JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2008, p. 1494–1497 Vol. 46, No. 4
0095-1137/08/$08.00+0 doi:10.1128/JCM.01945-07

Assessment of Rapid Tests for Detection of Human Immunodeficiency Virus-Specific Antibodies in Recently Infected Individuals[▽]

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Received 2 October 2007/Returned for modification 11 January 2008/Accepted 22 January 2008

We have evaluated four current Food and Drug Administration-cleared rapid tests for human immunodeficiency virus (HIV)-specific antibodies with a panel of specimens from recently infected individuals. Recent infection was detected by RNA-based screening coupled with enzyme immunoassay-based testing. We found that the sensitivities of the various rapid tests vary greatly with regard to their ability to detect HIV-specific antibodies in recently infected individuals.

The persistence of the human immunodeficiency virus (HIV) pandemic is in part the result of the inability to comprehensively test all at-risk individuals. Even when at-risk individuals submit to laboratory testing, the inherent limitations of laboratory-based testing can lead to the failure to identify or inform infected individuals. Such limitations include the window periods associated with antibody-based testing (the time between infection and the generation of detectable antibodies in the blood) and the limited sensitivities of certain antibody tests (2, 3, 11, 22). Moreover, the turnaround time associated with the logistics of laboratory-based testing can result in patients not obtaining their test results (6, 7, 9, 17, 20). Point-of-care testing (rapid testing) for HIV infection seeks to broaden the capacity of the public health and medical communities to identify and to inform infected individuals. Rapid tests are easy to perform and can give conclusive results within minutes, making them amenable for use in outreach centers, emergency rooms, doctor's offices, and clinics.

Currently, several rapid testing product options exist. In the United States, three such tests are cleared and classified as "waived" with regard to their complexity by the Food and Drug Administration (FDA): the OraQuick Advance rapid HIV-1/2 antibody test (OraSure Technologies, Bethlehem, PA), the Uni-Gold Recombigen HIV test (Trinity, Berkeley Heights, NJ), and the Clearview HIV 1/2 Stat-Pak test (Inverness, Louisville, CO). Those tests use a lateral flow device, whereby patient samples are drawn over HIV antigen-containing strips upon mixture with antibody detection reagents. A fourth FDA-cleared test, the Multispot HIV-1/HIV-2 rapid test (Bio-Rad, Hercules, CA), uses a flowthrough cartridge module system and is considered "moderately complex" by the FDA; as such, the test cannot be performed by a nonlaboratorian.

In communities with a high prevalence of individuals infected with HIV or in communities where at-risk individuals submit to testing on a regular basis, the ability to detect HIV infection in recently infected individuals is of paramount importance. For that reason, we have initiated, as others have done elsewhere (4, 13, 14, 15, 18), a strategy to identify recently infected individuals through the use of pooled RNA testing. In doing so, we have generated a panel of specimens from individuals who have recently been infected, as evidenced by the patient's history and the presence of HIV RNA simultaneously with a negative serological status for HIV-specific antibodies. This panel can serve a key function for the evaluation of antibody tests because it allows different HIV-specific antibody tests to be assessed for their sensitivities with specimens that may have relatively low anti-HIV immunoglobulin G (IgG) titers or that may contain only IgM. Previous studies have evaluated the sensitivities of various rapid tests, including analyses with seroconversion panels (1, 5, 8, 10, 12, 16, 19). However, no work to date has comprehensively evaluated the performance characteristics of all FDA-approved devices. In the present study, we assessed the relative sensitivities of several available rapid tests for HIV-specific antibodies using a panel of specimens from recently infected individuals. The ability of each of four different commercially available and FDA-cleared rapid tests to discern HIV serologic status was compared to that of laboratory-based enzyme immunoassays (EIAs), in addition to an RNA-based test (the Versant [version 3.0] branched DNA assay; Siemens, Berkeley, CA). We have found that the rapid testing options currently available in the United States possess significantly different sensitivities with regard to their abilities to discern HIV infection in recently infected individuals.

Between October 2003 and June 2007, surveillance for recent HIV infection through a strategy of pooled HIV RNA testing (13) led to the identification of 42 specimens (of 13,121 specimens tested) that contained HIV RNA but that were nonreactive by an initial antibody screening test. Initial negative antibody screening test results were achieved by either the OraQuick Advance (OraSure Technologies) rapid test (in 18 cases), the Vironostika HIV-1 Microelisa (bioMerieux Inc., Durham, NC) (a first-generation EIA; 22 cases), and the Genetic Systems HIV-1/HIV-2 PLUS O EIA (Bio-Rad, Redmond, WA) (a third-generation EIA; 2 cases). A portion of

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▽ Published ahead of print on 30 January 2008.

this panel (the first 19 specimens, specimens A through S) had been used to evaluate the capability of a third-generation EIA (the Genetic Systems HIV-1/HIV-2 PLUS O EIA), and the results were published previously (11). Since the time of that publication, the size of this panel has increased to 42 specimens. We sought to use this expanded panel of specimens from recently infected individuals to evaluate the sensitivities of four current, FDA-approved rapid tests for the detection of HIV-specific antibodies.

For all samples shown in Table 1, plasma was prepared from freshly drawn specimens and was either tested immediately by a screening test or stored at −80°C. The specimens remained at −80°C until they were analyzed by all rapid tests. This method was within the parameters specified by each manufacturer's documented recommendations with the exception of those for the OraQuick Advance test, for which the storage conditions are not indicated. The rapid antibody tests that were used included the OraQuick Advance rapid HIV-1/2 antibody test (with 5 μl of plasma tested), the Clearview HIV 1/2 Stat-Pak test (Inverness) (with 5 μl of plasma tested), the Uni-Gold Recombigen HIV test (Trinity) (with 50 μl of plasma tested), and the Multispot HIV-1/HIV-2 rapid test (with 30 μl of plasma tested). The OraQuick Advance, Clearview HIV 1/2 Stat-Pak, and Uni-Gold Recombigen tests are all considered to have low levels of complexity when they are used with whole blood. However, when they are used according to the package insert with stored plasma specimens (as we used them in the present study), the tests are considered to be moderately complex. The Multispot HIV-1/HIV-2 test is a flowthrough rapid test that is categorized as moderately complex in every instance in which it is used. In parallel, the same specimens were evaluated by two EIAs, the Vironostika HIV-1 Microelisa and the Genetic Systems HIV-1/HIV-2 PLUS O EIA, and with the Cambridge Biotech HIV-1 Western blot kit (Calypte Biomedical, Rockville, MD) or the Genetic Systems HIV-1 Western blot kit (BioRad, Redmond, WA).

Of the 42 HIV RNA-containing specimens evaluated, 14 were found to be reactive for HIV-specific antibodies by the Genetic Systems HIV-1/HIV-2 PLUS O EIA (a third-generation EIA), while none of the specimens were found to be reactive by the Vironostika HIV-1 Microelisa (a first-generation EIA). We next tested the panel of 42 specimens from recently infected individuals using a variety of commercially available rapid HIV-specific antibody tests. When the panel of 42 specimens was tested by either the OraQuick Advance test or the Clearview HIV-1/2 Stat-Pak test, one specimen (specimen I) was found to be reactive. The Multispot HIV-1/HIV-2 test detected HIV-1 antibody in 7 of the 42 specimens (specimens F, I, P, S, W, AF, and AP), and for all seven specimens, follow-up specimens were confirmed to be positive by Western blotting. The Uni-Gold Recombigen HIV-specific antibody rapid test was reactive with 11 of the 42 specimens (specimens C, F, I, K, L, P, S, W, AF, AL, and AP). These 11 specimens included the specimen which tested positive by all rapid tests (specimen I) and all of the specimens which were positive by the Multispot HIV-1/HIV-2 test (specimens F, I, P, S, W, AF, and AP). Two of these 11 specimens (specimens L and AF) were nonreactive by the third-generation EIA (the Genetic Systems HIV-1/HIV-2 Plus O EIA). All 11 specimens were confirmed to be positive when follow-up specimens were tested.

To confirm the initial findings of the rapid tests, as described above, we determined the performance of the rapid tests with samples from several of the members of the primary infection panel who returned for follow-up testing. Follow-up specimens were submitted from 10 to 225 days (median, 20 days) after submission of the initial reactive specimens. Confirmation of infection required either a positive immunofluorescent antibody assay (IFA) or Western blotting result. Overall, follow-up testing confirmed infection in 36 of the 42 individuals in the panel; however, follow-up specimens for rapid test analyses were available only from 30 of the 42 patients. The Uni-Gold Recombigen and the Multispot HIV-1/HIV-2 tests were reactive for all 30 follow-up specimens. The OraQuick Advance test was reactive for 26 of the 30 follow-up specimens, while the Clearview HIV 1/2 Stat-Pak test was reactive for 29 of the 30 specimens. Note that for specimen D, actual infection was called into question, given the fact that no follow-up specimen from this patient was ever made available. Moreover, the extremely low viral load found in specimen D further calls into question the actual infection status of this patient.

To establish quality control for all of the rapid tests used in this study, we tested each of the FDA-cleared rapid tests used here with 100 specimens that were established to be negative for HIV-1 antibody by a first-generation EIA (Vironostika) and that contained no detectable HIV RNA. In this assessment, none of the complexity-waived rapid tests (the OraQuick Advance, Clearview HIV 1/2 Stat-Pak, and Uni-Gold Recombigen tests) showed any reactivity. However, the Multispot HIV-1/HIV-2 test reacted with five specimens nonspecifically. All waived rapid tests were also tested with 55 specimens confirmed to be positive for HIV-specific antibodies by a first-generation EIA (Vironostika) and IFA. All tests were reactive with all 55 specimens.

The data described here indicate potentially substantial differences in the sensitivities of rapid HIV-specific antibody tests for the detection of HIV infection during early antibody seroconversion. The Uni-Gold Recombigen test was the most sensitive rapid test. While all of the tests evaluated were capable of detecting IgG specific for HIV, only the Uni-Gold Recombigen HIV test could potentially detect both IgG and IgM, as it uses a sandwich-based capture and detection system (21). This distinction also differentiates the first and second generations of the EIA tests from the so-called third generation of EIA tests for HIV-specific antibody detection. The ability to detect IgM may account for the Uni-Gold Recombigen HIV test's ability to detect HIV-specific antibody in more of the recently infected individual specimens than the other rapid tests. However, it is noteworthy that the ability to detect IgM may not solely account for the observed disparity in sensitivity between the Uni-Gold Recombigen HIV test and the OraQuick Advance and the Clearview HIV 1/2 Stat-Pak tests. The Multispot HIV-1/HIV-2 test (which detects only IgG) was capable of detecting HIV-specific antibody in 7 of the 11 specimens that were found to be reactive by the Uni-Gold Recombigen HIV test. A potentially important consideration regarding these findings is the difference in the volumes of blood/plasma specimens required by each test. While the OraQuick Advance and Clearview HIV 1/2 Stat-Pak tests each use 5 μl of

TABLE 1. Comparative HIV antibody test results with acute infection panel[a]

Patient identifier	Initial specimen								Follow-up specimen							
	Result by the following test:							Viral load (no. of copies/ml)	Days[b]	Result by the following test:						
	Vironostika EIA	WB	HIV-1/2/O EIA	OQ RT	UG RT	SP RT	MS RT			Vironostika EIA	IFA	WB	HIV-1/2/O EIA	OQ RT	UG RT	SP RT	MS RT
A	NR	I	NR	NR	NR	NR	NR	5,770	17			P	R				
B	NR	I	NR	NR	NR	NR	NR	≥500,000	26	R	P	P	R	R	R	R	R1
C	NR	I	R	NR	R	NR	NR	12,183	26	R	P	P	R	R	R	R	R1
C									42	R		P					
D	NR	N	NR	NR	NR	NR	NR	77									
E	NR	I	NR	NR	NR	NR	NR	6,373	41	R	P	P	R	R	R	R	R1
F	NR	I	R	NR	R	NR	R1	≥500,000	24	R		P					
G	NR	N	NR	NR	NR	NR	NR	12,852	24	NR	I	I	R	R	R	R	R1
H	NR	I	NR	NR	NR	NR	NR	14,062	25	R	P	P	R	R	R	R	R1
I	NR	I	R	R	R	R	R1	≥500,000	14	R	P	P	R	R	R	R	R1
J	NR	N	NR	NR	NR	NR	NR	3,921	53	R		P					
K	NR	N	R	NR	R	NR	NR	≥500,000	14	R	P	P	R	R	R	R	R1
L	NR	N	NR	NR	R	NR	NR	≥500,000	30	R	P	P	R	R	R	R	R1
M	NR	N	R	NR	NR	NR	NR	≥500,000	14	NR	I	I	R	NR	R	R	R1
M									28	R		P					
N	NR	N	NR	NR	NR	NR	NR	1,177	14	NR	I	I	R	NR	R	NR	R1
N									27	R		P					
O	NR	N	NR	NR	NR	NR	NR	≥500,000	61	R	P	P	R	R	R	R	R1
P	NR	N	R	NR	R	NR	R1	≥500,000	225	R	P	P	R	R	R	R	R1
Q	NR	N	NR	NR	NR	NR	NR	43,173	21	R	P	P	R	R	R	R	R1
R	NR	I	NR	NR	NR	NR	NR	30,734	13	NR	N	P	R	R	R	R	R1
S	NR	N	R	NR	R	NR	R1	≥500,000	49	R		P					
T	NR	N	R	NR	NR	NR	NR	≥500,000	22	R	P	P	R	R	R	R	R1
U	NR	N	NR	NR	NR	NR	NR	≥500,000	14	R	P	P	R	R	R	R	R1
V	NR	N	NR	NR	NR	NR	NR	≥500,000	21	R		I					
W	NR	I	R	NR	R	NR	R1	≥500,000	59	R		P					
W									68	R	P	P	R	R	R	R	R1
X	NR	N	NR	NR	NR	NR	NR	≥500,000	78	R	P	P	R	R	R	R	R1
Y	NR	N	R	NR	NR	NR	NR	≥500,000	10	NR	P	P	R	R	R	R	R1
Z	NR	N	NR	NR	NR	NR	NR	102,288									
AA	NR	N	NR	NR	NR	NR	NR	327,333	19	R	P	P	R	R	R	R	R1
AB	NR	N	NR	NR	NR	NR	NR	≥500,000	12	R	P	I	R	R	R	R	R1
AC	NR	I	NR	NR	NR	NR	NR	≥500,000	32	R		P					
AD	NR	N	NR	NR	NR	NR	NR	≥500,000	13	R	P	P	R	R	R	R	R1
AE	NR	N	NR	NR	NR	NR	NR	389,850	14	NR	I	I	R	NR	R	R	R1
AE									24	R	P	P	R	R	R	R	R1
AF	NR	I	NR	NR	R	NR	R1	413,186	94	R	P	P	R	R	R	R	R1
AG	NR	I	NR	NR	NR	NR	NR	446,770	24	R	P	P	R	R	R	R	R1
AH	NR	N	NR	NR	NR	NR	NR	358,030									
AJ	NR	N	R	NR	NR	NR	NR	≥500,000									
AK	NR	N	NR	NR	NR	NR	NR	427,490	18	R	P	P	R	R	R	R	R1
AL	NR	N	R	NR	R	NR	NR	210,204	14	NR	I	I	R	NR	R	R	R1
AL									34	R	P	P	R	R	R	R	R1
AM	NR	N	NR	NR	NR	NR	NR	≥500,000									
AN	NR	N	NR	NR	NR	NR	NR	≥500,000	13	R	P	P	R	R	R	R	R1
AO	NR	N	R	NR	NR	NR	NR	≥500,000									
AP	NR	N	R	NR	R	NR	R1	≥500,000	32	R	P	P	R	R	R	R	R1
AR	NR	N	NR	NR	NR	NR	NR	≥500,000	11	NR	P	I	R	R	R	R	R1

[a] Abbreviations: WB, Western blotting; HIV-1/2/O EIA, Genetic Systems HIV-1/HIV-2 PLUS O EIA; OQ RT, Oraquick Advance HIV-1/2 rapid test; UG RT, Uni-Gold Recombigen HIV-1 rapid test; SP RT, Stat Pak HIV-1/2 rapid test; MS RT, Multispot HIV-1/2 rapid test; R, reactive; R1, reactive for HIV-1; NR, nonreactive; P, positive; N, negative; I, indeterminate.

[b] Days, number of days between collection of the initial and the follow-up specimens.

specimen, the Multispot HIV-1/HIV-2 and the Uni-Gold Recombigen HIV tests require 30 and 50 μl of specimen, respectively. Hence, it is conceivable that the ability to test a relatively larger amount of specimen results in an increase in test sensitivity. It is also possible that the different rapid tests use different antigens/epitopes and that such differences may render certain tests more or less sensitive for the detection of the initial antibody response. It should be recognized that the manner in which these rapid tests were evaluated in the present study does not precisely mimic how the tests are used clinically. We used stored plasma specimens, which is acceptable for these tests, although the use of plasma is considered to be of moderate complexity by the FDA. The more common protocol for the use of these tests uses finger-stick blood, which is a low-complexity test.

The differences between the abilities of rapid tests to discern HIV serologic status in recently infected individuals may have important implications. Should multiple rapid test algorithms be developed for the screening and for the confirmation of HIV infection at the point of care, such algorithms may be affected by the differences in the sensitivities of the tests. This would be particularly important when recently infected individuals are subjected to algorithms in which the tests used are all rapid tests, including tests from different manufacturers. A discordant set of results, such as when the Uni-Gold Recombigen HIV rapid test is found to be reactive and other rapid tests are found to be nonreactive, might indicate acute HIV infection rather than an HIV-negative serologic status. In communities where the incidence of HIV is high, caution in the use of multiple rapid HIV detection test-containing algorithms that use high-sensitivity rapid tests as a primary screening test may be advised. These data may also provide some insight into the manufacture of future rapid tests. Tests that support the use of larger amounts of patient specimen may possess sensitivities higher than those of tests that seek to use relatively smaller volumes.

We thank HIV testing coordinator Andrew Reynolds, the staff of the San Francisco City Clinic, the staff of the San Francisco Department of Public Health Laboratory, and Shelley Facente for their work in collection and preparation of the specimens used and described in this report. We thank Katherine Ahrens and the UCSF Options Clinic for the provision of test results for 10 of the follow-up specimens described here.

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