekh BS, et al. Alternate algorithms for human immunodeficiency virus infection diagnosis using tests that are licensed in the United States. J Clin Microbiol. 2008; 46:1494–7. [PubMed: 18234875]

5. 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; 46:1588–95. [PubMed: 18322061]

6. Fiebig EW, Wright DJ, Rawal BD, Garrett PE, Schumacher RT, Peddada L, et al. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. AIDS. 2003; 17:1871–9. [PubMed: 12960819]

7. Pilcher CD, Fiscus SA, Nguyen TQ, Foust E, Wolf L, Williams D, et al. Detection of acute infections during HIV testing in North Carolina. N Engl J Med. 2005; 352:1873–83. [PubMed: 15872202]

8. Patel P, MacKellar D, Simmons P, Uniyal A, Gallagher K, Bennett B, et al. Detecting acute human immunodeficiency virus infection using 3 different screening immunoassays and nucleic acid amplification testing for human immunodeficiency virus RNA, 2006–2008. Arch Intern Med. 2010; 170:66–74. [PubMed: 20065201]

9. Delaney KP, Branson BM, Uniyal A, Philips S, Candal D, Owen SM, et al. Evaluation of the performance characteristics of 6 rapid HIV antibody tests. Clin Infect Dis. 2011; 52:257–63. [PubMed: 21288853]

10. Stekler J, Wood RW, Swenson PD, Golden M. Negative rapid HIV antibody testing during early HIV infection. Ann Intern Med. 2007; 147:147–8.

11. Wawer MJ, Gray RH, Sewankambo NK, Serwadda D, Li X, Laeyendecker O, et al. Rates of HIV-1 transmission per coital act, by stage of HIV-1 infection, in Rakai, Uganda. J Infect Dis. 2005; 191:1403–9. [PubMed: 15809897]

12. Brenner BG, Roger M, Routy JP, Moisi D, Ntemgwa M, Matte C, et al. High rates of forward transmission events after acute/early HIV-1 infection. J Infect Dis. 2007; 195:951–9. [PubMed: 17330784]

13. Stekler JD, Swenson PD, Coombs RW, Dragavon J, Thomas KK, Brennan CA, et al. HIV testing in a high incidence population: is antibody testing alone good enough? Clin Infect Dis. 2009; 49:444–53. [PubMed: 19538088]

14. Hutchinson AB, Patel P, Sansom SL, Farnham PG, Sullivan TJ, Bennett B, et al. Cost-effectiveness of pooled nucleic acid amplification testing for acute HIV infection after third-generation HIV antibody screening and rapid testing in the United States: a comparison of three public health settings. PLoS Med. 2010; 7(9):e10000342.

15. Chavez P, Wesolowski L, Patel P, Delaney K, Owen SM. Evaluation of the Performance of the Abbott ARCHITECT Ag/Ab Combo® Assay. J Clin Virol. 2011; 52(Suppl 1):S51–5. [PubMed: 21983253]

16. Branson BM. The future of HIV testing. J Acq Immune Def Synd. 2010; 55:S102–5.

Appendix A

CDC AHI Study Group consists of the authors: Apurva Uniyal, Peter Kerndt, Michael Chien, Staeci Morita, La Shawnda Royal and Ali Stirland from the Los Angeles Department of Health Services, Los Angeles, CA; Pat Simmons, Marlene LaLota, and Melissa Cox from the Florida Department of Health, Tallahassee, FL; Sally Fordan, Olanike David, Petrice Stephens, and Berry Bennett from the Florida Bureau of Laboratories, Jacksonville, FL; Kathy Gombel, Judith Wethers, Timothy J. Sullivan, and Monica Parker from the New York State Department of Health Wadsworth Center, Albany, NY; Kathleen Gallagher, Alexis Kowlaski, Susan Blank, and Steve Rubin from the New York City Department of Health and Mental Hygiene and Pragna Patel and Steven Ethridge, Centers for Disease Control and Prevention, Atlanta, GA.
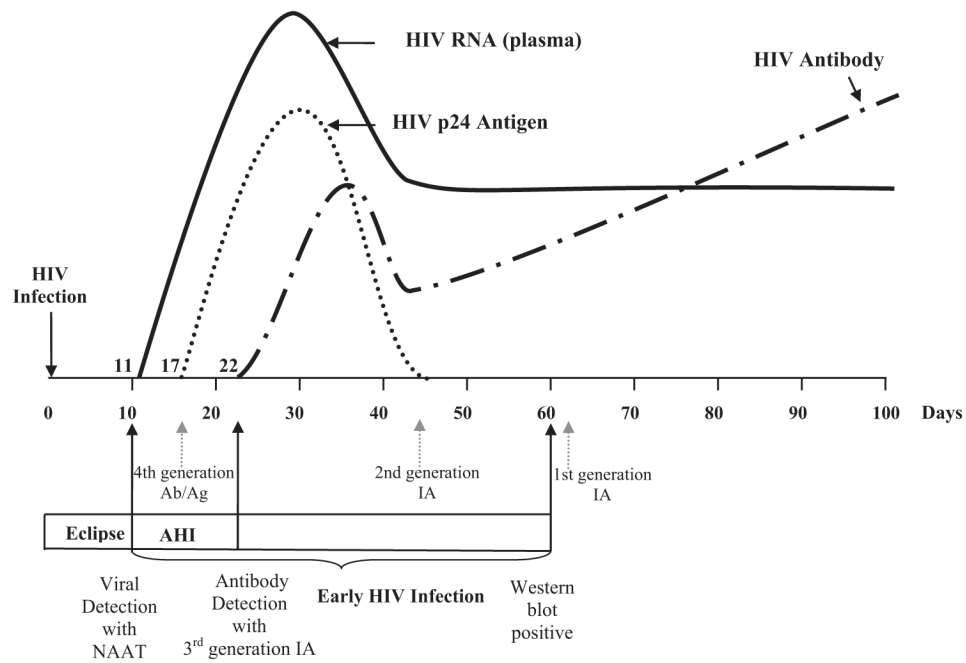
Fig. 1.
Window of detection of HIV markers early in HIV infection and window period of different generations of immunoassays (IAs) compared to nucleic acid amplification testing (NAAT) for HIV RNA and Western blot positivity. AHI, acute HIV infection; IA, immunoassay; Ab, antibody; Ag, antigen; NAAT, nucleic acid amplification test. Eclipse period: time after HIV acquisition when HIV RNA may be present in very small quantities but is undetectable. Acute HIV infection: phase of early HIV infection when HIV RNA and p24 antigen are detectable but HIV antibodies are not. Early HIV infection: stage of infection prior to HIV seroconversion or Western blot positivity. Fourth-generation assay: detects p24 antigen and IgM/IgG HIV antibodies; third-generation assay detects IgM/IgG HIV antibodies; second-generation and first-generation assays detect IgG HIV antibodies.
*Adapted from Fiebig et al. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. *AIDS* 2003, 17:1871–79 and slide courtesy of S. Kleinman (written permission obtained 03/08/2009).
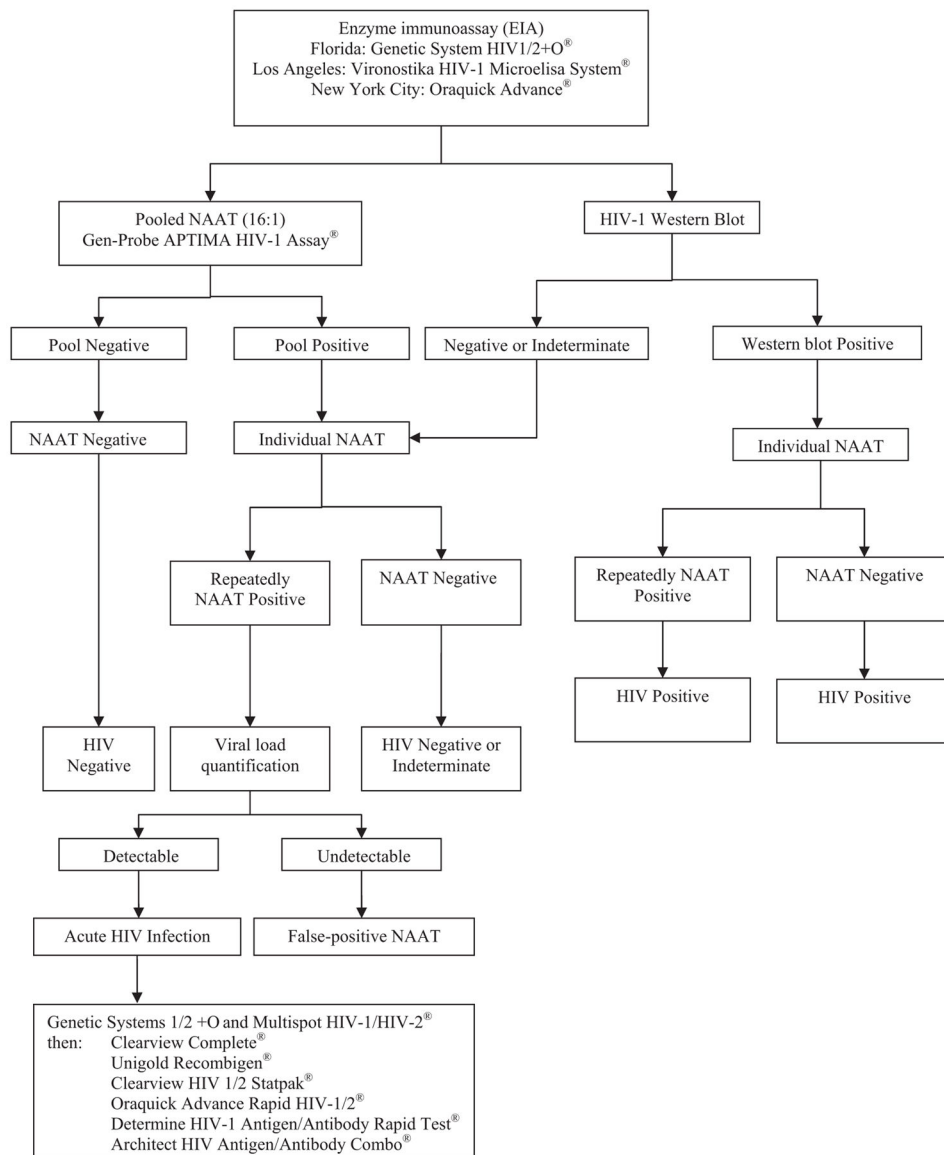
Fig. 2.
Specimen testing algorithm for early HIV infection.

Table 1

Summary of characteristics of lab-based assays and rapid testdevices for HIV detection.

HIV screening assay	Test type	Clinical laboratory improvement amendment (CLIA) category	Specimen type	Sensitivity[a] % (95% CI)	Specificity[a] % (95% CI)
Architect HIV-1 Ag/Ab Combo®	Fourth-generation[b]	High complexity	Serum and plasma	100 (94.3–100)	99.8 (99.2–99.9)
Determine HIV-1 Ag/Ab Rapid Test	Fourth-generation[c]	N/A[d]	Whole blood (fingerstick or venipuncture), serum, plasma	100[e]	99[e]
Genetic Systems HIV 1/2 + O®	Third-generation	High complexity	Serum and plasma	100 (99.8–100)	99.9 (99.8–99.96)
Multispot HIV-1/HIV-2 Rapid Test	Flow-through rapid	Moderate complexity	Serum	100 (99.9–100)	99.9 (99.8–100)
		Moderate complexity	Plasma	100 (99.9–100)	99.9 (99.8–100)
Clearview Complete HIV 1/2 Assay	Lateral flow rapid	Waived	Whole blood (fingerstick or venipuncture)	99.7 (98.9–100)	99.9 (99.6–100)
		Moderate complexity	Serum and plasma	99.7 (98.9–100)	99.9 (99.6–100)
Unigold Recombigen® HIV	Lateral flow rapid[f]	Waived	Whole blood (fingerstick or venipuncture)	100 (99.5–100)	99.7 (99.0–100)
		Moderate complexity	Serum and plasma	100 (99.5–100)	99.8 (99.3–100)
Clearview HIV 1/2 Stat-Pak Assay	Lateral flow rapid	Waived	Whole blood (fingerstick or venipuncture)	99.7 (98.9–100)	99.9 (99.6–100)
		Moderate complexity	Serum and plasma	99.7 (98.9–100)	99.9 (99.6–100)
Oraquick Advance Rapid HIV-1/2 Antibody Test	Lateral flow rapid	Waived	Oral fluid	99.3 (98.4–99.7)	99.8 (99.6–99.9)
		Waived	fingerstick whole blood	99.6 (98.5–99.9)	100 (99.7–100)
		Moderate complexity	Plasma	99.6 (98.9–99.8)	99.9 (99.6–99.9)

[a] Sensitivity and specificity values were taken from the package inserts of each test.

[b] Architect HIV-1 Ag/Ab Combo® is a combination lab-based assay that detects p24 antigen and HIV antibody using third-generation technology (IgM/IgG).

[c] The Determine HIV-1 Ag/Ab Rapid Test is a combination lab-based assay that has separate p24 antigen detection and HIV antibody detection using a third-generation immunoassay.

[d] The Determine HIV-1 Ag/Ab Rapid Test is not yet FDA-approved.

[e] Confidence intervals for performance characteristic were not provided in the package insert.

[f] Unigold Recombigen® HIV uses a sandwich technique similar to a third-generation immunoassay.

Table 2

Sensitivity for early HIV infection of rapid HIV tests compared with third- and fourth-generation assays.

HIV screening assay	Number of specimens that tested positive	Total number of specimens tested	Sensitivity for early HIV infection (%)	95% Confidence interval
Architect HIV-1 Ag/Ab Combo® *	29	33	87.8	(73.3–96.0)
Determine HIV-1 Ag/Ab Rapid Test †,‡	25	33	75.8	(59.1–88.1)
Genetic Systems HIV 1/2 + O®	19	33	57.5	(40.4–73.5)
Multispot HIV-1/HIV-2 Rapid Test	11	33	33.3	(18.9–50.4)
Clearview Complete HIV 1/2 Assay	8	27	29.6	(14.8–48.6)
Unigold Recombigen® HIV§	8	33	24.2	(11.9–40.9)
Clearview HIV 1/2 Stat-Pak Assay	7	31	22.6	(10.5–39.6)
Oraquick Advance Rapid HIV-1/2 Antibody Test	7	32	21.9	(10.1–38.6)

*
Architect HIV-1 Ag/Ab Combo® is a combination lab-based assay that detects p24 antigen and HIV antibody using third-generation technology (IgM/IgG).

†
The Determine HIV-1 Ag/Ab Rapid Test has separate p24 antigen detection and HIV antibody detection using a third-generation immunoassay.

‡
The Determine HIV-1 Ag/Ab Rapid Test is not yet FDA-approved.

§
Unigold Recombigen® HIV uses a sandwich technique similar to a third-generation immunoassay.