Evaluation of the Performance Characteristics of 6 Rapid HIV Antibody Tests

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Background. Since 2002, the US Food and Drug Administration has approved 6 rapid human immunodeficiency virus (HIV) tests for use in the United States. To date, there has been no direct comparison of the performance of all 6 tests.

Methods. Persons known to be HIV-infected and persons who sought HIV testing at 2 clinical sites in Los Angeles, California, were recruited for evaluation of 6 rapid HIV tests with whole blood, oral fluid, serum, and plasma specimens. Sensitivity and specificity of the rapid tests were compared with viral lysate and immunoglobulin (Ig) M–sensitive peptide HIV enzyme immunoassays (EIAs).

Results. A total of 6282 specimens were tested. Sensitivity was >95% and specificity was >99% for all rapid tests. Compared with the IgM-sensitive EIA, rapid tests gave false-negative results with an additional 2–5 specimens. All rapid tests had statistically equivalent performance characteristics, based on overlapping confidence intervals for sensitivity and specificity, compared with either conventional EIA.

Conclusions. All 6 rapid tests have high sensitivity and specificity, compared with that of conventional EIAs. Because performance was similar for all tests and specimen types, other characteristics, such as convenience, time to result, shelf life, and cost will likely be determining factors for selection of a rapid HIV screening test for a specific application.

In 1998, when the Centers for Disease Control and Prevention (CDC) encouraged the use of rapid human immunodeficiency virus (HIV) tests to increase the receipt of results among persons tested for HIV [1], only the Single Use Diagnostic System for HIV-1 (SUDS) was commercially available in the United States [2]. Since 2002, the US Food and Drug Administration (FDA) has approved 6 rapid HIV tests [3] that have become integral to initiatives designed to promote more widespread HIV testing [4–10].

Rapid HIV antibody tests provide results in <30 min [3]. FDA-approved rapid HIV tests (Table 1) employ either immunochromatography (lateral flow) or immunoconcentration (flow-through) techniques [11] and contain antigens that correspond to envelope regions of HIV-1 (gp41, gp120, or both). Some tests also have an HIV type 2 (HIV-2) envelope (gp36) antigen. However, recent studies have documented that rapid HIV tests have lower sensitivity, especially during early infection, than that of some conventional assays [12–14]. False-negative test results have also been observed in individuals with advanced disease [15] and in some persons who are receiving effective antiretroviral therapy (ART) [16, 17]. Because test manufacturers do not explicitly identify which reference tests were used to calculate sensitivity and specificity (Table 1), this study was undertaken to compare contemporary rapid HIV tests and conventional enzyme immunoassays (EIAs) when performed on specimens from the same persons.

METHODS

The two-phase field study was conducted at the Los Angeles Gay and Lesbian Center (LAGLC), an HIV testing
clinic primarily for high-risk men who have sex with men (MSM), and the Altamed Clinic, a primary health care center in Los Angeles primarily serving Hispanic men and women at high risk for HIV infection. During both phases, rapid HIV tests were offered to residents of Los Angeles County >18 years of age.

During phase I (June–September 2003), persons with a previous diagnosis of HIV infection were recruited to obtain a sufficient sample size to assess rapid test sensitivity. These HIV-infected participants were asked about current ART usage. During phase II (June 2003–August 2005), persons of unknown HIV status were offered rapid HIV tests. Demographic and risk characteristics of participants were collected at the time of testing by clinic staff and entered into the Los Angeles County HIV Information Resources System.

After enrollment and consent for HIV testing and specimen storage, all participants provided capillary (fingerstick) whole blood, anticoagulated (EDTA) venous whole blood, serum, and oral fluid specimens. After testing, remnant serum and plasma specimens were stored at −70°C at the Los Angeles County Health Department Laboratory during the study and, after identifiers were removed, transferred to the CDC at the end of the study.

The OraQuick Advance HIV 1/2 Antibody test (OraSure Technologies) was performed on oral fluid, capillary and anticoagulated whole blood, and plasma specimens. The Uni-Gold Recombigen HIV test (Trinity Biotech) was performed on whole blood and either serum or plasma samples. The OraQuick HIV-1 Antibody Test (MedMira) and Multispot HIV-1/HIV-2 Rapid Test (Bio-Rad Laboratories) were performed on serum and plasma. Two tests manufactured by Chembio Diagnostics, the HIV Stat-Pak and Surecheck HIV, were performed on whole blood and plasma specimens. (The Stat-Pak and SureCheck tests are now distributed as the Clearview HIV 1/2 STAT-PAK and Clearview COMPLETE HIV 1/2 rapid tests, respectively). The 2 Clearview tests have different configurations but identical test strips and antigens.

When possible, all rapid tests were performed on fresh specimens by trained staff. However, not all tests were available throughout the study; Multispot and Uni-Gold tests were performed on some stored specimens at the CDC HIV serology laboratory from August 2007 through August 2008. Rapid tests that produced false-positive or false-negative results were also repeated on stored specimens at the CDC to establish whether the discordant rapid test result was reproducible.

Reference tests were performed in the Los Angeles County Public Health Laboratory on a simultaneously collected serum specimen. Specimens were screened with the Vironostika HIV-1 Microelisa System (bioMérieux), a whole viral lysate EIA. Specimens with repeatedly reactive Vironostika results were tested with the GS HIV-1 Western blot (Bio-Rad Laboratories).
At the time of the study, the Vironostika was the screening EIA most widely used by public health laboratories [18], but it was withdrawn from the market in the summer of 2008. To compare rapid HIV test performance with contemporary EIAs that detect both immunoglobulin (Ig) G and IgM antibodies, stored plasma specimens were tested with the Bio-Rad GS HIV 1/2 PLUS O EIA (Bio-Rad Laboratories) at the CDC HIV serology laboratory.

Sensitivity and specificity of the rapid tests with exact 95% confidence intervals (CIs) [19] were first calculated on the basis of the results of the Vironostika and Western blot assays. For phase I, sensitivity was calculated separately for persons who were and were not receiving ART. For phase II, the sensitivity and specificity of the rapid HIV tests and Vironostika were also calculated, compared with the Bio-Rad test. This IgM-sensitive assay has been shown to detect HIV antibody up to 15 days earlier than the Vironostika and Western blot and at least 5 days before some rapid HIV tests [13]. Specimens with negative Vironostika results but repeatedly reactive Bio-Rad results were tested with Western blot, and specimens with repeatedly reactive Vironostika or Bio-Rad results and negative or indeterminate Western blots were excluded from calculations of rapid test sensitivity and specificity but were later tested with the APTIMA HIV-1 Qualitative RNA assay (Gen-Probe).

This study protocol was approved by the CDC and study site institutional review boards. Participants only received results of FDA-approved tests.

RESULTS

A total of 6282 study participants had results for at least 1 rapid test. Median age of participants was 32 years; 84% were male, 43% were non-Hispanic white, and 35% were Hispanic. Male sex was reported as the main risk factor for HIV infection by 69% of participants. Eighty-five percent of participants reported a previous HIV test, and 16% reported a previous rapid HIV test.

During phase I, 493 persons with known HIV infection were enrolled. Sensitivity for each of the 6 rapid tests ranged from 97.67% to 100.0% (Table 2). A total of 13 specimens from persons receiving ART had a false-negative result on at least 1 rapid test. For 3 tests (COMPLETE, OraQuick, and STAT-PAK), sensitivity was slightly lower for persons receiving ART than for those who were not, but the differences were not statistically significant (Table 2).

During phase II, 5789 participants were enrolled. Of these, 280 (4.8%) tested positive and 5505 tested negative by the Vironostika/Western blot algorithm. Four specimens with repeatedly reactive Vironostika results and indeterminate Western blot results were excluded from analysis. Sensitivity of the rapid tests performed on either fresh or stored specimens ranged from 96.76% to 100.0% (Table 3). Although the point estimate of sensitivity was lower than the manufacturer’s claim for several rapid tests, the only test for which the upper limit of the confidence interval for sensitivity (Table 3) was less than the lower limit of the confidence interval reported in the package insert (Table 1) was Uni-Gold, with whole blood and plasma specimens. Sufficient plasma was available to retest 6 of the 8 specimens with false-negative Uni-Gold results, and 5 of these were reactive on repeat testing, increasing Uni-Gold’s sensitivity to 99.59% (95% CI, 97.75%–99.99%). Of 19 phase II specimens with any false-negative rapid test results, 15 (79%) were false-negative on only 1 test, and 4 (21%) were false-negative on at least 2 different rapid tests: 1 on STAT-PAK, Uni-Gold, and Reveal, 1 on OraQuick and Uni-Gold, and 1 on STAT-PAK and Uni-Gold. The fourth specimen had false-negative results on all rapid tests performed on-site (OraQuick, Uni-Gold, Reveal, and both Clearview tests). Specimens from this participant tested positive by Vironostika and Western blot at the Los Angeles County Public Health Laboratory, and stored plasma tested positive with Uni-Gold, Multispot, Bio-Rad GS HIV 1/2 PLUS O EIA, and Western blot at the CDC.

Of the 5505 phase II participant specimens with negative Vironostika results, the number of fresh specimens tested on-site ranged from 3590 (Uni-Gold with whole blood) to 5475 (Reveal with plasma). Specificity of the rapid tests performed on either fresh or stored specimens ranged from 99.32% to 99.98% (Table 3). Of the 47 specimens with false-positive test results on at least 1 rapid test, 43 (91%) had false-positive results on a single rapid test, and 4 (9%) had false-positive results on 2 different tests: 1 each of Multispot and Reveal, Multispot and Uni-Gold, and Multispot and STAT-PAK, and 1 on OraQuick (oral fluid and blood) and STAT-PAK (blood). Thirty-three specimens had false-positive test results with Multispot (Table 3), including 2 fresh specimens that were tested on-site and 31 stored specimens that were tested at the CDC. When the 33 specimens were retested, only 11 had repeatable false-positive results, increasing the specificity of Multispot to 99.77% (95% CI, 99.59%–99.89%).

Of the 5789 phase II specimens, 5405 had sufficient plasma to be tested with the Bio-Rad GS HIV 1/2 PLUS O EIA at the CDC. All 259 specimens that had results positive by Vironostika and Western blot gave positive Bio-Rad results, but 18 of 5142 specimens with negative Vironostika results were repeatedly reactive according to the Bio-Rad test. At the CDC, 4 of these 18 specimens had positive results, 5 had indeterminate results, and 9 had negative results by Western blot. Lastly, among the 4 specimens with a repeatedly reactive Vironostika result and an indeterminate Western blot when tested in Los Angeles, 3 had positive Bio-Rad GS HIV 1/2 PLUS O EIA and Western blot results and 1 had negative Bio-Rad GS HIV 1/2 PLUS O EIA results. Thus, the rapid test
results could be compared with 266 specimens that were positive and 5125 specimens that were negative according to the Bio-Rad/Western blot algorithm.

Based on the Bio-Rad EIA/Western blot algorithm, point estimates for rapid test sensitivity ranged from 95.38% to 99.44% and from 99.35% to 99.98% for specificity (Table 3). Confidence intervals for these measures overlapped for all rapid tests. Additionally, confidence intervals for sensitivity and specificity using the Bio-Rad EIA/Western blot overlapped those calculated using the Vironostika/Western blot standard for each of the rapid tests.

The APTIMA test was performed on the 14 Bio-Rad EIA–reactive specimens that had negative results on all rapid tests and had negative or indeterminate Western blot results. Two of 5 specimens with indeterminate results and 2 of 9 specimens with negative results had detectable RNA.

**DISCUSSION**

This study provides the first head-to-head comparison of all 6 rapid HIV tests recently approved by the FDA using specimens from the same persons. The sensitivity and specificity of all tests were high with overlapping 95% CIs for both different tests and different specimen types. Sensitivity of some rapid tests was slightly lower for specimens from participants receiving ART, but the differences did not reach statistical significance, and one would not expect rapid HIV tests to be used to test HIV-positive persons taking ART. We also compared the sensitivity of the rapid tests to a contemporary IgM-sensitive EIA, which detected 2 Western blot–positive specimens that had negative results on all rapid tests. The decrease in sensitivity of the rapid tests relative to the IgM-sensitive assay was not statistically significant in this sample despite both high prevalence and high incidence of HIV infection, but observations in samples from other high-risk populations suggest that it may be clinically relevant [14, 20]. Persons with a recent potential exposure and those with ongoing high risk for HIV infection who have negative rapid test results should be counseled to be retested [21].

Two of the tests initially showed performance that was less than that claimed by the manufacturer’s package inserts. Uni-Gold’s sensitivity with whole blood and plasma specimens was outside the 95% CI specified in the package insert, but our sample size of prospectively identified HIV-positive specimens used for sensitivity calculation was small, and the 95% CIs for Uni-Gold’s sensitivity overlapped those of nearly all of the other tests. Multispot’s specificity was also below the lower bound of the 95% CI specified in the package insert and, in our sample, statistically significantly lower than that of the other tests. Multispot’s specificity was also below the lower bound of the 95% CI specified in the package insert and, in our sample, statistically significantly lower than that of the other tests. However, 31 of 33 of these false-positive results occurred with test kits from 1 lot used to test previously frozen specimens retrospectively at the CDC. When the Multispot test was repeated on these 33 stored specimens with a new lot of test kits, only 11 had false-positive results. Thus, the false-positive rate that we observed might be partially related both to the test kits

<table>
<thead>
<tr>
<th>Rapid test, specimen type</th>
<th>Reference positive (n=387)</th>
<th>False negative</th>
<th>Sensitivity, % (95% CI)</th>
<th>Reference positive (n=106)</th>
<th>False negative</th>
<th>Sensitivity, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearview COMPLETE</td>
<td>Whole blood</td>
<td>384</td>
<td>5</td>
<td>98.70 (96.99–99.58)</td>
<td>103</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>383</td>
<td>4</td>
<td>98.96 (97.35–99.71)</td>
<td>103</td>
<td>0</td>
</tr>
<tr>
<td>Clearview STAT-PAK</td>
<td>Plasma</td>
<td>383</td>
<td>6</td>
<td>98.43 (96.62–99.42)</td>
<td>106</td>
<td>0</td>
</tr>
<tr>
<td>OraQuick Advance HIV-1/2</td>
<td>Whole blood</td>
<td>386</td>
<td>3</td>
<td>99.22 (97.75–99.84)</td>
<td>106</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Oral fluid</td>
<td>386</td>
<td>9</td>
<td>97.67 (95.62–98.93)</td>
<td>106</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>258</td>
<td>0</td>
<td>100.0 (98.85–100.0)</td>
<td>69</td>
<td>0</td>
</tr>
<tr>
<td>Multispot</td>
<td>Plasma</td>
<td>376</td>
<td>0</td>
<td>100.0 (99.21–100.0)</td>
<td>103</td>
<td>0</td>
</tr>
<tr>
<td>Reveal G3</td>
<td>Serum</td>
<td>383</td>
<td>0</td>
<td>100.0 (99.22–100.0)</td>
<td>103</td>
<td>0</td>
</tr>
<tr>
<td>Uni-Gold Recombigen</td>
<td>Plasma</td>
<td>384</td>
<td>0</td>
<td>100.0 (99.22–100.0)</td>
<td>106</td>
<td>0</td>
</tr>
</tbody>
</table>

**NOTE.** CI, confidence interval.
### Table 3. Performance of 6 Rapid Human Immunodeficiency Virus (HIV) Tests Compared with the 2 Reference Standards: bioMérieux Vironostika HIV-1 Microelisa with GS HIV-1 Western blot and Bio-Rad GS HIV 1/2 PLUS O EIA with GS HIV-1 Western blot

<table>
<thead>
<tr>
<th>Test</th>
<th>Specimen type</th>
<th>bioMérieux Vironostika HIV-1 Microelisa with GS HIV-1 Western Blot</th>
<th>Bio-Rad GS HIV 1/2 PLUS O EIA&lt;sup&gt;a,b&lt;/sup&gt; with GS HIV-1 Western Blot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Reference positive</td>
<td>False negative</td>
</tr>
<tr>
<td>Clearview COMPLETE</td>
<td>Whole blood</td>
<td>213</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>213</td>
<td>0</td>
</tr>
<tr>
<td>Clearview STAT-PAK</td>
<td>Whole blood</td>
<td>246</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>246</td>
<td>4</td>
</tr>
<tr>
<td>OraQuick Advance HIV-1/2</td>
<td>Whole blood</td>
<td>277</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Oral fluid</td>
<td>280</td>
<td>5</td>
</tr>
<tr>
<td>Reveal G3</td>
<td>Plasma</td>
<td>278</td>
<td>3</td>
</tr>
<tr>
<td>Uni-Gold Recombigen</td>
<td>Whole blood</td>
<td>178</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>247</td>
<td>8</td>
</tr>
<tr>
<td>Vironostika HIV-1 Microelisa</td>
<td>Serum</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

<sup>a</sup> Based on testing stored specimens at the Centers for Disease Control and Prevention HIV Serology laboratory. Includes 7 specimens re-classified as reference positive on the basis of repeatedly reactive Bio-Rad GS HIV 1/2 PLUS O EIA and positive GS HIV-1 Western blot results. One specimen that had repeat reactive results using Vironostika and an indeterminate Western blot result was reclassified as reference negative on the basis of negative Bio-Rad GS HIV 1/2 PLUS O EIA results.

<sup>b</sup> Excludes 5 specimens that were repeatedly reactive on the Bio-Rad GS HIV 1/2 PLUS O EIA but had indeterminate GS HIV-1 Western blot results and 9 specimens that were repeatedly reactive on the Bio-Rad GS HIV 1/2 PLUS O EIA but had negative GS HIV-1 Western blot results.

<sup>c</sup> Specimens with initially reactive bioMérieux Vironostika HIV-1 Microelisa results. Twenty-one of 26 specimens were nonreactive when tests were repeated, 4 were repeatedly reactive but GS HIV-1 Western blot negative, and 1 was repeatedly reactive but GS HIV-1 Western blot indeterminate. All 26 specimens had negative Bio-Rad GS HIV 1/2 PLUS O EIA results.
that were used and the specimens that were tested. Other clusters of false-positive rapid HIV test results have been reported [22–26], but this is the first report of such a cluster with Multispot.

The high sensitivity and specificity of these rapid tests appear to be adequate to justify their use for screening. The high specificity of initial reactive rapid test results (higher than that of a single reactive Vironostika result) also supports providing patients with results from a single reactive rapid test. Nevertheless, the preliminary nature of a single reactive rapid test and the need for additional testing should be emphasized [1]. The identification of 2 specimens with reactive rapid test and positive Western blot results but negative Vironostika results corroborates earlier observations and supports the CDC’s recommendation for appropriate supplemental tests to confirm reactive rapid tests even if a subsequent screening EIA has negative results [27]. Additionally, 3 specimens with reactive rapid test, Vironostika, and Bio-Rad EIA results with indeterminate Western blot results from the Los Angeles laboratory were interpreted as having positive Western blot results when analyzed subsequently at the CDC. This underscores the subjective nature of the Western blot and the importance of follow-up testing for all reactive rapid test results that have discordant confirmatory test results [27, 28].

Four specimens had negative results on all rapid tests, a repeatedly reactive Bio-Rad EIA result, and a negative or indeterminate Western blot but detectable RNA. These results highlight the pitfalls inherent with use of the Western blot as the gold standard for confirmation, as well as the pitfalls of relying on sensitivity claims without knowing which assay was used for comparison. Strategies that detect RNA before antibodies develop have been evaluated [14, 20, 29–31], and tests that detect both p24 antigen and antibodies to HIV are now available [14, 20, 32–35]. As initial HIV screening tests continue to evolve and become more sensitive for early HIV infection, alternative testing algorithms [36, 37] that include RNA are needed to accurately establish or rule out a diagnosis of HIV infection.

This study is subject to several limitations. First, all tests were performed by trained staff with strict attention to recommended procedures and quality assurance. Personnel with less experience or training might not achieve comparable results. Second, most rapid tests are designed for use with fresh specimens. Because not all tests were consistently available throughout the study, some of our results include specimens that had been frozen, stored, and tested later. This allowed us to maximize comparisons of different tests conducted on specimens from the same participants, but it may not accurately represent the performance of these rapid tests as they are intended to be used. However, the only observed difference in test performance comparing stored and fresh specimens occurred with the Multispot test, which was not reproducible when the stored specimens were retested. Third, some of our point estimates for performance are based on different denominators. Percentages calculated using small denominators might appear to exaggerate differences in performance that are not statistically significant. For example, the sensitivity of the OraQuick test performed on oral fluid specimens from phase 1 patients receiving ART (97.67%) appears to be worse than the sensitivity claimed by the package insert, despite the fact that the 95% CI of this estimate is wide. Finally, appraising the performance of HIV screening tests is complicated by the need to use a composite reference standard [38]. Sensitivity is compared with a single assay, but specificity is compared with an algorithm of a repeatedly reactive initial test and a positive supplemental test result. Of note, using this composite reference standard, the specificities of almost all of the rapid tests that we evaluated exceeded that of the Vironostika assay, which was, until recently, the test most commonly used for initial screening.

Compared with rapid tests used to detect other diseases, which often have sensitivities or specificities <90% [39], performance expectations for HIV tests are extremely high. All 6 rapid HIV tests demonstrated excellent performance with different types of specimens. Other characteristics that we did not evaluate, such as convenience, time to result, shelf-life, and cost [40], will likely prove to be more important than differences in test performance when selecting a rapid HIV test for a specific application.


