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BRIEF REPORT

Assessment of HIV Screening Tests for Use in Preexposure Prophylaxis Programs

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Preexposure prophylaxis programs involve frequent human immunodeficiency virus (HIV) testing. We evaluated the sensitivity of 2 antigen/antibody immunoassays (Architect and Bioplex), 2 antibody-based rapid tests (Vikia-HIV-1/2 and Autotest-VIH), and 1 antigen/antibody rapid test (Alere HIV Combo) for the diagnosis of HIV infection. Among the 31 HIV-1–infected participants in the ANRS-IPERGAY trial, HIV-1 RNA was detected alone in only 2. The sensitivities of the Architect and Bioplex assays were 83% (95% confidence interval [CI], 76%–99%) and 82% (95% CI, 63%–94%), respectively. The sensitivities of the Vikia, Autotest, and Alere tests were 54% (95% CI, 34%–72%), 50% (95% CI, 31%–69%), and 78% (95% CI, 58%–91%), respectively. Antigen/antibody tests should be preferred to avoid missing cases of acute HIV infection and to decrease the related risks of viral transmission and emergence of drug resistance.

Keywords. HIV-1; acute infection; recent infection; antigen/antibody test; rapid test; HIV diagnosis; PrEP; HIV-1 RNA.

Recently, randomized trials have demonstrated that preexposure prophylaxis (PrEP) with oral tenofovir/emtricitabine is safe and effective for preventing HIV infection in uninfected men who have sex with men (MSM), transgender women who have sex with men, and heterosexual men and women [1–6]. In view of this recent evidence, the World Health Organization (WHO) has recommended since 2015 that antiretroviral therapy be used as PrEP in populations with high-level exposure to HIV [7].

PrEP recipients are tested for HIV both before starting PrEP and regularly during PrEP, as drug resistance may occur if PrEP takes place despite ongoing infection. The WHO recommends use of quality-assured HIV tests and those most sensitive for recent infection, according to local availability [7]. HIV screening is generally based on 2 types of serological tests: antigen/antibody immunoassays (EIA-4Gs) or antibody rapid tests [8]. For individuals with symptoms of acute HIV infection, the use of an assay to measure the HIV-1 load is warranted.

The poor availability of EIA-4Gs in many healthcare settings has led to more widespread use of antibody-based rapid tests in PrEP programs. Here, we evaluated the performance of several HIV diagnostic tests, including an antigen/antibody rapid test, among high-risk MSM who contracted HIV-1 during the ANRS IPERGAY PrEP trial.

PATIENTS AND METHODS

Patients

The ANRS IPERGAY study was a double-blinded, randomized trial of PrEP for HIV-seronegative MSM with a high level of exposure to HIV. In November 2014, an open-label phase was begun, which lasted until June 2016 [5, 9].

HIV testing was performed at the screening visit and at each trial visit (M1, M2, and every 2 months thereafter), using one of the following HIV-1/2 EIA-4Gs: the Architect HIV Ag/Ab Combo assay (Abbott, Rungis, France) or the Liaison XL Murex HIV Ab/Ag HT assay (Diasorin, Antony, France). In case of suspected primary HIV infection, plasma HIV-1 RNA load was measured concomitantly, using the AmpliPrep/Cobas TaqMan HIV-1 test, version 2.0. The protocol required collection of serum and plasma samples at each study visit for storage at –80°C.

Methods

In case of a positive EIA-4G result, the HIV-1 RNA load was retrospectively measured in plasma stored at the previous visit, to estimate the timing of infection. All patients with a diagnosis of HIV infection (based on detection of HIV-1 RNA or positive results of an EIA-4G) were included in this substudy.

Frozen samples were retested with the Architect assay (mean p24 antigen sensitivity, 18.39 pg/mL; reactive index, >1). We also tested samples by use of the BioPlex 2200 HIV Ag-Ab assay (Bioplex; Biorad, Marnes-La-Coquette, France; mean p24 antigen sensitivity, 5.2 pg/mL; reactive index, >1).

We evaluated 2 CE-labeled HIV-1/2 antibody-based rapid tests: Vikia HIV-1/2 (Vikia; Biomerieux, Marcy-l'Etoile, France)

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and Autotest VIH (Autotest; AAZ, France). Sera were also tested with the new version of the antigen/antibody rapid test Alere HIV Combo (Alere; Alere, Jouy-en-Josas, France).

Using an HIV-1 Western blot (Biorad, Marnes-La-Coquette, France), we created 3 groups: (1) negative for antibodies to HIV-1, defined as patients with no detectable antibody; (2) incomplete antibody response to HIV-1, defined as patient with 1–6 antibodies; and (3) complete antibody response to HIV-1, defined as patient with ≥7 antibodies. Patients were also categorized according to adapted Fiebig infection stages.

Statistical Analysis

The sensitivity of the EIA-4G and rapid tests was determined by calculating the percentage of positive results yielded by these tests among all samples that tested positive for HIV (based on detection of HIV-1 RNA and positive result of the Architect test). Exact confidence intervals (CIs) were calculated according to the binomial distribution. An exact McNemar test, based on the binomial distribution, was used to compare side by side the sensitivities of the tests.

We searched for factors associated with rapid test positivity by comparing the following characteristics of HIV-positive and HIV-negative sera, using an exact Wilcoxon test for evaluation of continuous characteristics (ie, EIA index, number of antibodies detected by Western blot, Fiebig stage, $\log_{10}$ HIV-1 RNA level, and $CD4^+$ T-cell count) and an exact Fisher test for analysis of qualitative characteristics (ie, viral subtype).

RESULTS

Patients in Whom HIV-1 Infection Was Diagnosed in the IPERGAY Trial

Thirty-one patients received a diagnosis of HIV-1 infection among 478 high-risk MSM who were screened in the IPERGAY trial (Supplementary Table 1). Ten HIV-1–positive patients received a diagnosis at the screening visit, while 21 patients (19 during the blind phase and 2 during the open-label phase) acquired HIV-1 infection during the trial. During the blind phase, 3 participants received a diagnosis of HIV-1 infection after random assignment but before tenofovir disoproxil fumarate (TDF)/emtricitabine (FTC) or placebo uptake, and 16 (14 in the placebo arm and 2 in the TDF/FTC arm) received a diagnosis after TDF/FTC or placebo uptake. During the open phase, 2 participants received a diagnosis of HIV-1 infection (1 after screening but before TDF/FTC uptake and 1 during follow-up)

We retrospectively measured HIV-1 RNA levels in 19 available frozen plasma samples obtained at the visit preceding the first positive EIA-4G result. The HIV-1 load was detectable in only 2 cases (450 and 110 copies/mL in patients 1 and 2, respectively; Supplementary Table 1).

Overall, at the time of HIV diagnosis (ie, the time of the first positive EIA-4G result or the first detectable viral load), 7 patients (6 at screening and 1 later) had a complete antibody response on Western blot (median, 9 antibodies) and a median HIV-1 RNA load of 5.16 $\log_{10}$ copies/mL (range, 3.86–6.12 $\log_{10}$ copies/mL). Nine patients (2 at screening and 7 later) had an incomplete response (median, 3 antibodies) and a median load of 4.59 $\log_{10}$ copies/mL (range, 3.42–6.43 $\log_{10}$ copies/mL). A negative response was found for 14 patients (1 at screening and 13 after), with a median load of 6.69 $\log_{10}$ copies/mL (range, 2.04–8.18 $\log_{10}$ copies/mL). Western blot findings were missing for 1 screening visit.

Fiebig stage I was observed in 2 patients; stage II, in 9; stage III, in 3; stage IV, in 6; stage V, in 3; and stage VI, in 7; the disease stage for 1 patient could not be classified. Twenty of 31 patients were infected with HIV subtype B (65%), 7 with subtype CRF_02-AG (23%), and 4 (13%) with other non-B subtypes.

HIV-1/2 Antigen/Antibody Immunoassay Performance

We studied the ability of the EIA-4G to detect HIV-1 infection at the date of diagnosis. Stored samples were available for 28 of 31 diagnosed cases (7 of 7 with a complete antibody response on Western blot, 8 of 9 with an incomplete response, and 13 of 14 with a negative response). The results of the EIA-4Gs are shown in Table 1. For patients with an incomplete or complete antibody response on Western blot (patients 15–30; Supplementary Table 1), the sensitivity of both EIA-4Gs was 100%. For the 13 patients with a negative response (patients 1–14), the Architect test was positive in 11 cases (85%; 95% CI, 55%–98%), and the Bioplex test was positive in 8 cases (62%; 95% CI, 32%–86%). The difference in sensitivity between the Architect and Bioplex assays was not statistically significant ($P = .25$). Discordant results between the 2 EIA-4Gs were found in 3 cases (patients 3–5): the Architect results were positive (index values, 1.08, 1.25, and 2.24, respectively), and the Bioplex results were negative; HIV-1 RNA loads were 1 571 675, 138 442, and 130 030 copies/mL, respectively.

HIV-1/2 Rapid Test Performance

In 6 of 7 patients with a complete antibody response on Western blot, results of all 3 rapid tests were positive, for a sensitivity of 100% (95% CI, 54%–100%; Table 2). In the last case, results of the Vikia and Autotest assays were positive, but no serum was available for analysis by the Alere test. In the 8 patients with an incomplete response, the sensitivity was 75% (95% CI, 35%–97%) for the Vikia test, 88% (95% CI, 47%–100%) for the Autotest assay, and 100% (95% CI, 63%–100%) for the Alere assay. In the 13 patients with a negative response, the sensitivity was 15% (95% CI, 2%–45%) for the Vikia assay, 0% (95% CI, 0%–25%) for the Autotest assay, and 54% (95% CI, 25%–81%) for the Alere assay. Sensitivity was significantly better with the Alere test than with Vikia test (7 sera had Alere-positive and Vikia-negative results; none had Vikia-positive and Alere-negative results; $P = .016$). The Alere assay was also more sensitive than the Autotest assay (8 sera had Alere-positive

Table 1. Human Immunodeficiency Virus 1/2 Antigen/Antibody Immunoassay Performance, Based on Analysis of Stored Specimens From 28 Patients in the IPERGAY Trial

	Architect		Bioplex, Positive Results			
Western Blot Result	Positive Results	Index	For Ag and/or Ab	For Ag Only	For Ag and Ab	For Ab Only
Complete (n = 7)						
Specimens, no. or no. (%)	7	…	7	0	0	7 (100)
Sensitivity, % (95% CI)	100 (59–100)	…	100 (59–100)	…	…	…
Index, median (IQR)	…	416 (61–639)	…	…	…	…
Incomplete (n = 8)						
Specimens, no. or no. (%)	8	…	8	0	2 (25)	6 (75)
Sensitivity, % (95% CI)	100 (63–100)	…	100 (63–100)	…	…	…
Index, median (IQR)	…	9.8 (3.1–28.5)	…	…	…	…
Negative (n = 13)						
Specimens, no. or no. (%)	11	…	8	7 (54)	1 (8)	0
Sensitivity, % (95% CI)	85 (55–98)	…	62 (32–86)	…	…	…
Index, median (IQR)	…	52.2 (.1–1079)	…	…	…	…
Overall (n = 28)						
Specimens, no. or no. (%)	26	…	23	7 (25)	3 (11)	13 (46)
Sensitivity, % (95% CI)	83 (76–99)	…	82 (63–94)	…	…	…
Index, median (IQR)	…	40.4 (.1–1079)	…	…	…	…

See Methods for definitions of Western blot results. The sensitivity of the Bioplex test was calculated according to findings of the Architect test.

Abbreviations: Ab, antibody; Ag, antigen; Architect, Architect HIV Ag/Ab Combo test (Abbott); Bioplex, Bioplex 2200 HIV Ag-Ab assay (Biorad); CI, confidence interval; IQR, interquartile range.

and Autotest-negative results; none had Autotest-positive and Alere-negative findings; $P = .008$).

Sensitivity and Risk Factor Analysis

We compared the sensitivity of the 3 antigen/antibody assays: the Alere assay, the Architect EIA-4G, and the Bioplex assay. Four sera (from patients 3–5 and 7) had Architect-positive and Alere-negative results ($P = .125$), and 1 serum specimen (from patient 7) had Bioplex-positive and Alere-negative results ($P = 1$). None had Alere-positive and Architect- or Bioplex-negative results.

We then analyzed factors associated with negative rapid test results (Supplementary Table 2). A false-negative rapid test result was significantly associated with a lower number of antibodies detected on Western blot and an early Fiebig stage (for the Vikia, Autotest, and Alere tests) and with a lower Architect index (for the Vikia and Alere assays).

Discussion

Reliable HIV screening is critical both before PrEP initiation and regularly during PrEP (every 3 months in implementation

Table 2. Human Immunodeficiency Virus 1/2 Rapid Test Performance, Based on Analysis of Stored Specimens From 28 Patients in the IPERGAY Trial

			Alere, Positive Results			
Western Blot Result	Vikia, Positive Results	Autotest, Positive Results	For Ag and/or Ab	For Ag Only	For Ag and Ab	For Ab Only
Complete (n = 7)						
Specimens, no. or no. (%)	7	7	6[a]	0	0	6 (100)
Sensitivity, % (95% CI)	100 (59–100)	100 (59–100)	100 (54–100)	…	…	…
Incomplete (n = 8)						
Specimens, no. or no. (%)	6	7	8	0	0	8 (100)
Sensitivity, % (95% CI)	75 (35–97)	88 (47–100)	100 (63–100)	…	…	…
Negative (n = 13)						
Specimens, no. or no. (%)	2	0	7	4 (31)	3 (23)	0
Sensitivity, % (95% CI)	15 (2–45)	0 (0–25)	54 (25–81)	…	…	…
Overall (n = 28)						
Specimens, no. or no. (%)	15	14	21	4 (15)	3 (11)	14 (52)
Sensitivity, % (95% CI)	54 (34–72)	50 (31–69)	78 (58–91)	…	…	…

See Methods for definitions of Western blot results. The sensitivities of the Vikia, Autotest, and Alere tests were calculated according to findings of the Architect HIV Ag/Ab Combo test (Abbott).

Abbreviations: Ab, antibody; Ag, antigen; Alere, Alere HIV Combo (Alere; Autotest, Autotest VIH test (AAZ); CI, confidence interval; Vikia, Vikia HIV-1/2 test (Biomérieux).

[a]Data are missing for 1 patient with a complete Ab response detected by Western blot.

programs). Optimal HIV testing algorithms are needed to avoid missing cases of acute HIV infection acquired before or during PrEP and, thus, to lessen the risk of both ongoing transmission and selecting drug resistance.

During the ANRS IPERGAY trial, HIV-1 infection was diagnosed in 31 high-risk MSM. Ten men were HIV positive at the screening visit, of whom 6 had a complete antibody response on Western blot, suggestive of chronic infection, although this could not be confirmed in the absence of analysis of sera collected earlier. Among the 25 incident cases, 24 corresponded to acute or recent infection. Thus, regular HIV testing during the PrEP program led not only to the diagnosis of HIV infection in exposed persons who were unaware of their HIV status, but also to very early diagnosis. This is important, as nearly two thirds of new infections are attributed to transmission from patients who are unaware of their status [10]. Moreover, the risk of transmission during primary infection could account for up to 30%–70% of new infections among MSM.

Acute HIV infection represents a particular challenge for screening tests. In our study, the Architect test detected 85% of acute infections and failed to detect only 2 early infections, which had very low viral loads (<500 copies/mL). Using the Bioplex EIA-4G, which claims better sensitivity for p24 antigen detection (5 ng vs 17 ng), we missed 3 more infections, all with high viral loads (>5 $\log_{10}$ copies/ml). In a large prospective study of HIV testing in a high-prevalence US population, Peters et al reported that the Architect assay diagnosed 82% of acute HIV infections detectable by pooled HIV RNA testing and recommended its use in these populations [11]. EIA-4Gs can be performed rapidly (in 0.5–2 hours) and are cost-effective, but they require specific equipment and laboratory procedures.

Both antibody-based rapid tests (the Vikia and Autotest assays) gave positive results for 100% of patients with a complete antibody response on Western blot and for most patients with an incomplete response on Western blot; estimated sensitivities were 78% and 89% for the Vikia and Autotest assays, respectively. As previously observed [12, 13], these antibody-based rapid tests performed poorly during acute infection with a negative antibody response on Western blot (sensitivities of 15% and 0% for the Vikia and Autotest assays, respectively). Indeed, most cases of de novo drug resistance during PrEP trials occurred in patients with undiagnosed infection and negative rapid test results who initiated PrEP during the acute phase of infection [1, 4, 6].

As the first version of the Alere antigen/antibody rapid test (which is approved by the Food and Drug Administration) failed to identify most cases of acute infection in a high-risk population [12, 14], we tested the new version Alere HIV Combo (which has a CE mark) of the Alere test, which had positive results for 100% of sera with incomplete and complete antibody responses on Western blot and for 54% with negative responses on Western blot. We confirm that this test is more sensitive than other rapid tests for detecting acute infection, as previously shown on a seroconversion panel [15]. In our hands, the performance of the Alere test was not significantly different from that of the EIA-4Gs, but our panel only included 13 samples with negative responses on Western blot.

Our study has several limitations. First, it was not designed to prospectively evaluate the performance of the different diagnostic tests. We compared the sensitivities of the Bioplex and rapid tests by using cases for which the Architect test yielded positive results or HIV-1 RNA was detected in plasma. It should be noted that our results, which were obtained with frozen sera, may overestimate the performance of rapid tests when applied to whole-blood specimens collected by finger prick, the usual sample type [12]. Finally, we tested a relatively small number of HIV-positive samples, although diagnoses and investigations for some occurred at a very early infection stage (Fiebig stages I or II).

We confirm that screening tests (EIAs or rapid tests) that combine p24 antigen and antibody detection seem most appropriate for the diagnosis of acute HIV infection. With widespread implementation of PrEP, specific local guidelines for HIV testing are needed and will depend on the availability, feasibility, simplicity, and cost of these tests in each setting. Importantly, as the majority of primary infections are asymptomatic, especially when diagnosed early, retesting 4 weeks after the initiation of PrEP with the same test should be highly recommended, to detect the rare primary infection missed with the first test.

STUDY GROUP MEMBERS

Members of the ANRS IPERGAY Study Group are specified in the Supplementary Materials.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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