Original Investigation

Screening Yield of HIV Antigen/Antibody Combination and Pooled HIV RNA Testing for Acute HIV Infection in a High-Prevalence Population

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IMPORTANCE Although acute HIV infection contributes disproportionately to onward HIV transmission, HIV testing has not routinely included screening for acute HIV infection.

OBJECTIVE To evaluate the performance of an HIV antigen/antibody (Ag/Ab) combination assay to detect acute HIV infection compared with pooled HIV RNA testing.

DESIGN, SETTING, AND PARTICIPANTS Multisite, prospective, within-individual comparison study conducted between September 2011 and October 2013 in 7 sexually transmitted infection clinics and 5 community-based programs in New York, California, and North Carolina. Participants were 12 years or older and seeking HIV testing, without known HIV infection.

EXPOSURES All participants with a negative rapid HIV test result were screened for acute HIV infection with an HIV Ag/Ab combination assay (index test) and pooled human immunodeficiency virus 1 (HIV-1) RNA testing. HIV RNA testing was the reference standard, with positive reference standard result defined as detectable HIV-1 RNA on an individual RNA test.

MAIN OUTCOMES AND MEASURES Number and proportion with acute HIV infections detected.

RESULTS Among 86 836 participants with complete test results (median age, 29 years; 75.0% men; 51.8% men who have sex with men), established HIV infection was diagnosed in 1158 participants (1.33%) and acute HIV infection was diagnosed in 168 participants (0.19%). Acute HIV infection was detected in 134 participants with HIV Ag/Ab combination testing (0.15% [95% CI, 0.13%-0.18%]; sensitivity, 79.8% [95% CI, 72.9%-85.6%]; specificity, 99.9% [95% CI, 99.9%-99.9%]; positive predictive value, 59.0% [95% CI, 52.3%-65.5%]) and in 164 participants with pooled HIV RNA testing (0.19% [95% CI, 0.16%-0.22%]; sensitivity, 97.6% [95% CI, 94.0%-99.4%]; specificity, 100% [95% CI, 100%-100%]; positive predictive value, 96.5% [95% CI, 92.5%-98.7%]; sensitivity comparison, $P < .001$). Overall HIV Ag/Ab combination testing detected 82% of acute HIV infections detectable by pooled HIV RNA testing. Compared with rapid HIV testing alone, HIV Ag/Ab combination testing increased the relative HIV diagnostic yield (both established and acute HIV infections) by 10.4% (95% CI, 8.8%-12.2%) and pooled HIV RNA testing increased the relative HIV diagnostic yield by 12.4% (95% CI, 10.7%-14.3%).

CONCLUSIONS AND RELEVANCE In a high-prevalence population, HIV screening using an HIV Ag/Ab combination assay following a negative rapid test detected 82% of acute HIV infections detectable by pooled HIV RNA testing, with a positive predictive value of 59%. Further research is needed to evaluate this strategy in lower-prevalence populations and in persons using preexposure prophylaxis for HIV prevention.

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Acute HIV infection contributes disproportionately to HIV transmission.[1-4] A high viral load,[5] which often peaks at levels greater than 10 000 000 copies/mL, and homogeneity of viral variants recently selected for transmission[3,6] both contribute to this highly infectious phase. Individuals with acute HIV infection have de facto engaged in high-risk behaviors and are often unaware that they are infected with HIV. Identifying individuals with acute HIV infection is critical to prevent further HIV transmission,[7] as diagnosis can lead to several effective HIV prevention interventions. Acute HIV infection is characterized as the interval between the appearance of HIV RNA and detection of HIV-specific antibodies.[8] Acute HIV infection can be diagnosed with assays that detect either HIV RNA (the reference standard) or the p24 antigen (an HIV core protein), which are both detectable early after HIV infection and before an antibody response develops (eFigure in the Supplement).[8,9] Although HIV RNA testing using a pooled protocol (in which multiple HIV antibody–negative specimens are combined and tested together) can effectively detect acute HIV infection, it has not been widely implemented because only 1 RNA assay is US Food and Drug Administration (FDA)–approved for HIV diagnosis, the pooling protocol is logistically complex and time intensive, and it may not be cost-effective.[10] HIV immunoassays that detect both the p24 antigen and anti-HIV antibody (fourth generation antigen/antibody [Ag/Ab] combination immunoassays) are currently being implemented as the initial screening test in the 2014 Centers for Disease Control and Prevention (CDC) and Association of Public Health Laboratories (APHL)–recommended HIV diagnostic algorithm,[8] can be performed rapidly, and are likely cost-effective.[11] However, HIV Ag/Ab combination assays have a lower sensitivity compared with pooled HIV RNA testing, and its performance at detecting acute HIV infection has not been established. In a prospective study we evaluated the performance of an HIV Ag/Ab combination assay to detect acute HIV infection compared with pooled HIV RNA testing in a high-prevalence population.

Methods

Study Design and Participants

This study was approved by local institutional review boards, as indicated by local policies, for the University of California at San Francisco, the University of North Carolina at Chapel Hill, and the New York City Department of Health and Mental Hygiene, and was approved by CDC through a research determination in accordance with federal human participants protection regulations and CDC policies and procedures.[13,14] Participants provided medical consent for HIV testing (including screening for acute HIV infection), and the study had a waiver of participant consent for research based on an exempt status determined by local institutional review boards. The Screening Targeted Populations to Interrupt On-going Chains of HIV Transmission with Enhanced Partner Notification (STOP) study was a prospective study examining implementation of HIV Ag/Ab combination testing to detect acute HIV infection linked to enhanced partner services in New York City, New York; San Francisco, California; and Raleigh, Durham, and Winston-Salem, North Carolina.[12] Participants were 12 years or older and receiving HIV testing at 1 of 12 facilities, including sexually transmitted infection clinics and community-based programs. Participants self-reported demographic (eg, age, race, and ethnicity) and behavioral information based on fixed categories. Race was included as a variable because HIV disproportionately affects certain racial and ethnic groups. Acute HIV testing was offered to consecutive sexually active men who have sex with men (MSM) at testing venues in New York and California, and to all consecutive patients (including MSM) receiving HIV testing at 3 sexually transmitted infection clinics in North Carolina (**Table 1**).

Test Methods

Specimens from participants were initially screened with a point-of-care rapid HIV test (OraQuick ADVANCE Rapid HIV-1/2 Antibody Test, OraSure Technologies or Clearview HIV 1/2 STAT-PAK assay, Alere). Specimens with a negative test result for the rapid test were tested for acute HIV infection with the index test, a laboratory-based, fourth-generation HIV Ag/Ab combination immunoassay (Abbott Architect HIV Ag/Ab Combo Assay; Abbott Diagnostics) according to the manufacturer's specifications[15] (eAppendix 1 in the Supplement) and with pooled human immunodeficiency virus 1 (HIV-1) RNA testing. The HIV Ag/Ab combination immunoassay was considered reactive if the signal:cutoff ratio result was 1.00 or more on the initial test and on at least 1 of 2 additional repeat tests (ie, all reactive specimens had 3 signal:cutoff ratio results).[15]

A combination of pooled and individual HIV-1 RNA testing was used as the reference standard for acute HIV infection because HIV RNA is the first laboratory marker to become detectable in the plasma after HIV infection (eFigure in the Supplement).[5,8,9] Because HIV RNA testing is expensive and time intensive, multiple specimens that had tested negative for HIV antibody (ie, do not have established HIV infection) were combined into a pool and the HIV RNA assay was performed on the pool.[16] Although pooling increases the lower limit of quantification (LLQ) of the assay by diluting the contribution of each specimen, patients with acute HIV infection often have markedly elevated HIV viral loads (often exceeding 1 000 000 copies/mL). In the pooled HIV RNA testing protocol, if the pooled result was undetectable for HIV RNA, all of the specimens in the pool had an undetectable result. If the pooled test detected HIV RNA, each specimen that contributed to the pool was tested individually to determine the HIV RNA detectable specimen. HIV-1 RNA testing was performed with either Aptima HIV-1 RNA qualitative assay (Gen-Probe), a qualitative method with a lower limit of detection of approximately 30 copies/mL, or Abbott m2000 RealTime HIV-1 quantitative assay (Abbott Diagnostics), a quantitative method with a LLQ of 40 copies/mL according to the manufacturer's specifications.[17] Pool size (ie, the number of specimens with a

Table 1. Characteristics and Risk Behavior Information Stratified by Site[a]

Variable	No. (%) New York (n = 20 884)	California (n = 29 335)	North Carolina (n = 36 617)	Total (N = 86 836)[b]
Age, y				
<25	4716 (22.6)	3520 (12.0)	15 400 (42.1)	23 636 (27.2)
25 to 34	10 691 (51.2)	12 144 (41.4)	11 378 (31.1)	34 213 (39.4)
35 to 44	3495 (16.7)	7221 (24.6)	5109 (14.0)	15 825 (18.2)
≥45	1982 (9.5)	6405 (21.8)	3649 (10.0)	12 036 (13.9)
Missing	0	45 (0.2)	1081 (2.6)	1126 (1.3)
Median (IQR)	29 (25-35)	33 (27-43)	26 (22-34)	29 (24-38)
Gender				
Male	19 670 (94.2)	28 541 (97.3)	16 875 (46.1)	65 086 (75.0)
Female	1141 (5.5)	641 (2.2)	18 708 (51.1)	20 490 (23.6)
Transgender or other	73 (0.4)	123 (0.5)	6 (0.02)	202 (0.2)
Missing	0	30 (0.1)	1028 (2.8)	1058 (1.2)
Race/ethnicity				
Black/African American	4005 (19.2)	1404 (4.8)	25 572 (69.8)	30 981 (35.7)
Hispanic/Latino	4518 (21.6)	5475 (18.7)	4147 (11.3)	14 140 (16.3)
White	9703 (46.5)	16 929 (57.7)	4801 (13.1)	31 433 (36.2)
Asian	1425 (6.8)	3486 (11.9)	134 (0.4)	5045 (5.8)
Other	22 (0.1)	616 (2.1)	63 (0.2)	701 (0.8)
Unknown	1211 (5.8)	1425 (4.9)	1900 (5.2)	4536 (5.2)
Risk Behaviors in Previous 12 Months[c]				
Male participants only (n = 65 086)				
Sex with men	17 910 (91.1)	25 355 (88.8)	1690 (10.0)	44 955 (69.1)
Sex with women only	0	1136 (4.0)	11 978 (71.0)	13 114 (20.2)
Female participants only (n = 20 490)				
Sex with MSM	147 (12.9)	103 (16.1)	60 (0.3)	310 (1.5)
Sex with men (but not MSM)	0	460 (71.8)	14 942 (79.9)	15 402 (75.2)
Sex with women only	0	22 (3.4)	367 (2.0)	389 (1.9)
All participants (n = 86 836)				
Sex with an HIV-infected sex partner	1145 (5.5)	6758 (23.0)	337 (0.9)	8240 (9.5)
Sex in exchange for money or drugs	0	17 (0.1)	234 (0.6)	251 (0.3)
Injection drug use	134 (0.6)	519 (1.8)	88 (0.2)	741 (0.9)

Abbreviations: IQR, interquartile range; MSM, men who have sex with men.

[a] Characteristics and risk behavior information are self-reported at the time of testing. The test result (and information collected at that test event) is the unit of analysis. Participants could be diagnosed with HIV infection (either acute or established) only once in this study, but participants with a negative HIV test result could be tested again.

[b] Thirty-one participants without complete HIV testing (missing a combination Ag/Ab combination assay or pooled HIV RNA result) were excluded.

[c] Each risk behavior (eg, sex with men) was self-reported as "yes" by checking a box and "no" by not checking a box. Therefore, among all male participants, 44 955 men (69.1%) indicated that they have sex with men and the remaining 20 131 men (30.9%) did not indicate that they have sex with men.

negative HIV rapid test result pooled together for an HIV RNA test) ranged from 10 in California (LLQ = 400 copies/mL), to 16 in New York (LLQ = 480 copies/mL), and to 80 in North Carolina (LLQ = 2400 copies/mL).

The HIV Ag/Ab combination immunoassay and the pooled HIV RNA testing were performed in parallel and a final result was assigned for each assay without information on the other assay's result. If the HIV Ag/Ab combination immunoassay and the pooled HIV RNA testing were discordant, an additional individual HIV RNA test was performed. In summary, if HIV Ag/Ab combination immunoassay was nonreactive, the HIV-1 RNA reference standard was classified negative if the pooled HIV RNA result was undetectable. If either the HIV Ag/Ab combination immunoassay or the pooled HIV RNA test result was positive, the HIV-1 RNA reference standard result (positive or negative) was determined by the additional individual HIV RNA result (detectable or undetectable).

Reactive HIV Ag/Ab combination assay specimens were also tested with 2 antibody-based confirmatory tests including the Multispot HIV-1/HIV-2 Rapid Test (Bio-Rad Laboratories) and either an HIV-1 Western blot (Bio-Rad Laboratories) or an HIV-1 immunofluorescent assay (Sanochemia) per package insert.[18] The Multispot was included in this analysis because it is now the recommended confirmatory test for HIV diagnosis in the 2014 CDC/APHL HIV laboratory testing recommendations[8] (eAppendix 1 in the Supplement).

Acute HIV infection was defined by a negative result for the rapid HIV test followed by a reactive result for the HIV Ag/Ab combination assay, or detectable HIV RNA on pooled HIV RNA testing confirmed with an individual HIV RNA test. Established HIV infection was defined as HIV infection detected by a rapid HIV test and confirmed by a reactive Multispot or positive HIV-1 Western blot result, with discordant confirmatory results resolved with HIV-1 RNA testing. Because the majority of testing occurred among MSM at 2 of

the sites, an analysis of risk for acute HIV infection was conducted among MSM only.

Statistical Analysis

The primary analysis was a within-individual comparison of HIV Ag/Ab combination and pooled HIV RNA test results. The unit of analysis was a test; although an individual could contribute only 1 positive result (subsequent tests were excluded after an HIV diagnosis), individuals with negative test results were encouraged to return every 3 months for testing. The frequencies of acute HIV infection detected with these 2 assays were described and compared. The McNemar test was used to test for differences in proportions (categorical variables). Indeterminate HIV Ag/Ab combination or pooled HIV RNA test results were repeated and participants missing either test were excluded from the analysis. The Wilcoxon rank sum test (continuous variables), the χ^2 and Fisher exact tests (2-level categorical variables), and a log-linked binomial regression model (Proc Genmod; >2-level categorical variables[19]) were used to evaluate for associations between acute HIV infection and demographic and behavioral information.[20] Missing demographic data (eg, race) was categorized into a "missing" category if not present on the data collection form or if the participant refused to provide an answer, and this "missing" category was included in the analysis. Statistical significance was indicated by a 2-sided *P* value less than .05. All analyses, including calculations of sensitivity, specificity, and positive predictive value and their respective 95% confidence intervals by exact (Clopper-Pearson) methods, were performed using SAS (SAS Institute), version 9.3. The intended sample size for this study was 109 acute HIV infections, which would provide adequate power (β = .1 and α = .05) to detect a 13% difference in the proportion of acute HIV infections detected by the HIV Ag/Ab combination assay compared with pooled HIV RNA testing.

Results

Between September 2011 and October 2013, 86 867 HIV tests were performed (**Figure 1**). Among 86 836 with complete HIV testing results, the median age was 29 years (interquartile range [IQR], 24-38), the majority (75.0%) of those testing were men (Table 1), and 51.8% were MSM. Among male participants (n = 65 086), 69.1% reported having sex with men. The majority of female participants (n = 18 708 of 20 490; 91.3%) and male participants who reported having sex with women only (n = 11 978 of 13 114; 91.3%) were tested in North Carolina (Table 1).

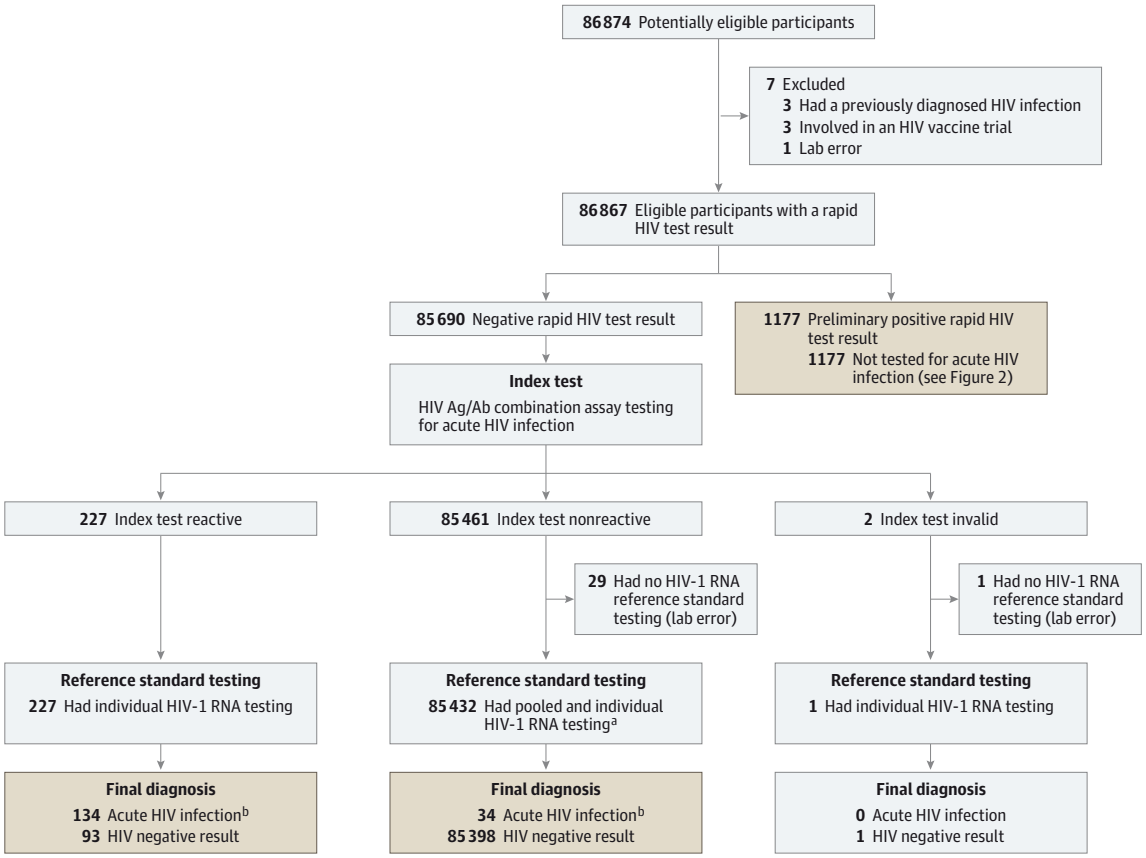
Established HIV infection (rapid HIV test reactive and confirmed) was diagnosed in 1158 individuals (1.33%), with few rapid HIV test results (n = 19; 0.02% of all tests, 1.6% of all positive tests) determined to be false-positive (**Figure 2**). The HIV Ag/Ab combination assay result was repeatedly reactive for all 1158 with established HIV infection and negative for all 19 rapid specimens with a false-positive result. Western blot and immunofluorescent assay test results were positive in 1143 of the 1158 specimens (98.7%) determined to have established HIV infection but were indeterminate (n = 9; 0.8%) or negative (n = 6; 0.5%) in 15 established HIV infection specimens (Figure 2). Multispot confirmatory testing was HIV-1 reactive in 1140 of the 1158 specimens (98.4%) determined to have established HIV infection but was indeterminate (n = 10; 0.9%) or negative (n = 4; 0.3%) in 14 established HIV infection specimens (Figure 2).

Acute HIV infection was diagnosed in 168 individuals (0.19%) by testing specimens that were negative by rapid HIV testing (n = 85 690) with HIV Ag/Ab combination and pooled HIV RNA assays (eTable 1 in the Supplement). The Ag/Ab combination assay was reactive in 227 of these specimens, of which 134 (0.15% [95% CI, 0.13%- 0.18%]) were confirmed as acute HIV infection on individual HIV RNA testing, and 93 (0.11% [95% CI, 0.09%-0.13%]) were determined to be false-positive test results (Figure 1). The positive predictive value for acute HIV infection of a reactive Ag/Ab combination assay result after a negative rapid HIV test was 59.0% (95% CI, 52.3%-65.5%). Pooled HIV-1 RNA testing was reactive in 170 of 85 690 rapid negative specimens (0.20%), of which 164 (0.19%) were determined to be acute HIV infection on individual HIV-1 RNA testing. Four results from pooled HIV-1 RNA testing (0.005% of the rapid negative specimens [95% CI, 0.00%- 0.02%]) were determined to be false-negative, and 6 results (0.007% [95% CI, 0.00%-0.02%]) were determined to be false-positive.

Of 168 acute HIV infections diagnosed in this study, HIV Ag/Ab combination testing detected 134 acute infections (acute HIV infection: sensitivity, 79.8% [95 CI, 72.9%-85.6%]; specificity, 99.9% [95% CI, 99.9%-99.9%]; positive predictive value, 59.0% [95% CI, 52.3%-65.5%]). Pooled HIV RNA testing detected 164 acute infections (sensitivity, 97.6% [95% CI, 94.0%-99.4%]; specificity, 100% [95% CI, 100%-100%]; positive predictive value, 96.5% [95% CI, 92.5%-98.7%]) (sensitivity comparison, $P < .001$). Overall, HIV Ag/Ab combination testing detected 82% of acute HIV infections detectable by pooled HIV RNA testing. Acute infections detectable by pooled HIV RNA testing only (n = 34) had a lower median HIV-1 viral load (6019 copies/mL [IQR, 1225-25 866]) than those reactive on HIV Ag/Ab combination testing (750 000 copies/mL [IQR, 224 152-2 033 422]; $P < .001$). The full testing results of the 4 acute infections (2.4%) that were reactive on the Ag/Ab combination testing but negative on pooled HIV RNA testing are available online (eTable 2 in the Supplement). All acute infections detected by HIV Ag/Ab combination testing were also tested with the Multispot antibody assay (eAppendix 2 in the Supplement).

Based on the number of established HIV infections diagnosed with rapid HIV testing alone (n = 1158; 1.33%), the addition of Ag/Ab combination testing increased the absolute diagnostic yield by 0.15% (95% CI, 0.13%-0.18%) and the relative diagnostic yield by 10.4% (95% CI, 8.8%-12.2%) (**Table 2**). Pooled RNA testing increased the absolute and relative diagnostic yields by 0.19% (95% CI, 0.16%-0.22%)

Figure 1. Flow of Specimens Screened for Acute HIV Infection With an HIV Ag/Ab Combination Assay (Index Test) and HIV-1 RNA Testing (Reference Standard Test)



Ag indicates antigen; Ab, antibody; HIV-1, human immunodeficiency virus 1. Brown boxes indicate specimens with complete HIV test results and included in the primary analysis.

[a] An additional individual HIV-1 RNA assay was performed if the pooled HIV-1 RNA testing had a positive result.

[b] Acute HIV infection was diagnosed with (1) a reactive HIV Ag/Ab combination assay or detectable HIV RNA on pooled HIV RNA testing and (2) detectable HIV RNA on an individual HIV RNA test.

and 12.4% (95% CI, 10.7%-14.3%), respectively, compared with rapid HIV testing (Table 2), and by 0.04% (95% CI, 0.03%-0.05%) and 2.6% (95% CI, 1.8%-3.6%), respectively, compared with HIV Ag/Ab combination testing. This increase in absolute diagnostic yield from Ag/Ab combination testing ranged from an increase of 0.04% in North Carolina and 0.15% in New York, to 0.21% in California. The increase in relative diagnostic yield from Ag/Ab combination testing ranged from 7.6% in North Carolina and 8.5% in New York, to 14.7% in California (Table 2).

Because HIV testing focused on sexually active MSM in both New York City, New York, and San Francisco, California (86% of all tests), a subanalysis of MSM was conducted to evaluate risk factors for acute HIV infection (**Table 3**). Among 44 955 MSM tested at all 3 sites, 1107 (2.46%) were diagnosed with HIV infection, including 956 (2.13%) with established HIV infection and 151 (0.34%) with acute HIV infection. Among only MSM, the addition of Ag/Ab combination testing to rapid HIV testing alone increased the relative diagnostic yield by 11.2% (95% CI, 9.4% to 13.3%), and pooled HIV RNA testing increased the relative diagnostic yield by 13.4% (95% CI, 11.5% to 15.6%). Younger (<25 years of age) MSM had a higher frequency of acute HIV infection compared with older (≥45 years) MSM, but the difference was not statistically significant (0.40% for younger MSM vs 0.28% for older MSM; risk difference [RD], 0.12% [95% CI, −0.06% to 0.31%]; P = .19). Compared with white MSM, there were higher frequencies of acute HIV infection among African American or black MSM (0.54% for African American or black MSM vs 0.28% for white MSM; RD, 0.26% [95% CI, 0.05% to 0.48%]; P = .01) and Hispanic or Latino MSM (0.43% for Hispanic or Latino MSM vs 0.28% for white MSM; RD, 0.16% [95% CI, 0.01% to 0.31%]; P = .04). Overall, only 16.9% (n = 7603) of MSM reported having an HIV-infected sex partner at the time of testing, but these MSM had a higher frequency of acute HIV infection than MSM who did not report an HIV-infected sex partner (0.47% for MSM with HIV-infected sex partner vs 0.31% for MSM without HIV-infected sex partner; RD, 0.16% [95% CI, 0.00% to 0.33%]; P = .048).

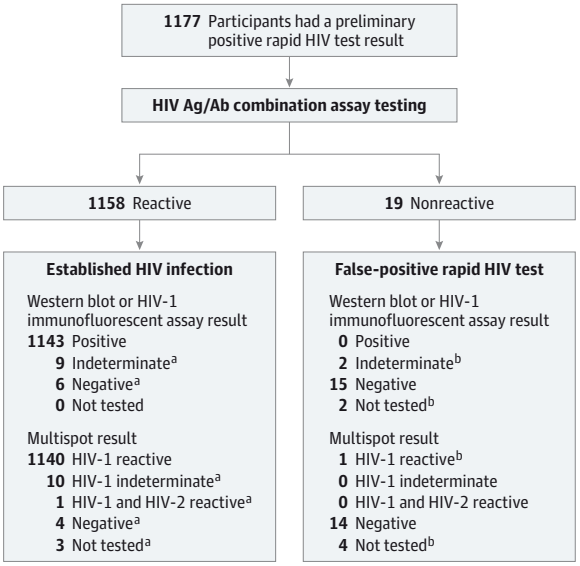
Discussion

In this prospective study evaluating HIV testing in a high-HIV prevalence population, the HIV Ag/Ab combination assay in place of rapid HIV testing increased the absolute HIV diagnostic yield by 0.15% (a 10.4% increase in the relative diagnostic yield) and diagnosed 82% of the acute HIV infections detectable by pooled RNA testing. Because rapid HIV testing detected HIV infection in only 87.3% of HIV-infected participants, alternative strategies such as using a laboratory-based HIV Ag/Ab combination assay that can detect acute infection should be considered in high-prevalence populations in the United States.

These data are novel in several respects. First, this study detected more acute HIV infections (n = 168) than have been previously diagnosed in a study in the United States to our knowledge. Diagnosing acute HIV infection has been considered a rare event; preliminary data from the US National HIV Surveillance System on a subset of new HIV diagnoses indicated that only 3.1% of HIV infections were diagnosed in the acute phase from 2008 through 2012.[21] In our study, almost 12% of HIV infections were detected in the acute phase, demonstrating that acute infection can be diagnosed if the testing strategy has adequate acute HIV infection sensitivity. Second, to our knowledge, this is the only US prospective study to evaluate the performance of the HIV Ag/Ab combination assay compared with the referent standard, HIV RNA testing. Previous studies have evaluated the relative performance of these tests in vitro with retrospective testing of stored specimens[22,23] or have been performed in Asia, where the proportion of HIV diagnoses in the acute phase of infection was low (1.4%), and pooled HIV RNA testing was only performed in participants with a negative HIV Ag/Ab combination assay result.[24] Third, this study prospectively compared the performance of the HIV Ag/Ab combination test and HIV RNA testing following rapid point-of-care testing. In 2013, 58.5% of all CDC-funded HIV tests performed in persons at high risk for HIV infection were rapid HIV antibody tests[25] and in our study greater than 12% of HIV diagnoses were not detected with rapid HIV testing alone.

Pooled HIV-1 RNA testing detected almost all of the acute infections in this study, consistent with data from seroconversion studies demonstrating that HIV RNA can be detected approximately 6 days before the earliest detection of p24 antigen.[22] However, few HIV testing sites have access to a laboratory with the logistic capacity to perform timely pooled testing at a sustainable cost; including labor costs, HIV-1 RNA testing costs approximate $160.07 per test compared with $4.23 per test for Ag/Ab combination testing.[11] In

Figure 2. Flow of Additional HIV Testing on Specimens With a Reactive Rapid HIV Test Result



Ag indicates antigen; Ab, antibody.

[a] These specimens were reactive on HIV-1 RNA testing confirming HIV infection.

[b] These specimens had a negative result with additional testing.

Table 2. Diagnostic Yield With the Addition of Assays That Detect Acute HIV Infection

Testing Following a Rapid HIV Test	HIV Tests, No.	HIV Infections Detected With Rapid HIV Test, No. (%)	Additional HIV Infections Diagnosed (Absolute Increased Diagnostic Yield), No. (%) [95% CI]	Relative Diagnostic Yield Following a Rapid HIV Testing, % (95% CI)[a]
HIV Ag/Ab Combination Testing				
Overall	86 836	1158 (1.33)	134 (0.15) [0.13-0.18]	10.4 (8.8-12.2)
MSM only	44 955	956 (2.13)	121 (0.27) [0.22-0.32]	11.2 (9.4-13.3)
New York	36 617	603 (1.65)	56 (0.15) [0.12-0.20]	8.5 (6.4-10.9)
California	29 335	360 (1.23)	62 (0.21) [0.16-0.27]	14.7 (11.5-18.4)
North Carolina	36 617	195 (0.53)	16 (0.04) [0.02-0.07]	7.6 (4.4-12.0)
Pooled HIV RNA Testing				
Overall	86 836	1158 (1.33)	164 (0.19) [0.16-0.22]	12.4 (10.7-14.3)
MSM only	44 955	956 (2.13)	148 (0.33) [0.28-0.39]	13.4 (11.5-15.6)
New York	36 617	603 (1.65)	69 (0.19) [0.15-0.24]	10.3 (8.1-12.8)
California	29 335	360 (1.23)	77 (0.26) [0.21-0.33]	17.6 (14.2-21.5)
North Carolina	36 617	195 (0.53)	18 (0.05) [0.03-0.08]	8.5 (5.1-13.0)

Abbreviations: Ab, antibody; Ag, antigen; MSM, men who have sex with men.

[a] The relative diagnostic yield was calculated as the number of additional acute HIV infections diagnosed divided by the total number of HIV infections (sum of HIV infections detected with the rapid HIV test and additional acute HIV infection diagnosed).

Table 3. Characteristics and Risk Behavior Information of Men Who Have Sex With Men Stratified by HIV Status[a]

Variable	HIV Infection Status, No. (%)			Relative Risk of Acute HIV Infection (95% CI)[b]	*P* Value
	Acute (n = 151 [0.34%])	Established (n = 956 [2.13%])	Negative (n = 43 848 [97.54%])		
Site					
New York	62 (0.35)	542 (3.03)	17 306 (96.63)	1 [Reference]	
California	78 (0.31)	331 (1.31)	24 946 (98.39)	0.87 (0.63-1.22)	.42
North Carolina	11 (0.65)	83 (4.91)	1596 (94.44)	1.92 (1.01-3.63)	.046
Age, y					
<25	32 (0.40)	230 (2.89)	7704 (96.71)	1.46 (0.84-2.55)	.18
25-34	70 (0.34)	426 (2.09)	19 914 (97.57)	1.24 (0.75-2.03)	.40
35-44	29 (0.31)	184 (1.96)	9180 (97.73)	1.11 (0.63-1.96)	.72
≥45	20 (0.28)	115 (1.60)	7032 (98.12)	1 [Reference]	
Missing	0	1 (0.10)	18 (0.04)	NC	NC
Race/ethnicity					
Black/African American	28 (0.54)	262 (5.07)	4875 (94.39)	2.03 (1.31-3.15)	.002
Hispanic/Latino	39 (0.43)	230 (2.56)	8700 (97.00)	1.59 (1.07-2.35)	.02
White	66 (0.28)	328 (1.38)	23 379 (98.34)	1 [Reference]	
Asian	11 (0.26)	60 (1.41)	4172 (98.33)	0.93 (0.49-1.77)	.83
Other	2 (0.36)	12 (2.13)	549 (97.51)	1.29 (0.32-5.25)	.72
Unknown	5 (0.22)	64 (2.85)	2173 (96.92)	0.82 (0.33-2.02)	.66
Risk behaviors[c]					
Sex with a known HIV-positive partner	36 (0.47)	199 (2.62)	7368 (96.91)	1.55 (1.07-2.25)	.02
Injection drug use	8 (1.75)	29 (6.33)	421 (91.92)	5.68 (2.81-11.51)	<.001
Exchange sex for money or drugs	0	1 (2.94)	33 (97.06)	NC	NC
Sex while high on drugs or intoxicated	20 (0.23)	138 (1.56)	8662 (98.21)	0.62 (0.39-0.99)	.047

Abbreviation: NC, not calculated (1 cell included a 0).

[a] Characteristics and risk behavior information is self-reported at the time of testing. The test result (and information collected at that test event) is the unit of analysis. Participants can only be diagnosed with HIV infection (either acute or established) once in this study but participants who have an HIV test negative result can be tested again.

[b] Relative risk of acute HIV infection compared participants with acute HIV infection and participants with a negative HIV test result only (excluding established HIV infection in the denominator) and was calculated using a log-linked binomial regression model (GENMOD Procedure).

[c] The reference group for each self-reported risk behavior is all participants who did not report (by checking a box on the form) that particular risk behavior.

addition, health insurance can be billed for HIV Ag/Ab combination testing under a specific *Current Procedural Terminology* code and the cost is covered without patient copay by Affordable Care Act insurance plans and Medicaid because HIV screening has a US Preventive Services Task Force grade A recommendation.[26] In addition, HIV Ag/Ab combination testing can be performed in 30 minutes (if results are negative) to 60 minutes (if results are reactive), whereas the HIV RNA test requires 6 hours and the pooling process can delay results by 4 to 7 days.

Although rapid HIV testing has certain advantages such as the immediate provision of a result and the ability to test in nonclinical outreach settings, our findings indicate that the lower sensitivity for acute HIV infection is a major limitation.[23,27] The sensitivity of rapid, point-of-care HIV testing for acute HIV infection may improve with the introduction of rapid tests that can detect both HIV antigen and antibody[28] (approved by the FDA in 2014) or point-of-care HIV-1 RNA tests[29] (in development), although field testing of point-of-care HIV antigen/antibody assays have hitherto not had sufficient sensitivity for acute HIV infection.[30]

To address the low yield of currently available rapid HIV antibody tests for acute HIV infection, 3 testing strategies could increase the likelihood that high-risk individuals such as MSM are accurately diagnosed. In the first strategy, laboratory-based Ag/Ab combination testing would replace the rapid HIV test as the initial screening test. This strategy is consistent with the CDC/APHL HIV diagnostic testing algorithm, sensitive for acute HIV infection, and cost-effective, but patients would not receive an immediate result. This strategy could be particularly useful for MSM who (1) are testing on a regular basis, (2) are aware of the advantages of Ag/Ab combination testing and willing to be tested using phlebotomy, and (3) have reliable contact information to receive their results.

In a second strategy, the rapid HIV test would continue to be the initial screening test, and if preliminarily positive, confirmatory testing and linkage to care would be performed. If the result were negative, MSM and others at substantial risk of HIV would be offered an additional laboratory-based Ag/Ab combination test to diagnose acute HIV infection potentially missed with rapid testing. Although this approach is predicted to be cost-effective,[31] the positive predictive value of a reactive HIV Ag/Ab combination test after a negative rapid HIV test was only 59% in our study, highlighting the importance of confirming the diagnosis with an individual HIV RNA test and indicating that this approach would be unsuited to low-HIV prevalence populations.

In a third strategy, a rapid HIV test would be the initial screening test, and if negative, HIV RNA testing would be offered to MSM and others at substantial risk. This strategy is resource intensive but also the most sensitive for acute

HIV infection, and accordingly the most useful in settings with the highest rates of acute HIV infection. Of note, HIV RNA testing alone is not recommended, because approximately 3% to 5% of people with HIV will have a negative RNA test result due to undisclosed antiretroviral use or elite control of their HIV infection.[32] Each strategy has advantages in different settings, but all allow for acute HIV infection diagnosis, which is an important advantage over rapid HIV antibody testing alone.

This study has several limitations. First, HIV testing was conducted in high-risk populations (sexually transmitted infection clinics and testing venues that focus on HIV testing in MSM) and a majority of acute infections were detected in MSM. These results may not be generalizable to low-risk and non-MSM populations such as women. Nevertheless, MSM remain disproportionately affected by HIV,[18] and implementation of effective strategies to diagnose acute HIV infection in MSM and other at-risk populations is needed. Second, preexposure prophylaxis and postexposure prophylaxis were not widely used at the time of this study. Although there have not been false-negative results with assays that detect HIV antibody or HIV RNA in persons who become HIV infected while taking preexposure prophylaxis in clinical trials,[33] HIV detection assays should be further evaluated in this context as antiretroviral medications can suppress HIV RNA and delay seroconversion when given during acute infection.[34]

Conclusions

In a high-prevalence population, HIV screening using an HIV Ag/Ab combination assay following a negative rapid test detected 82% of acute HIV infections detectable by pooled HIV RNA testing, with a positive predictive value of 59%. Further research is needed to evaluate this strategy in lower-prevalence populations and in persons using preexposure prophylaxis for HIV prevention.

ARTICLE INFORMATION

Author Contributions: Dr Peters had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Peters, Westheimer, Patel, Hightow-Weidman, Gay.
Acquisition, analysis, or interpretation of data: Peters, Westheimer, Cohen, Hightow-Weidman, Moss, Tsoi, Hall, Fann, Daskalakis, Beagle, Patel, Radix, Foust, Kohn, Marmorino, Pandori, Fu, Samandari, Gay.
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