Screening and Diagnostic Performance of Enzyme Immunoassay for Antibody to Lymphadenopathy-Associated Virus

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In a multicenter cooperative study, an enzyme immunoassay (EIA) using purified antigen of lymphadenopathy-associated virus was compared with radioimmunoprecipitation (RIP) for detection of antibody to human immunodeficiency virus (HIV) in 634 patients with acquired immunodeficiency syndrome or related conditions, 687 apparently healthy persons at risk for HIV infection, 93 controls with cancer or autoimmune diseases, and 10,038 blood or plasma donors. Excluding the donors, the EIA was reactive in 875 (61.9%) of 1,414 subjects; compared with RIP, the sensitivity and specificity of EIA both were 99.8%. There was one false-positive EIA among 148 intravenous drug abusers and two false-negative EIAs among 472 apparently healthy homosexual men; no other discordant results between EIA and RIP occurred in these subjects. The EIA was repeatedly reactive in 20 donors (0.2%), among whom 13 (65%) were positive by RIP; none of 529 randomly selected EIA-negative donors was RIP positive. In addition to its utility as a screening test in low-risk populations, the EIA for antibody to lymphadenopathy-associated virus is useful as a diagnostic test in persons with clinical evidence of or at risk for HIV infection.

The discovery of the retrovirus(es) associated with acquired immune deficiency syndrome (AIDS) was a signal advance in the potential control of the syndrome. The first two isolates were termed lymphadenopathy-associated virus (LAV) (2) and human T-cell lymphotropic virus type III (8), but it is now clear that these and other similar viruses (10) are closely related to one another (10, 11, 13, 15). The term human immunodeficiency virus (HIV) has been proposed (J. Coffin, A. Hasse, J. A. Levy, L. Montagnier, S. Oroszlan, N. Teich, H. Temin, K. Toyoshima, H. Varmus, P. Vogt, and R. Weiss, Letter, Science 232:477–484, 1986) and is used in this paper as a generic term for all of these agents; LAV and human T-cell lymphotropic virus type III designate the specific prototype isolates.

Identification of HIV led rapidly to the development of enzyme immunoassays (EIA) for the detection of antibody to the virus and to evidence that the majority of seropositive persons harbor HIV (6). These assays were promptly applied to screening of potential blood donors to prevent transmission of infection via blood and blood products. The early EIAs were designed to maximize sensitivity at the potential specificity of infection. In addition, the ability of EIA to detect antibody to specific viral antigens, as determined by Western blotting or radioimmunoprecipitation (RIP), has been challenged, as has the ability of EIA to predict the presence of HIV itself in persons with clinical diagnosis of AIDS or related conditions (9). Reduced specificity of these assays has been shown to be due in part to contamination of HIV antigen preparations with class II human leukocyte antigens present in the H-2 cell line used to grow the virus (17, J. B. Hunter and J. E. Menitove, Letter, Lancet ii:397, 1985; P. Kuhl, S. Seidl, and G. Holzberger, Letter, Lancet i:1222–1223, 1985). Accordingly, the early EIAs were initially approved by the U.S. Food and Drug Administration for screening low-prevalence populations but not for diagnostic use in persons suspected of being infected with HIV.

We developed an EIA for antibody to HIV based on isolation and purification of antigen from LAV grown in the CEM cell line, which lacks class II human leukocyte antigens (12). The present study was undertaken to assess the performance of this test in persons with clinical diagnoses of AIDS or other AIDS-related conditions, apparently healthy members of populations at risk for HIV infection, and blood or plasma donors. The results showed this assay to be highly accurate both for screening blood donors and other low-risk populations and as a diagnostic test for HIV infection in persons at risk for AIDS or with clinical findings suggestive of HIV infection.

MATERIALS AND METHODS

Study population. Serum or plasma specimens were provided by 11 clinical or screening centers throughout the United States. These study sites included three clinics for the treatment or assessment of persons with AIDS, AIDS-related conditions, or sexually transmitted diseases; a hemo-
TABLE 1. Demographic characteristics of study subjects

<table>
<thead>
<tr>
<th>Study group (no. of subjects)</th>
<th>Mean (±SD) age (yr)</th>
<th>Male</th>
<th>% with the following sexual orientation: Homosexual or bisexual Heterosexual or homosexual Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS (200)</td>
<td>36.2 ± 8.9</td>
<td>93</td>
<td>87</td>
</tr>
<tr>
<td>ARC (123)</td>
<td>35.5 ± 7.0</td>
<td>87</td>
<td>77</td>
</tr>
<tr>
<td>PGL (311)</td>
<td>32.4 ± 6.5</td>
<td>99</td>
<td>96</td>
</tr>
<tr>
<td>PTR* (28)</td>
<td>32.6 ± 11.8</td>
<td>89</td>
<td>86</td>
</tr>
<tr>
<td>HMM (472)</td>
<td>32.9 ± 7.9</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>IVDA (148)</td>
<td>30.3 ± 7.9</td>
<td>55</td>
<td>0</td>
</tr>
<tr>
<td>BPR* (39)</td>
<td>29.3 ± 12.1</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>AIM (93)</td>
<td>56.1 ± 15.6</td>
<td>45</td>
<td>0</td>
</tr>
</tbody>
</table>

* PTR, Sexual partners of persons with AIDS-related conditions.

φ BPR, High-risk blood product recipients.

philic treatment program; a drug abuse rehabilitation clinic; a general medical clinic at a private medical center; four voluntary blood banks; and a plasma collection center for paid donors. All specimens were collected between 1982 and mid-1985.

Standardized clinical definitions for AIDS, AIDS-related complex (ARC), and persistent generalized lymphadenopathy (PGL) were used in all centers. AIDS and ARC were diagnosed according to the initial epidemiologic definitions of the Centers for Disease Control (4). PGL was defined by the presence of abnormally enlarged lymph nodes in ≥2 noninguinal sites that persisted for ≥3 months (two centers) or ≥6 months (one center) in the absence of additional clinical or laboratory criteria to warrant a diagnosis of ARC or AIDS. Diagnoses of AIDS, ARC, and PGL were made independently of any specific laboratory tests for HIV infection. Apparently healthy homosexual men (HMM) were consecutive or randomly selected asymptomatic, sexually active homosexual or bisexual men from three urban areas with moderate or high incidences of AIDS; persons known to have PGL, ARC, or AIDS were excluded. Sera from sexual partners of persons with clinical diagnoses of AIDS or ARC were collected at two centers; the majority had repeated sexual contact with the index cases and were asymptomatic, but these features were not systematically determined. Specimens from asymptomatic current or recent intravenous drug abusers (IVDA) were collected at a drug rehabilitation clinic and an AIDS research clinic in two cities with substantial incidences of AIDS in this population. Consecutive high-risk blood product recipient patients were recruited from a hemophilia treatment program. To assess the possible nonspecific influence of chronic illness on the EIA, sera were collected from patients at a private medical clinic; these subjects had well-characterized autoimmune disorders or malignancies (AIM) and were not in populations at risk for AIDS. Table 1 summarizes these populations and selected demographic characteristics.

Serum or plasma specimens from 6,928 volunteer blood donors and 3,110 paid plasma donors also were studied. The donation centers requested voluntary self-exclusion of prospective donors at risk for AIDS, as recommended by the Centers for Disease Control (5). Demographic and behavioral data on these subjects were not collected.

Subjects with clinical evidence of HIV infection and those in populations at risk for AIDS gave written informed consent to participate in the present study or, in some cases, had previously given written consent for the use of stored serum samples for subsequent experimental assays. The AIN patients and volunteer blood donors gave specific informed consent to participate in this study. Specimens from paid plasma donors were salvaged from the collection center before being discarded, without identifying information. The study was approved by the institutional review boards of all cooperating institutions.

Laboratory methods. (i) EIA. All specimens were tested by the LAV EIA (Genetic Systems Corp., Seattle, Wash.) per manufacturer instructions using microtiter plates with wells coated with purified LAV antigen grown in the CEM cell line (7). In summary, a 100-μl sample of a 1:401 dilution of serum or plasma was added to each well. After incubation at 37°C for 1 h, the wells were washed, 100 μl of horseradish peroxidase-labeled goat anti-human immunoglobulin was added to each well, and the plates were reincubated at 37°C for 1 h. After repeat washing, 100 μl of a tetramethylbenzidine chromogen solution was added to each well; after 30 min of incubation in the dark at room temperature, 100 μl of 3 N H2SO4 was added to terminate the chromogenic reaction. A492 was measured with a 630-nm reference filter in an automatic microplate reader (Genetic Systems Corp.).

For each microtiter plate, a cutoff optical density (OD) value was determined by adding 0.225 to the mean of three negative controls assayed on the same plate. To standardize comparisons of OD values between plates, the ratio of the specimen OD to the cutoff OD for the controls for each plate was used for such comparisons. By definition, therefore, an OD ratio of ≥1.0 represented a reactive result. Initially negative results were considered definitive. Initially reactive specimens were retested and retested in duplicate with a new cutoff value determined for the new plate; only those specimens with at least one reactive repeat test were considered positive.

(ii) Confirmatory assays. RIP assays were performed by a previously described method (14), and Western blot assays were carried out as described by Towbin et al. (16). RIP and Western blot test results were interpreted by a modification of the U.S. Food and Drug Administration criteria for the Western blot assay (6). Specifically, positivity was defined by antibody to antigen gp41 (with or without antibody to other antigens) or by antibody to p25 plus antibody to p18, p34, p55, p68, gp110, or gp150. All other patterns of reactivity were considered negative.

Study design. The initial and repeated EIAs were performed in the cooperating investigators’ laboratories. The EIA was repeated once again in our laboratory (Genetic Systems Corp.) if there was a discrepancy between the EIA result obtained by the investigator and the RIP assay. Except where specifically indicated, only the investigator’s EIA result was used in analyzing the data. RIP assays were carried out in our laboratory (Genetic Systems Corp.) on all specimens from all study groups except the blood and plasma donors. For the blood and plasma donors, RIP assays were performed on all initially or repeatedly EIA-reactive specimens and on a computer-generated random sample of 529 EIA-negative specimens, including a minimum of 100 specimens from each donation center. Western blot assays were performed in the Genetic Systems Corp. laboratory on all specimens with discrepancies between EIA and RIP results. All RIP and Western blot assays were carried out and interpreted by personnel who were blind to all prior test results and to the reason for conducting a confirmatory assay.

Statistical methods. Dichotomous values were compared by chi-square analysis with Yates’ continuity correction or by the two-tailed Fisher exact test. Continuous variables
### RESULTS

Table 2 summarizes the EIA results compared with RIP results in patients with clinical evidence of or at risk for HIV infection and in AIM patients. All patients with AIDS or ARC were seropositive by both methods. Among patients with PGL, 86.5% were seropositive by both techniques, with no discrepancies between EIA and RIP. Discrepant EIA and RIP results occurred in two apparently EIA-negative, RIP-positive HHM (0.4% of this group). In addition, there was a single EIA-positive, RIP-negative IVDA subject (0.7% of this group). All 93 subjects with AIM were seronegative by both methods. Among these 1,414 patients, the sensitivity of the EIA compared with RIP was 99.8% (874/875), the specificity was 99.8% (537/538), the positive predictive value (PVpos) was 99.9% (874/875), and the negative predictive value (PVneg) was 99.6% (537/539).

Among the 10,038 blood or plasma donors, 20 (0.2%) were repeatedly reactive by EIA; 13 (65%) of these were positive by RIP (Table 3). Among the 13 other specimens that were initially but not repeatedly reactive, none was positive by RIP or Western blotting, and none had any bands suggestive of reactivity to any HIV antigen by either of these tests. Among the 529 randomly selected EIA-negative specimens, none was RIP positive or contained any RIP bands consistent with antibody to HIV. Thus, in this population, the sensitivity of a repeatedly reactive EIA compared with RIP was 100%. The specificity was at least 98.7% (542/549) and would be 99.9% if there were no positive RIP assays among the 9,476 untested specimens. The PVpos and PVneg were 65 (13/20) and 100%, respectively.

### DISCUSSION

Soon after the discovery of the HIV prototypes, LAV (2) and human T-cell lymphotropic virus type III (8), several investigators described serological methods for detection of anti-HIV antibody by Western blotting, RIP, immunofluorescence, and EIA. Although Western blotting and RIP had the apparent advantage of high specificity due to their ability to detect antibody to specific viral antigens, their labor-intensive characteristics and expense make them impractical for rapid screening of large numbers of specimens. EIA as inherently suited to this need and were shown to be highly sensitive in patients with overt AIDS or with specific anti-HIV antibody by Western blotting or RIP. However, the early EIA results appeared to be relatively nonspecific (1) because reactivity was commonly present in asymptomatic persons with negative antigen-specific assays. It has been shown (17; Hunter and Menitove, Letter; Kuhnl et al., Letter) that

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**Table 2. Results of LAV EIA and RIP assay in 1,321 persons with clinical evidence of or at risk for HIV infection and 93 AIM patients**

<table>
<thead>
<tr>
<th>Study group</th>
<th>EIA +</th>
<th>EIA +</th>
<th>EIA -</th>
<th>EIA -</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RIP+</td>
<td>RIP-</td>
<td>RIP+</td>
<td>RIP-</td>
</tr>
<tr>
<td>AIDS</td>
<td>200 (100)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ARC</td>
<td>123 (100)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PGL</td>
<td>269 (86.5)</td>
<td>0</td>
<td>0</td>
<td>42 (13.5)</td>
</tr>
<tr>
<td>PTR*</td>
<td>17 (61)</td>
<td>0</td>
<td>0</td>
<td>11 (39)</td>
</tr>
<tr>
<td>HHM</td>
<td>206 (43.6)</td>
<td>0</td>
<td>2 (0.4)</td>
<td>264 (55.9)</td>
</tr>
<tr>
<td>IVDA</td>
<td>33 (22.3)</td>
<td>1 (0.7)</td>
<td>0</td>
<td>114 (76.5)</td>
</tr>
<tr>
<td>BPR*</td>
<td>26 (67)</td>
<td>0</td>
<td>0</td>
<td>13 (33)</td>
</tr>
<tr>
<td>AIM</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>93 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>874 (61.8)</td>
<td>1 (0.07)</td>
<td>2 (0.14)</td>
<td>537 (38.0)</td>
</tr>
</tbody>
</table>

* a PTR, Sexual partners of persons with AIDS-related conditions.
* b BPR, High-risk blood product recipients.

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**Table 3. Results of LAV EIA and RIP assay in 10,038 blood or plasma donors**

<table>
<thead>
<tr>
<th>RIP result</th>
<th>Repeatedly reactive</th>
<th>Initially reactive only</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>13 (65)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>-</td>
<td>7 (35)</td>
<td>13 (100)</td>
<td>529 (100)*</td>
</tr>
</tbody>
</table>

* Among 10,005 EIA-negative specimens, RIP was performed on a random sample of 529 (see Materials and Methods).
many false-positive reactions are related to the presence of class II human leukocyte antigens in the H-9 cell line used to grow the virus for the EIA antigen. In addition, the ability of antibody to detect persons with transmissible HIV infection was uncertain. For these reasons, the U.S. Food and Drug Administration licensed the initial commercial EIAs for screening low-risk populations but not for diagnostic use.

The present study demonstrates that the EIA with purified LAV antigen grown in the CEM cell line, which lacks class II human leukocyte antigens, is sufficiently specific for use diagnostically and for evaluation of persons at risk for HIV infection without sacrificing the sensitivity required for screening prospective blood donors and other low-risk populations. In persons with clinical evidence of or at risk for HIV infection and in the AIM subjects, the sensitivity and specificity both were 99.8%. Only 3 of these 1,414 subjects had discrepant EIA and RIP results, and one of these is likely to have resulted from a technical error in test performance. The $PV_{pos}$ and $PV_{neg}$ in these subjects were 99.9 and 99.6%, respectively, and a confirmatory assay, therefore, rarely will be required in similar populations. Based on a subset of 562 blood or plasma donors tested by RIP as well as EIA, the apparent sensitivity and specificity of EIA in this group were 100% and at least 98.7%, respectively. Despite the low prevalence of RIP-positive donors (0.13%), the $PV_{pos}$ of the LAV EIA was 65% in this group.

For any quantitative test, when overlap exists between the values in affected and unaffected persons, the definition of positive and negative results requires either maximization of sensitivity at the expense of specificity or the reverse. However, as illustrated in Fig. 1 and 2, the LAV EIA achieved excellent separation between reactive and nonreactive specimens. Most other experimental or commercial EIAs have had reported sensitivities similar to those we found for the LAV EIA but, compared with Western blotting, RIP, or indirect immunofluorescence, most have had lower reported specificities (1, 3). The limited data included in the package inserts of five commercial EIAs (from Abbott Laboratories, Burroughs Wellcome Co., Du Pont Co., Electro-Nucleonics, Inc., and Litton Bionetics) also suggest lower specificities and $PV_{pos}$ than we documented. For all of these assays, however, the $PV_{pos}$ probably exceeds 95% in persons with clinical evidence of or at substantial risk for HIV infection. Definitive analysis of differences between currently available tests will require direct comparisons in the same populations.

RIP and Western blotting are alternative techniques for detection of antigen-specific antibodies, and the choice between them is an empirical one. RIP has the theoretical advantage of greater sensitivity for detection of antibodies to high-molecular-weight glycoproteins, whereas Western blotting may have greater sensitivity for antibodies to core proteins of lower molecular weight. No published studies have systematically compared RIP with Western blotting for detection of antigen-specific antibodies in substantial numbers of persons of risk for HIV infection. However, we have analyzed several hundred sera from both high- and low-risk populations by both techniques, and no significant differences in sensitivity or specificity have been observed; although rare specimens have met criteria for reactivity by
only one test or the other, the discrepancies have been equally frequent in either direction (unpublished data). Our decision to perform Western blot tests on specimens with discrepant EIA and RIP results, therefore, does not imply that RIP is less satisfactory than Western blotting as a basis for analyzing EIA performance.

Among the eight serum samples with apparently false-positive EIA results, Western blotting demonstrated that six contained antibodies with reactivity to individual HIV core antigens such as p25 or p18. Although such results may be considered false-positive with respect to the presence or absence of HIV infection, the LAV EIA is virtually 100% specific for detecting antibody with specificity to HIV antigens, even in populations at very low risk for AIDS. This phenomenon might be due to atypical reactivity to HIV or infection with one or more related retroviruses (or other agents), or it may have other explanations. In the absence of a definitive means to exclude HIV infection, LAV EIA-seropositive persons with similar atypically reactive RIP or Western blotting results should continue to be deferred as blood or tissue donors. Studies that might help to elucidate the interpretation of such results include analysis of sera from persons with recent acquisition of HIV, virus cultures and perhaps antigen detection assays, studies of patients infected with other retroviruses, and detailed analysis of the patterns of antigen-specific antibody reactivity in larger numbers of patients.

In summary, the LAV EIA is useful for screening low-risk populations such as prospective donors of blood, plasma, tissues, or organs and as a diagnostic assay for HIV infection in patients with clinical evidence of HIV infection and persons at risk for HIV infection. Repeatedly reactive specimens from persons at low risk for HIV infection should be retested with a presumptively specific assay, but confirmatory tests are rarely necessary in those with clinical evidence of or at substantial risk for HIV infection.

ACKNOWLEDGMENTS

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LITERATURE CITED


