Human Immunodeficiency Virus Western Blot Tests: Comparisons and Considerations

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ABSTRACT

Human immunodeficiency virus (HIV) Western Blot (WB) tests are used as an aid in the diagnosis of acquired immunodeficiency disease syndrome (AIDS) and related disorders. The object of this study is to examine and compare the results of four commercially available HIV WB methods. Sera from 974 persons were tested by four (Kits I, II, III, and IV) HIV WB methods from different vendors. The HIV WB were interpreted by recognized criteria published in Morbidity and Mortality Weekly Report. Results: 65 percent of the referred specimens received were HIV enzyme immunoassay (EIA) repeatedly reactive (R), and 35 percent of the referred specimens were HIV EIA nonreactive (NR). The EIA (NR) sera showed the greatest variability for HIV WB results, especially in the indeterminant (I) category. The HIV WB tests commercially available vary in sensitivity and specificity. Standardization of materials and controls and the use of uniform interpretation criteria are needed.

Introduction

The Western Blot test for confirmation of the presence of antibodies against the human immunodeficiency virus (HIV) has been recommended from a variety of sources. An important consideration for application of this confirmatory test is that some screening test, e.g., an enzyme immunoassay (EIA) for HIV, be performed first. The HIV EIA screening is very sensitive but not particularly spe-
specific for true HIV infection.2,4 The HIV Western Blot (WB) test is perceived, at least in part, to be a more definitive test and to eliminate potential false positive HIV EIA’s. Questions have been raised from the transfusion medicine community related to the sensitivity and specificity of HIV WB testing in donor populations with HIV EIA non-reactive (NR) or reactive results.3,9

The purpose of this study is two-fold. The first is to examine the criteria for HIV WB interpretation. The second is to compare results of HIV WB methods, taking into account analytical and interpretive differences.

Materials and Methods

Nine hundred seventy-four different referred serum specimens and 17 sera from outside proficiency testing agencies were tested. Some had been pre-screened for HIV antibodies by EIA. All sera were retested in the reference laboratory by one of two commercially available HIV EIA methods and then subjected to HIV WB by kits of one of four companies (Dupont, Kit I; Organon Teknika, Kit II; BioRad, Kit III; and Genetics Systems, Kit IV). In each instance, the directions, protocols, and, if applicable, the interpretive criteria recommended by the manufacturers were followed.

The interpretive criteria that were used included those of the Centers for Disease Control (CDC)/Association of State and Territorial Public Health Laboratory Directors (ASTPHLD), Dupont’s FDA licensed test, the American Red Cross (ARC), or the Consortium for Retrovirus Serology Standardization. A summary of HIV WB interpretive criteria follows:5 (1) CDC/ASTPHLD interpretive criteria: Any two of p24, gp41, gp120/gp160; (2) Dupont FDA licensed test criteria: p24 & p31 and gp41 or gp120/gp160; (3) American Red Cross criteria: ≥3 Bands—one from each gene product, e.g., GAG, POL & ENV and (4) Retrovirus Serology Standardization: ≥2 Bands = p24 or p31 plus gp41 or gp120/gp160.

The interpretation is given as positive (P) if the criteria are met, indeterminant (I) if some but not all criteria are present, and negative (N) if no bands are present.

Results

The specimens were retested for HIV EIA in the reference laboratory. Of the referred specimens, 65 percent (633/974) were positive for HIV antibodies by EIA, and 35 percent were negative by HIV EIA.

The HIV WB results indicated that, depending upon the kit and criteria for interpretation used, between 82 percent and 98 percent of the HIV EIA reactive specimens were also Western Blot positive. The indeterminant WB’s ranged from two to 18 percent. The Western Blot negatives ranged from 0 to five percent (table I). For Kit IV, the CDC criteria for evaluation were applied; for Kits I, II, and III, the FDA licensed Kit or ARC criteria were used.

On the HIV EIA nonreactive sera, HIV WB negatives ranged from 84 percent to 100 percent, and the indeterminant WB’s ranged from 0 to 49 percent. One HIV WB of Kit II would have been positive if the CDC criteria were used and negative by Kit III.

The results of HIV WB testing on 17 proficiency test specimens are presented in table II. All seven HIV EIA non-specific specimens were negative by Kits I, III, and IV. Note that the different kits demonstrated various bands. Although there was not agreement of the band identified, each of the kits would have properly identified and HIV WB as positive if appropriate interpretive criteria were used.
TABLE I

<table>
<thead>
<tr>
<th></th>
<th>Kit I (n = 353)</th>
<th>Kit II (n = 257)</th>
<th>Kit III (n = 333)</th>
<th>Kit IV (n = 31)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WB(+) WB(I) WB(-)</td>
<td>WB(+) WB(I) WB(-)</td>
<td>WB(+) WB(I) WB(-)</td>
<td>WB(+) WB(I) WB(-)</td>
</tr>
<tr>
<td>EIA (R)</td>
<td>82% 16% 2%</td>
<td>80% 18% 2%</td>
<td>90% 10% 0%</td>
<td>98% 2% 0%</td>
</tr>
<tr>
<td>EIA (NR)</td>
<td>0% 16% 84%</td>
<td>0% 25% 75%</td>
<td>0% 49% 51%</td>
<td>0% 0% 100%</td>
</tr>
</tbody>
</table>

**WB(+) = Western Blot Positive**  
**WB(I) = Western Blot Indeterminant**  
**WB(-) = Western Blot Negative**  
**EIA (R) = HIV Enzyme Immunoassay Reactive**  
**EIA (NR) = HIV Enzyme Immunoassay Non- Reactive**  

### Discussion

Dependence is often placed upon the HIV WB test for confirmation of HIV EIA reactive patients. This study and others have demonstrated that there are variable results on HIV WB testing when different vendors are used. The reasons for this are many. They included different culture sources for the antigens, strains of HIV used, application of antigens to the plates, alternative plate materials, and electrophoresis conditions among the possibilities.

The criteria for HIV WB interpretations are not standardized. Using the CDC criteria with Kit IV resulted in the smallest percentage of indeterminants. Kit III had an unacceptably high level of indeterminants with HIV EIA nonreactive sera using the FDA licensed kit criteria.

What is the standard for making up the Western Blot antigens? What is the "correct" or expected antibody response of a wide variety of patients? Presently, there is only one FDA licensed kit (Kit I), and it had fairly rigid criteria. Application of these criteria resulted in a relatively small percentage (16 percent) of indeterminants for either HIV EIA reactive or nonreactive specimens. Kit III had good sensitivity, showing an HIV WB positive in 90 percent of the HIV EIA reactive specimens, but gave an unacceptably high number of indeterminants (49 percent) for HIV EIA nonreactive specimens.

It would appear that the recommendations to screen patients with a sensitive test (HIV EIA) and then do follow up
HIV WB's on only those patients that are EIA positive is an acceptable approach. This may miss some patients who do not have circulating antibodies of either sufficient titer or specificity to give a positive HIV EIA. The HIV WB interpretive criteria should be standardized in order to avoid having an unduly high number of indeterminants which result in confusion to attending physicians who are searching for a definitive diagnosis or follow-up. Application of other molecular biological techniques, including a search for specific HIV antigen or antigens, would add to the diagnostic possibilities.

SUMMARY

HIV Western Blot results vary by vendor and the interpretive criteria that are used. In the absence of analytical and clinical standards, the definitive laboratory diagnostic approach to HIV infection remains to be determined. Our present recommendation is that a combination of screening for antibodies with a sensitive method (e.g., HIV EIA) followed by a search for antibodies using CDC interpretive criteria on the Western Blot and some method for identifying HIV antigen or antigens is a reasonable course in the pursuit of laboratory substantiation of a clinically suspected HIV infection.

Acknowledgment

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References