Status of Current Clinical Tests for Human Immunodeficiency Virus (HIV): Applications and Limitations

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ABSTRACT

Two laboratory tests are currently used to detect the human immunodeficiency virus (HIV) specific antibodies that are produced when an individual has been infected by the virus at some time. These include the enzyme-linked immunosorbent assay (ELISA) as the screening test and the Western blot (WB) as the confirmatory test. They are not yet optimally effective and have brought with them some problems, especially when used to screen low risk populations such as asymptomatic blood donors. Currently licensed ELISA tests used to detect HIV have sensitivities that range between 93 percent and 99 percent, and all have specificities greater than 99 percent. An important concern is that the positive predictive value for the ELISA screening test is low in spite of the fairly high sensitivity and high specificity values. This poor predictive value is due to the low prevalence of individuals in the general population who have been infected with HIV. Multiple causes of false positive ELISA and Western blot tests have been identified. They can be eliminated by utilizing reagent antigens which are produced by recombinant deoxyribonucleic acid (DNA). The false negative ELISA and Western blot tests can be reduced by tests designed to detect IgM antibodies to HIV.

Introduction

Remarkable progress has been made in developing laboratory tests to detect the presence of antibodies against the causative agent of acquired immunodeficiency syndrome (AIDS)—human immunodeficiency virus (HIV) (previously referred to as human T cell lymphotropic virus type III/lymphadenopathy-associated virus). In the past two years, the widespread application of these tests has made it possible to determine whether or not a person has been infected by the virus at some time. However, the introduction of these tests has brought some problems.27

Up to now, the tests are not yet optimally reliable. There is still space for investigation and development of the tests with increased sensitivity, specificity, and predictive values.

Currently, there are three approaches to detect HIV infections: (1) detection of
the virus by culture, (2) detection of the antigens elaborated by the virus, and (3) detection of HIV–specific antibodies produced by the host immune system.

Both tests mainly used currently are of the third type. One of them is an enzyme-linked immunosorbent assay (ELISA) which reacts to the presence of antibodies in the blood and shows a more intense color when larger quantities of the antibodies are present in the serum. A positive reaction is recorded when the observed intensity exceeds a cutoff value set by the manufacturer. The other one is the Western blot test which is also an antibody detecting test. It differs from the ELISA in some ways. It uses electrophoresis to detect particular antibodies that HIV antigens may elicit. The presence of antibodies against the HIV antigens P24 and P41 are required for a positive Western Blot. It is less sensitive but more specific than the ELISA.

Several versions of the ELISA test are on the market. They differ in their comparative sensitivity to the various antigens to HIV and reflect different information about the antibodies in a given blood sample. Currently licensed tests to detect antibody to HIV range in sensitivity between 93 percent and 99 percent, and all are above 99 percent in specificity (table I). These kits utilize the enzyme-linked immunosorbent assay technique by using beads or microtiter wells coated with antigens obtained from disrupted whole virus growing in a human leukemic cell line, such as H9. The test serum is incubated with the antigen-coated surface; following serum removal and appropriate washing, goat anti-human (IgG) immunoglobulin linked to an enzyme (horseradish peroxidase) is added and allowed to incubate. During this incubation, the goat antibody combines with any human IgG antibody that has bound to viral and cellular antigens during the first incubation period. After removal of the goat antibody and washing of the well and beads, a substrate (O-phenylenediamine or azinobenzthiazolium sulfomate) is added that is acted on by the enzyme to produce a color of intensity proportional to the amount of HIV antibody. The amount of color developed is read spectrophotometrically.

The positive predictive value is defined as the likelihood a positive test represents a true positive result. The negative predictive value is the likelihood that a negative test represents a true negative result. Bayes' theorem states that these values depend upon the sensitivity and the specificity of a test, as well as the prevalence of a disease in the population tested before the test is performed.

Based upon the sensitivity, specificity and prevalence, the calculated positive predictive values for different groups of asymptomatic individuals varies greatly. Using the data pertaining to two well-studied high-risk groups and the general population, with an assumed prevalence of asymptomatic HIV infection of 0.044 percent for the general pop-
ulation, 65 percent for homosexual men, and 36 percent for IV drug abusers, the positive predictive value of the general population is extremely low for the general population and high for high prevalence groups \(^{24}\) (table II).

### Discussion

There is a strong correlation between strongly reactive ELISA serological test results and Western Blot positivities. Of 61,190 donors tested by the Center for Disease Control (CDC), 0.8 percent were initially reactive and 0.25 percent were repeatedly reactive. Of these donors, 31 percent were strongly reactive. Approximately 86 percent of these strongly reactive samples were positive by Western blot and 58 percent were viral culture positive. Samples that were reactive only initially were neither positive by Western blot nor by viral culture.\(^{18}\)

American Red Cross (ARC) data on testing done on 2.58 million blood donors showed one percent of donors to be initially reactive, 0.27 percent repeatedly reactive, and only 0.035 percent Western blot positive.\(^{18}\)

In West Germany (WG), over 80,000 donors were tested between May 1985 and September 1985. Approximately 0.2 percent were found to be repeatedly reactive and 0.01 to 0.02 percent to be Western blot positive.\(^{18}\)

The tabulation of the data from the three aforementioned sources (table III) demonstrates that only a fairly small percentage of the repeatedly ELISA-reactives were Western blot positives.

The Puget Sound Blood Center used the ELISA test method to screen 15,680 donors during a six-week period and found 14 repeatedly reactive donors. Only three of the 14 donors were confirmed positive by Western blot. Eight of the 11 false-positive samples were from women who had at one time been pregnant. Serum from each of these eight women was screened for complement-mediated cytotoxicity against a selected panel of 92 different lymphocyte specimens in an effort to find antibodies with HLA specificities. The sera were also tested for complement-mediated cytotoxicity against the human T-cell leukemia line HUT-78 (H-9 cells). This cell line has the HLA phenotype HLA-AL, BW62, DR4, DQW3. In seven of these women, the HLA antibodies were directed to class II antigens expressed on the cells used to grow

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>Predictive Values of an ELISA Test for Anti-HIV in Selected Populations*</th>
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<tbody>
<tr>
<td>Center</td>
<td>General Population</td>
</tr>
<tr>
<td>Assumed prevalence</td>
<td>0.044%</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>0.6%</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>99.999%</td>
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</tbody>
</table>

*Assumed prevalence = 0.044%
Positive predictive value = 0.6%
Negative predictive value = 99.999%
Sensitivity = 97.7%
Specificity = 92.6%
Minimum positive ELISA value = 3

<table>
<thead>
<tr>
<th>TABLE III</th>
<th>Correlation Between Reactive ELISA Serological Test Results and Western Blot Positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Center</td>
<td>American Red Cross</td>
</tr>
<tr>
<td>CDC</td>
<td>61,190</td>
</tr>
<tr>
<td>ELISA - initially reactive</td>
<td>0.8%</td>
</tr>
<tr>
<td>ELISA repeatedly reactive</td>
<td>0.25%</td>
</tr>
<tr>
<td>Western blot positive</td>
<td>0.067%</td>
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</table>

\(^{18}\) Intravenous drug abusers
HIV in the preparation of screening ELISA kits. This indicates that HLA antibodies can be a cause of false-positive reactions in the ELISA screening test.22

A serological survey of 250 outpatients in rural Zaire showed that the prevalence of antibody against HIV, as detected by ELISA, correlated strongly with the level of antibodies against *Plasmodium falciparum*.5 However, tests with control sera obtained from HIV seropositive homosexual men in the United States and American subjects repeatedly infected with malaria who had high antibody titers against *Plasmodium falciparum* indicated that there was no cross reactivity between *Plasmodium falciparum* and HIV.5

Another study shows the prevalence of HIV antibodies among the Amazonian Indians in Venezuela suggesting past or concurrent exposure to HIV or a highly-cross-reactive virus which may be naturally endemic in certain areas of South America. The antibodies were found among aboriginal Indians inhabiting a nearly impenetrable area of Amazonia, a group distinct from randomly chosen blood donors from Venezuelan cities. Despite the high prevalence among the Venezuelan Indians of up to 13 percent, no clinical symptoms of AIDS were observed. This indicates that antibodies detected in the Amazonian Indians might be due to another new member of the family of human retroviruses. Cross-reactive antibodies are thus another cause of false positives of ELISA screening test.20

Multiple other reported causes of false positive ELISA tests for HIV screening include anti-hepatitis A IgM, anti-hepatitis B core IgM, anti-thyroid microsomal, antinuclear, anti-smooth muscle, anti-parietal cell, anti-liver/kidney microsomal, and anti-mitochondrial.15

Western blot analysis is based on detection of antibody against specific molecular weight antigens of HIV21 (table IV).

The 24 and 65 kilodalton antigens are components of viral core protein. The 120 kilodalton antigen is an envelope glycoprotein. Molecular sequence analysis of HIV also shows a 41 kilodalton glycoprotein that is component of a transmembrane glycoprotein. A specimen is considered to be immunoblot reactive by the presence of bands at the 24 kilodalton and/or 41 kilodalton molecular weight region with or without the presence of other bands.9

Antibody against the 24 kilodalton core antigen was detected more frequently than against the 41 kilodalton transmembrane glycoprotein antigen in seropositive individuals9 (table V). Most other studies, however, suggest the opposite. It is thought that large amounts of core protein liberated from destroyed infected cells absorb antibody to core antigen.

The two human T-lymphotrophic retroviruses (HTLV) HTLV-I and HTLV-II

<table>
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<tr>
<th>TABLE IV</th>
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<tbody>
<tr>
<td>Anti-human Immunodeficiency Virus Specificity for Antigens on Western Blot</td>
</tr>
<tr>
<td>Kilodalton</td>
</tr>
<tr>
<td>24</td>
</tr>
<tr>
<td>53</td>
</tr>
<tr>
<td>65</td>
</tr>
<tr>
<td>41</td>
</tr>
<tr>
<td>120</td>
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<table>
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<tr>
<th>TABLE V</th>
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<tbody>
<tr>
<td>ELISA and Western Blot Antigen Activity</td>
</tr>
<tr>
<td>Status</td>
</tr>
<tr>
<td>Homosexual healthy men</td>
</tr>
<tr>
<td>Hemophiliac</td>
</tr>
<tr>
<td>IV drug abuser</td>
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TABLE VI

Multiple Monoclonal Antibodies Specific for Human Immunodeficiency Virus Antigens

<table>
<thead>
<tr>
<th>Monoclonal Antibody</th>
<th>HIV Antigen Specificity</th>
</tr>
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<tbody>
<tr>
<td>HT462</td>
<td>p52</td>
</tr>
<tr>
<td>NDB-1</td>
<td>p24</td>
</tr>
<tr>
<td>GIN-7</td>
<td>p19</td>
</tr>
<tr>
<td>FR-45</td>
<td>p15</td>
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</tbody>
</table>

should be distinguished from the HIV. Several of the antigens of HIV detected by the Western blot technique are similar in size to those detected for HTLV-I and HTLV-II, but the banding pattern is different. The ELISA tests for detection of HTLV-I and HIV are specific for their respective viruses.¹,²⁵

**Summary**

Causes of false positive Western blot tests for antibodies to HIV are listed as follows:

1. Presence of antibody to another human retrovirus in the patient’s serum.
2. Presence of another human retrovirus in the H9 cells that the reagent antigen is obtained from.
3. Cross reactions with cell derived ribonucleoproteins or other non-viral proteins.

Concerns have been raised about false negative ELISA tests. A prospective investigation of 65 HIV ELISA IgG-seronegative drug addicts was conducted by using a modified ELISA technique to detect IgM antibodies. Among these 65 specimens, five IgG positives were confirmed by IgM Western blot. Four of these five switched from IgM to IgG after one, 14, 23, and 38 weeks, respectively.⁴ It is evident that, in some instances, HIV IgM antibody may be the only serological sign of infection. Tests designed to detect IgG antibody will not be positive early during the infection when primarily IgM antibody is produced.

Up to the present time, multiple distinct monoclonal antibodies specific for HIV have been produced and proposed for usage in identifying expression of HIV in infected cultures and in cells or tissues from patients²⁶ (table VI).

Monoclonal antibodies can be a pure, well defined and characterized reagent used to detect the HIV virus. These antibodies do, however, show cross reactivity with other antigens. The reasons for cross reactivity are listed as follows:

1. Proteins (table VII) found in HIV particles may be part of normal cellular polypeptides that are expressed only under particular circumstances, such as rapid cell proliferation where cellular polypeptides are entrapped with genuine HIV components.
2. Whole polypeptides which are unrelated but share short amino acid sequences with HIV can give rise to immunologic cross reactions. This is molecular mimicry.
3. Proteins encoded by the HIV genome and gene products of the human endogenous gag sequences are related to the extent that monoclonal antibodies to HIV react with both.

Among these proteins, there are three fractions 24–25 kilodalton, 25–28 kilo-

**TABLE VII**

Human Immunodeficiency Virus Proteins Characterized as Core (gag), Reverse Transcriptase (pol), and Envelope (env) Antigens*

<table>
<thead>
<tr>
<th>HIV Antigen</th>
<th>Proteins (Kilodaltons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core (gag)</td>
<td>15, 17, 19, 24, 38, 55</td>
</tr>
<tr>
<td>Reverse transcriptase (pol)</td>
<td>51, 53, 64</td>
</tr>
<tr>
<td>Envelope (env)</td>
<td>21, 22, 27, 41, 46-52, 61-68, 88, 120, 160</td>
</tr>
</tbody>
</table>

dalton, and 14–15 kilodalton which are encoded by three unique genes of HIV: (A) short open reading frame (sor), (B) 3' open reading frame (3' orf), and (C) transactivating function (tat). The two small open reading frames, sor and 3' orf, in the HIV genome have no equivalents in size and location in the genomes of other exogenous human retroviruses such as HTLV-I and HTLV-II. The sor region is located between the pol and env genes and can encode a protein of 192 amino acids with estimated molecular weight of 24–25 kilodalton. The 3'orf can encode a protein of 25–28 kilodalton.

Western blot analysis is useful in detection of HIV infection in neonates where HIV antibody is present in the neonate because of placental transfer of maternal antibody. Primary gene products are polypeptides with molecular weights of 55, 64, and 160 kilodalton. The parent polypeptides give rise to smaller derivative polypeptides. Gag specified core polypeptides P24, P15, P9, and P7 are derived from the parent P55, pol specified reverse transcriptase polypeptide P53 is derived from parent p64, and env specified gp120 and gp41 are derived from gp160. Appearance of antibody to parent polypeptides in a neonate with persistent IgG antibody to derivative proteins and absent IgM antibody signifies perinatal infection.

The importance of the polypeptide proteins must be considered in view of the discovery of HIV II. Both HIV I and HIV II share core protein epitopes: (1) p25, P18, P55, and p40 for HIV I, and (2) p26, p16, and p55 for HIV II. There is no cross reactivity between envelope glycoproteins: (1) gp110 and gp41 for HIV I, and (2) gp130-105 for HIV II. Differentiation of AIDS secondary to HIV I or HIV II infection can be differentiated by Western blot analysis. Serological studies using the ELISA test methodology area in progress, and it appears that HIV II infected patients are negative for HIV I by ELISA.

Conclusion

Improvements that will be made in testing for HIV are outlined as follows:

1. Specific confirmatory tests for anti-HIV capable of distinguishing false positive from true positive reactions utilizing reagent antigens produced by recombinant DNA will be performed in blood centers.

2. Direct methods of virus detection utilizing monoclonal antibodies to detect the HIV genome and virus-specific proteins in blood will be available for individuals with antibody level too low to detect with any test.

3. Detection of viral antigen by ELISA methodology is under investigation. At this time it appears to have limited use. Newly infected individuals may have detectable antigen prior to antibody formation. Individuals with high titer antibody are often negative for detectable antigen by ELISA methodology owing to immune complex formation.

4. Detection of infection by culture of patient mononuclear cells and use of nucleic acid in situ hybridization will be performed on a routine basis while viral culture will remain difficult owing to the complexity involved in the culture of this virus.

5. ELISA testing will continue to evolve as more is learned about the human retroviruses and new human retroviruses are discovered. HIV I is well delineated. The newly discovered HIV II and HTLV-IV may be closely related, yet HTLV-IV does not seem to be associated with disease.
References


