

Evaluation of a Human Immunodeficiency Virus Test Algorithm Utilizing a Recombinant Protein Enzyme Immunoassay

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Many truly human immunodeficiency virus (HIV) antibody-negative serum samples may be unnecessarily subjected to costly and time-consuming Western blots (immunoblots). An investigation was undertaken to evaluate the efficiency of using a recombinant protein-based enzyme immunoassay (EIA; Cambridge BioScience [CBC] Recombigen HIV EIA) as an adjunct to whole viral lysate EIA. A total of 2,212 serum samples which had been screened by viral lysate EIA were tested by CBC EIA in parallel with the Western blot. The sensitivity and specificity of the CBC kit were 99.9 and 99.7%, respectively. Positive and negative predictive values were 99.7 and 99.9%, respectively. The high sensitivity of this kit and its high negative predictive value make it an attractive addition to an HIV testing algorithm by reducing the number of Western blot tests on truly antibody negative serum samples.

Human immunodeficiency virus (HIV) infection is most often detected in the laboratory by demonstrating the presence of HIV-specific antibody. Test algorithms commonly used rely on an enzyme immunoassay (EIA) screen whereby specimens are classified as being either negative or repeatedly reactive. The antibody status of EIA repeatedly reactive serum samples should be verified by a supplemental assay, which frequently is the immunoblot. The sensitivity and specificity of viral lysate-based commercial EIA kits have been well documented (6, 9).

The number of repeatedly reactive EIA serum samples that can be verified by immunoblot correlates with the prevalence of HIV infection in the population being studied (5, 13). In low-prevalence populations, the majority of EIA reactive serum samples will test as immunoblot negative (3). A confounding issue that laboratories may encounter is the indeterminate antibody status of some serum samples when tested by immunoblot. These sera are neither antibody negative nor positive but demonstrate either reactivity to cellular proteins that are similar in molecular weight (MW) to viral proteins or show an incomplete profile of reactivity to HIV viral proteins.

In this study, we investigated a strategy in which predominantly EIA reactive sera (whole viral lysate), as verified by immunoblotting, were retested by a recombinant protein-based EIA (Cambridge BioScience [CBC] Recombigen HIV EIA).

This Recombigen EIA was compared with the whole viral lysate Western blot (immunoblot). An alternative algorithm is proposed which would reduce the number of truly negative serum samples being unnecessarily subjected to Western blotting.

MATERIALS AND METHODS

Specimens. All serum or plasma samples received at the Federal Centre for AIDS (FCA) which required confirmatory testing were also tested by using the CBC Recombigen HIV EIA (Cambridge BioScience, Worcester, Mass.). The regulatory status of this kit in Canada allows the manufacturer to sell its product to designated Canadian HIV testing laboratories. Of the samples submitted, 31% were from

provincial laboratories of public health and hospital laboratories. The majority of these specimens were repeatedly EIA reactive by the screen test used in these laboratories. Forty-seven percent of the samples tested were received from centers involved in special, ongoing, collaborative studies with the FCA. These studies involved individuals at high risk for HIV infection. In addition, samples submitted by the Haitian Red Cross, which were screen test positive in Haiti, were included in the evaluation and accounted for 22% of the samples tested.

EIA. The antigen sources of the CBC EIA kit are genetically engineered components of gp41 and gp120 envelope proteins and p24 gag proteins. This kit differs from that used in a study by Burke et al., in which only the envelope proteins were used as the antigen source (2). The envelope and core proteins are expressed in *Escherichia coli*, purified, and coated onto 96-well, polystyrene microdilution plates. The testing format of the Recombigen EIA is similar to standard whole viral lysate EIAs. All samples were tested in duplicate, and reactivity was calculated according to the protocol of the manufacturer.

Confirmatory tests. In-house Western blots were performed by using whole viral lysate (Organon Teknika, Scarborough, Ontario) as previously described (11). In addition, the human T-cell lymphotropic virus type III Western Blot kit (Du Pont Co., Wilmington, Del.) was used to analyze some samples. The criteria used for interpretation of Western blot reactivity were those established at a Canadian national consensus meeting (Canadian Laboratory Directors Meeting on Retrovirus Testing, Calgary, Alberta, Canada, November 1988). Samples which demonstrated antibody reactivity to *env* and *gag* proteins (or *env* proteins only if clinical history was indicative of HIV infection) were considered Western blot positive. Samples were negative if no bands were seen on the Western blot and were indeterminate if they failed to meet the criteria of either a positive or negative sample. Indeterminate samples were further tested by using an immunoblot which utilized genetically engineered HIV-specific proteins and/or the radioimmunoprecipitation assay (RIPA). The recombinant immunoblot protocol is the same as that described for the whole viral lysate blot. The HIV recombinant proteins (MicroGeneSys, West Haven, Conn.) were a 160,000 MW envelope protein and/or a

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TABLE 1. Comparison of Western blot and CBC EIA for 2,212 serum samples

CBC Recombigen EIA result	No. of Western blot (whole viral lysate) results		
	Positive	Negative	Indeterminate
Positive	1,148	2	22
Negative	0	637	402
Indeterminate	1	0	0

24,000 MW core protein. The RIPA procedure (10) was modified in that viral proteins were biosynthetically labeled with [³⁵S]methionine and [³⁵S]cysteine (ICN Biomedicals, St. Laurent, Quebec). Serum samples containing antibodies reacting with envelope proteins of the virus were considered RIPA positive.

RESULTS

The Western blot and CBC EIA results on 2,212 serum or plasma samples are shown in Table 1. A total of 1,149 (51.9%) of the samples tested were positive by immunoblotting. All but one of the blot positive samples were positive in the CBC EIA. This one sample, which was received from the Haitian Red Cross, was indeterminate by the CBC test. When tested in duplicate wells, the sample had one absorbance value above and one below the cutoff value of the test plate. Repeat testing produced similar results. A total of 639 (28.8%) of the samples tested were negative by Western blotting. Of these, two (also from Haiti) were positive in the CBC EIA. The remaining 424 samples (19.2%) were indeterminate by immunoblotting. Of these, 402 (almost 95%) were negative in the CBC EIA.

Further supplemental testing (recombinant immunoblot and/or RIPA) on the Western blot indeterminate samples found all but 22 of the 424 samples to be negative. Discordant supplemental test and CBC EIA results were observed for two specimens. One confirmed positive sample received from the Haitian Red Cross was negative in the CBC test. One sample which was negative in the supplemental tests was positive in CBC EIA.

By combining these data and those from Table 1 and by using the formulas of Ransohoff and Feinstein (8), the sensitivity and specificity of the CBC Recombigen EIA were found to be 99.9 and 99.7%, respectively. The positive predictive value and negative predictive value of the test were 99.7 and 99.9%, respectively.

DISCUSSION

The screening test of choice in many laboratories for the detection of antibodies to HIV is the EIA, which employs disrupted whole viral lysate as its diagnostic antigen. This relatively inexpensive test has evolved into a highly sensitive and specific assay which can usually be completed in under 4 h.

The predictive values of an EIA are dependent not only on the sensitivity and specificity of the assay but also on the prevalence of disease in the test population. In general, as prevalence of infection increases, so does the positive predictive value (13). At the same time, the negative predictive value declines. The opposite is true in populations at low risk for HIV infection.

As the uses for HIV testing have expanded to include insurance, employment, and visa screening, laboratories find

that many individuals whose serum samples are repeatedly reactive in the EIA may be indeterminate in the Western blot. Except in some cases, such as seroconversion or passive antibody transfer (through hepatitis B immunoglobulin administration, for example), subsequent HIV testing on these individuals may not resolve their immune status (4).

The CBC EIA was introduced into the FCA testing algorithm as a primary supplemental test to determine if, by this strategy, the number of indeterminate Western blot samples could be substantially reduced. To be of value, therefore, the test had to be at least as sensitive as currently available whole viral lysate EIAs and demonstrate higher specificity. The Western blot would still be considered the "gold standard," and HIV positivity would still require confirmation by the immunoblot procedure.

In this evaluation of 2,212 serum or plasma samples, the CBC Recombigen HIV EIA had a sensitivity of 99.9 and a specificity of 99.7%. In an earlier study in which a test utilized only recombinant envelope protein as the capture antigen, a sensitivity of 99.9 and specificity of 99.1% were obtained when 2,707 consecutive serum samples were tested (1).

One potentially false-negative serum sample by the Recombigen test was received from the Haitian Red Cross, and a follow-up sample was unavailable. More diverse strains of HIV may be circulating in the Haitian population than in North America since the widely divergent HTLV RF strain was originally isolated from a Haitian (12). Thus, a highly specific recombinant protein-based commercial kit designed for screening of North American sera may fail to detect antibodies elicited by HIV variants. Alternatively, the sample may have been cross-contaminated by another serum sample positive for HIV antibody. Data from CDC proficiency panels and in-house experience with diluted samples indicate that the CBC product does not detect diluted samples as efficiently as do viral lysate tests. Of the three CBC EIA potentially false-positive serum samples, two were from the Haitian Red Cross and follow-up testing could not be done. It is possible that these two specimens from a country with an HIV prevalence rate of 3 to 9% (7) may represent two early seroconversions. The Laboratory Services Branch, Ministry of Health, Toronto, Ontario, has reported that in some cases the CBC EIA kit was able to detect HIV antibody earlier than whole viral lysate EIA (C. Major, personal communication). In most of these cases, however, the immunoblots were indeterminate. The other CBC reactive sample was from an asymptomatic male who was not in any known risk group and may represent a true false-positive in the CBC EIA.

Over 19% of the total samples tested were indeterminate in Western blots. The band patterns observed with these specimens typically consisted of a 17,000 MW band (17K) only, a 24K band only, or 17K and 24K bands. Most of these specimens were received from Provincial Health Laboratories with HIV positivity rates ranging from 3 to 4%. Many of these specimens were CBC EIA negative and were confirmed negative by the supplemental tests.

If the EIA screen and Western blot use similar viral lysates, then EIA falsely reactive sera may not be resolved by Western blot since any nonspecific reactivity would be against the same nonviral proteins. There was no clinical or laboratory evidence which suggested that serum samples which were immunoblot indeterminate and CBC negative were from individuals infected with HIV.

The samples tested by the FCA represent a population for which the prevalence of HIV infection is high (52.9%), as

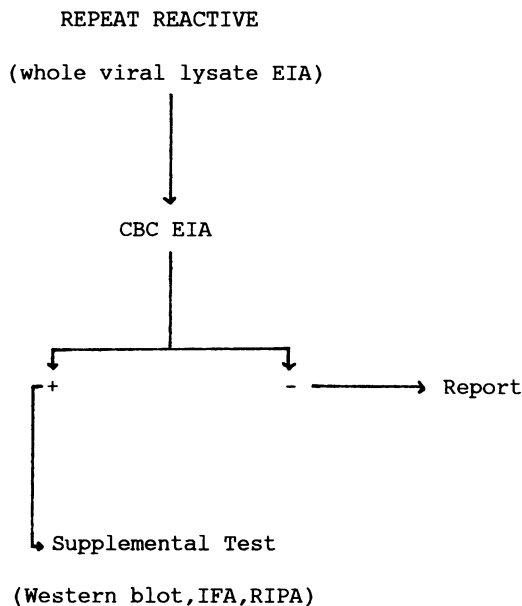


FIG. 1. Proposed HIV test algorithm. IFA, Indirect fluorescent-antibody test.

determined by Western blot positivity. In such populations, the negative predictive value of an EIA screen may be as low as 76.9% (13). The negative predictive value of the CBC kit in this study was 99.9%. Such a high negative predictive value could obviate the need for subsequent testing. Since this evaluation concentrated on a high-risk category, the value of the CBC kit in low-risk populations, such as voluntary blood donors, could not be determined.

The proposed testing hierarchy (Fig. 1) using the CBC EIA would reduce the number of serum samples requiring supplemental testing. EIA repeat reactive serum samples which are found negative by CBC EIA need not be subjected to Western blotting. Serum samples reactive in both the initial viral lysate screen test and CBC EIA, however, require confirmation by Western blotting or tests of equivalent sensitivity and specificity such as RIPA or the indirect fluorescent-antibody test. Until a consensus is reached, such a "gold standard" should continue to be used in diagnostic laboratories. However in blind or unlinked studies, such as seroepidemiological surveys, in which patients are not notified of test results, supplemental testing need not be required.

If this algorithm had been used in this study, the number of Western blots performed would have been reduced by 47%. In reagents alone, this could represent a savings of over \$50,000. In addition, 95% of the secondary supplemental testing would not have been necessary and the reporting time on specimens not requiring such testing would have been reduced by one-third.

In this study, the CBC Recombigen HIV EIA was highly sensitive (99.9%) and may be more specific (99.7%) than whole viral EIAs. It may be a valuable addition to the HIV testing algorithm of many laboratories.

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