Evaluation of the Recombigen HIV-1 Latex Agglutination Test

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The Recombigen HIV-1 Latex Agglutination (LA) Test was recently licensed by the U.S. Food and Drug Administration for use as a rapid screening assay for human immunodeficiency virus type 1 (HIV-1) antibodies. However, its performance in various settings and in different populations has not been firmly established. Consequently, we evaluated the test in the Cleveland Clinic Retrovirus Laboratory, a regional reference laboratory for HIV diagnostic testing and a testing laboratory for the Ohio Department of Health Anonymous HIV Testing and Counseling Program. Serum samples from 93 individuals presumed to be at high risk for HIV infection were evaluated. The sera were initially tested for HIV antibodies by enzyme-linked immunosorbent assay (ELISA). All repeatedly reactive sera were subjected to confirmatory Western blot (WB; immunoblot) testing. Of 97 serum specimens tested (5 were from one seroconverter), 44 were repeatedly reactive by ELISA and 53 were nonreactive. Of the reactive serum specimens, 31 were confirmed positive and 12 were indeterminate by WB. All of the sera were coded and then retested by the LA test. Of 53 serum specimens nonreactive by ELISA, 51 were also nonreactive in the LA test. Of the 44 serum specimens reactive by ELISA, 16 were nonreactive by LA; however, 3 of the latter were WB positive. No serum specimen with an ELISA ratio (specimen optical density/cutoff optical density) of less than 2.1 scored reactive in the LA test. The LA test was positive for only two of five consecutive serum specimens from a seroconverter despite the fact that all but the earliest of these were ELISA reactive and WB positive. Although the LA test appears to be an adequate first-line screening test when appropriately used according to the directions of the manufacturer, our data suggest that occasional sera with low levels of reactivity by ELISA may not be readily detected as reactive by the LA test.

Enzyme-linked immunosorbent assays (ELISA) are currently the standard mode of screening for antibodies to human immunodeficiency virus type 1 (HIV-1), T. C. Quinn, Letter, J. Am. Med. Assoc. 261:1148, 1989). They are highly sensitive and specific assays and have been extensively used in blood banks, in acquired immune deficiency syndrome testing and counseling programs, and as tests for diagnosis of HIV infection. In less developed countries, however, facilities, environmental conditions, and economics have not permitted the widespread application of ELISA-based tests, despite the fact that large numbers of persons in these countries are at high risk of infection (4, 5).

In December 1988, the U.S. Food and Drug Administration (FDA) licensed a new HIV antibody screening assay, the principle of which is based on latex agglutination. The Recombigen HIV-1 Latex Agglutination (LA) Test (Cambridge Bioscience, Worcester, Mass.) requires a minimum of equipment, can be performed in as little as 5 min, and is ideally suited for use in settings where sophisticated laboratory equipment is not available (6, 7).

Although the LA test has been evaluated in a limited number of trials (2, 6, 9, 11), questions remain concerning its performance under a variety of conditions in conventional laboratories and other health care settings (4). Since its licensure, concern has been expressed that this test might be used inappropriately in settings and for purposes for which ELISA would be the preferred screening test (6; J. Pachciarz, Letter, J. Am. Med. Assoc. 261:1147, 1989; S. C. Arya, Letter, J. Am. Med. Assoc. 261:1148, 1989; G. Freeman, Letter, AIDS Alert 4:26, 1989; G. M. Smith, Letter, Hosp. Employee Health 8:17, 1989). Here, we report the results of an evaluation of the LA test in comparison with ELISA, completed at The Cleveland Clinic Foundation Retrovirus Laboratory, a regional reference laboratory for HIV diagnostic testing and a testing laboratory for the Ohio Department of Health Anonymous HIV Testing and Counseling Program.

Fresh sera stored at 2 to 8°C for less than 1 week and sera stored frozen (−20°C) for less than 6 months were selected for testing. Specimens were from sexually transmitted disease clinics, drug treatment clinics, or patients referred to infectious disease physicians for evaluation of possible HIV infection. Clinical diagnoses were not available to the laboratory, but all sera were presumed to be from individuals at risk of infection with HIV. Selection of sera was based on initial ELISA screening for antibodies to HIV-1. Among the sera of 93 individuals tested were 5 consecutive serum specimens from a documented HIV seroconverter, 14 serum specimens reactive for hepatitis B virus core antibody but nonreactive for HIV, and 1 serum specimen reactive for the antibody to human T-lymphotropic virus type III. Heparizated whole blood of 12 patients from whom HIV had been isolated in culture was tested in addition to the sera described.

All sera were first tested for antibodies to HIV-1 by lymphadenopathy virus (LAV) ELISA (Genetic Systems, Seattle, Wash.). Initially reactive specimens were retested in duplicate to verify reactivity. A P/C ratio (patient specimen optical density/cutoff optical density) was determined for each specimen, and an average P/C ratio based on the three ratios was calculated for each reactive specimen (range, 1.2 to 10.2). Nonreactive specimens had initial P/C ratios of less than 1.0. Repeatedly reactive specimens were subjected to supplementary Western blot (WB; immunoblot) assay (FDA-licensed DuPont HIV-1 Western Blot). For interpret-
The Recombigen LA test kit contains a colloidal suspension of latex beads coated with purified recombinant gp41 and gp120 envelope polypeptides (7, 10). Vials of negative and positive controls are included in the kit. All kit reagents arrive prediluted and in plastic dropper bottles. Specimens from patients are diluted directly on an agglutination card that has six circular wells on a black background. Agglutination cards and an interpretation guide with photographs and descriptions of the agglutination reactions are included in the kit.

The FDA requires that users familiarize themselves with the LA test by training with an LA interpretation panel (Cambridge Bioscience). The panel, packaged and sold separately, contains negative and positive controls that yield HIV antibody reactivity levels ranging from 0 to 4+. For our evaluation, all tests were done by following the protocol of the manufacturer exactly. We ran only one card at a time, always with a positive and a negative control on the card. We also ran the interpretive panel for each series of tests done at one sitting. Briefly, 1 drop (approximately 25 μl) of the prediluted negative serum and 1 drop the positive control serum were placed on separate circles and spread over the area enclosed by the circles with a transfer loop (contained in the kit). A drop of sample diluent was then added to each of the four remaining circles, and separate transfer loops were used to pick up and deliver each patient specimen to the card, yielding a final specimen dilution of 1:10. With the transfer loop, the specimen was mixed with the diluent and spread over the entire surface of the circle. One drop (approximately 15 μl) of latex bead suspension was next added to each sample and control circle, and the cards were rotated on an orbital rotator at 30 to 60 rpm for 3 min at room temperature. A cover was placed over the card during rotation to prevent drying the reagents. The agglutination patterns were read immediately (within 30 to 60 s) with the assistance of a high-intensity lamp and a magnifier.

Positive specimens yielded agglutination reactions ranging from 1+ to 4+, whereas positive control agglutinations ranged from 1+ to 3+ (scale, 0 to 4+). A 1+ reaction was characterized by very fine aggregates against a milky-white background, and a 4+ pattern had larger clumps of beads with no milky-white background. Negative reactions were milky-white and exhibited no visible agglutination when compared with the negative control. Because we found it difficult to interpret a 1+ reaction (even as demonstrated by the interpretation panel) and often had to scrutinize the cards closely, gloves, a mask, and protective eyewear were worn by the technologist.

Ninety-seven serum specimens (from 93 individuals) were tested, and the results are summarized in Fig. 1. Among the 44 ELISA-reactive serum specimens, 70% were confirmed positive by WB, 27% were indeterminate (5 specimens had only a p17 band, 6 had only a p24 band, and 1 had both a p17 and a p24 band), and 2% were negative. For 81.4% of the specimens, ELISA and LA results were concordant, and 18.5% yielded discrepant results. For 16.5% of the specimens, LA was negative and ELISA was positive, and for 2.1% of the specimens, LA was positive and ELISA was negative. In our hands, neither 14 serum samples containing antibodies to the hepatitis B virus core antigen nor a single serum sample having ELISA reactivity for human T-lymphotropic virus type I/II had reactivity for HIV-1 by LA or ELISA, although data from the manufacturer (LA test kit brochure) and other sources (6, 11) suggest that one might expect some false LA reactivity with sera from patients with conditions unrelated to HIV infection.

Compared with ELISA, the sensitivity of the LA test was 63.6% and the specificity was 96.2%. However, when compared with sera confirmed reactive by WB, the sensitivity of the LA test was 90.3%. We were unable to calculate specificity in the latter case because we do not routinely do WB on sera that are ELISA nonreactive. When the two discrepant sera that were LA reactive (but ELISA nonreactive) were subsequently subjected to WB analysis, one was negative and the other was indeterminate (with an apparently nonspecific band at 70 kilodaltons). Why these would weakly agglutinate in the LA test is unknown; however, they were variably scored as both reactive and nonreactive in several repeat tests done by the same technologist. The 16 serum specimens that were ELISA reactive but LA nonreactive all had ELISA P/C ratios ranging from 1.2 to 2.9. Of particular significance is the fact that 3 of these 16 serum specimens were from a sexually transmitted disease clinic patient whose ELISA P/C ratio was 2.9, with WB bands at p24, gp41, p51, p66, and gp120/160. The other two were nonconsecutive serum specimens from a documented seroconverter (Table 1). ELISA
TABLE 1. Serologic reactivities of five consecutive serum samples from an HIV-1 seroconverter

<table>
<thead>
<tr>
<th>Date (mo/day/yr) of serum collection</th>
<th>ELISA result (P/C ratio)*</th>
<th>WB bands</th>
<th>LA test result*</th>
</tr>
</thead>
<tbody>
<tr>
<td>11/22/88</td>
<td>NR (0.3)</td>
<td>Not done</td>
<td>NR</td>
</tr>
<tr>
<td>12/30/88</td>
<td>R (2.4)</td>
<td>p24, p51, p66, gp160</td>
<td>NR</td>
</tr>
<tr>
<td>01/09/89</td>
<td>R (3.3)</td>
<td>p24, p66, gp160</td>
<td>1+</td>
</tr>
<tr>
<td>02/12/89</td>
<td>R (2.7)</td>
<td>p24, p51, p66, gp160</td>
<td>NR</td>
</tr>
<tr>
<td>03/07/89</td>
<td>R (2.1)</td>
<td>p24, p51, p66, gp160</td>
<td>1+</td>
</tr>
</tbody>
</table>

* NR, Nonreactive; R, reactive.

P/C ratios for these latter two serum specimens were 2.4 and 2.7, respectively, and both had WB bands at p24, p51, p66, and gp120/160. In addition to the 97 sera evaluated, heparinized whole-blood samples of 12 patients from whom HIV had been isolated in culture were also tested, and all had 3+ to 4+ LA reactions.

Because there appeared to be an ELISA P/C ratio below which reactivity in the LA test was difficult to discern, we calculated the ELISA P/C ratios of the individual samples constituting the LA interpretation panel provided by the manufacturer. The 4+, 3+, 2+, and 1+ samples had ratios of 8.5, 7.4, 6.6, and 5.7, respectively, whereas the LA-negative sample was ELISA nonreactive. Thus, the interpretation panel sample with the lowest LA reactivity had an ELISA P/C ratio well above the ratios of those sera that we were unable to classify as reactive in the LA test, further suggesting that some specimens minimally reactive by ELISA will be scored as nonreactive in the LA test.

The sensitivity of the Recombigen LA test as stated by the manufacturer is 99.4%, based on 100% prevalence of HIV-1 antibody in clinically diagnosed acquired immune deficiency syndrome patients. The specificity of the test is 99.5%, based on 0% prevalence of HIV-1 antibody in blood donors in the United States. This differs from the sensitivity and specificity observed in our study; however, our calculations were based on a comparison of LA with ELISA that used sera from individuals presumed to be at high risk for HIV infection rather than from patients with a clinical diagnosis of acquired immune deficiency syndrome or from blood donors. In preclinical studies, specificity in a doctor's office and two hospital emergency rooms ranged from 88.7 to 98.0% (LA test kit brochure). Data from the doctor's office revealed an LA-reactive rate of 10.9% compared with an ELISA-reactive rate of 0%. In one emergency room, the LA-reactive rate was 15.1% versus an ELISA-reactive rate of only 1.9%. These and more recent findings in sensitivity and specificity between the LA test and ELISA have been attributed both to a failure of users to follow kit instructions exactly and to less-than-satisfactory user training and proficiency (C. H. Riggin and R. M. Thorn, Letter, Lancet i:671, 1989). Whereas this may be true, in our hands the sensitivity of the LA test versus the Genetic Systems ELISA was comparable to sensitivities reported by others (4, 9, 11), emphasizing that specimens that are falsely negative by LA will occasionally be encountered. Our test concordance of only 81.4% for LA with ELISA serves to emphasize the need to use and interpret the LA test judiciously, especially since our testing was done under optimal laboratory conditions.

Although studies reported thus far, as well as our current study, give us confidence in the ability of the LA test to detect most HIV-infected individuals who have thoroughly seroconverted, our experience with five serum samples from a single HIV seroconverter suggests that the LA test may be unable to detect some early seroconverters. This may occur when the antibody titer is too low or when antibodies reactive with the two recombinant polypeptide envelope antigens (gp41 and gp120) in the test have yet to be produced in quantities sufficient to yield a 1+ or greater LA reaction. This was supported by the fact that sera from the individual in question showed no WB reactivity with gp41 or gp120, although weak reactivity to gp160 precursor glycoprotein and to nonenvelope proteins was evident in all but the earliest (negative) of his sera (Table 1). Our experience with this patient contrasts with the findings of the manufacturer, who found in tests of sera from several commercial seroconversion panels that the LA test is as sensitive as ELISA for detecting seroconversion (LA Test kit brochure) and should reinforce the point that no antibody test, rapid or otherwise, can identify every person who is infected. The subjective nature of this test also poses potential problems for its use in screening blood products when an objective alternative test such as ELISA is available. Although the FDA licensure of the LA test permits its use for screening blood products in the United States, the FDA has advised caution with its use for this purpose (P. D. Parkman, Center for Biologics Evaluation and Research, FDA, Memorandum to Registered Blood and Plasma Establishments, 1 August 1989).

Data provided in the LA kit brochure indicate that several clinical conditions unrelated to HIV-1 infection may be associated with falsely positive Recombigen LA tests. These include patients with antinuclear antibody, rheumatoid factor, or myeloma as well as those with Escherichia coli, rubella, hepatitis B virus, or human T-lymphotropic virus type II/III infections. Although the sample size of the sera surveyed in those studies was small, the possibility of false positivity must be considered if the LA test is to be used to screen individuals who have existing medical conditions (4). Whether antibodies associated with one of the conditions listed above or whether other factors could account for the two apparently falsely reactive specimens from the LA test in our study is not known. However, neither could be confirmed as HIV positive and both might have been ELISA reactive had the comparison with LA been made with the ELISA of another manufacturer (8; unpublished data).

In conclusion, the Recombigen HIV-1 LA Test is rapid, requires little equipment, and technically is easy to perform. Its major disadvantage lies in the need for the user to subjectively visualize an agglutination pattern that can be difficult to read at the weak end of the scale. This ensures occasional differences in test interpretation by different users (9) and necessitates strict attention being paid to good laboratory practice when the LA test is used (Riggin and Thorn, Letter). Use of this test on sera from individuals undergoing seroconversion or on sera from those with medical conditions that might cause falsely positive tests may be problematic. Certainly, further evaluations of such individuals are needed. For these reasons, if for no other, it would be prudent to be cautious in choosing to use the LA test to screen blood products for transfusion or tissues for transplantation under conditions in which an objective test could be done. Nevertheless, in the hands of technologists who are conscientious and well trained, the Recombigen HIV-1 LA Test appears to be an adequate first-line testing if other options are not available (e.g., for economic, environmental, or other reasons in third-world countries) or in rare instances in which an immediate test can be justified.
(e.g., an emergency heart transplant, emergency transfusions under battlefield conditions).

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LITERATURE CITED