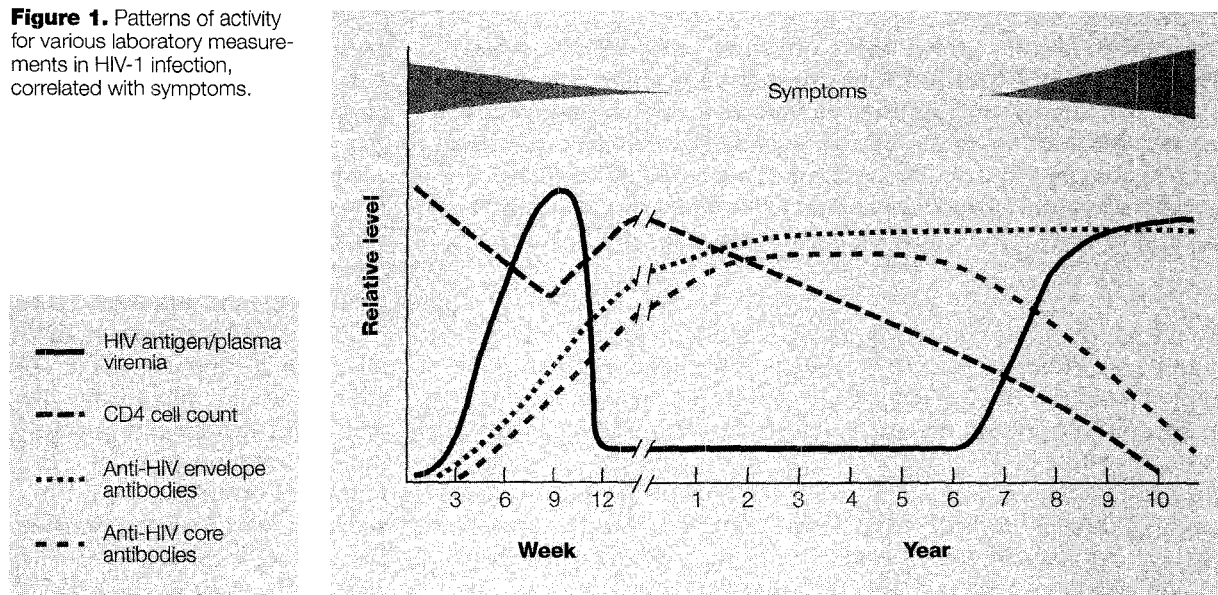


**Passively acquired antibodies to HIV have been found in persons who have received human globulin, but the effect is transient and no resulting HIV infections have been reported.**

**Figure 1.** Patterns of activity for various laboratory measurements in HIV-1 infection, correlated with symptoms.



in the sample population. The likelihood of a false-positive result is greater in a low-risk population than in a high-risk population. Although false-positive and false-negative results are rare, they do occur. The causes of false-positives are listed in table 1.

Antibodies to class II leukocytes are often present in recipients of multiple blood transfusions and in multiparous women.<sup>5</sup> These antibodies can cross-react with HLA class II antigens in HIV-1 preparations that are harvested from H9 cells. Use of synthetic or recombinant HIV peptides in

the ELISA eliminates this problem.

Passively acquired antibodies to HIV have been found in persons who have received human globulin, namely, certain lots of hepatitis B immune globulin containing antibodies to HIV.<sup>6</sup> This effect is transient, as it is in some infants who receive HIV antibody transplacentally. The half-life of passively acquired HIV antibody is 12 days.<sup>7</sup> No HIV infections have occurred as a result of infusions of intravenous gamma globulin.

A false-positive ELISA following influenza vaccination is prob-

ably due to cross-reaction with IgM antibodies to other viral epitopes.<sup>8,9</sup> This effect is short-lived (about 75 days), and results may vary with the type of ELISA kit used.<sup>9,10</sup> Because the incidence of this effect is less than 1.7%, recent vaccine recipients need not be discouraged from donating blood.<sup>9</sup>

A false-positive result can also be caused by laboratory error, such as inadequate washing of the specimen or incorrect dilution.<sup>11</sup> Repeating the test helps to eliminate this problem. Usually such false reactions are weak and can-

**Serum samples from patients at high risk for HIV infection who have repeatedly reactive ELISA results are usually unequivocally positive on Western blot analysis.**

not be confirmed on supplementary testing.<sup>5</sup> Often the reactive specimen is negative when tested with another manufacturer's ELISA kit. If an initial test is reactive and a subsequent test is nonreactive, a different ELISA test should be performed.

False-negative reactions can result from various factors (table 2). Powder from disposable gloves worn by technicians performing the test has been shown to be a potential cause. The powder acts as an absorbent of large molecules such as immunoglobulins.<sup>12</sup> Other factors can result in test situations in which antibody levels are insufficient for detection.

**Western blot assay**

The Western blot technique identifies antibodies to specific HIV proteins. Because it is costly, labor-intensive, and subjective in interpretation, Western blot is a poor screening test.<sup>5,13</sup>

The technique involves separating viral proteins electrophoretically by their molecular weights. After separation, the proteins are transferred, or "blotted," onto nitrocellulose paper, which is then incubated with the patient's serum sample.

After incubation and washing of the specimen, specific bound antibodies are detected by anti-

**Table 1. Causes of false-positive results for HIV on ELISA**

- Hematologic malignant disorders
- DNA viral infections
- Autoimmune disorders
- Multiple myeloma
- Primary biliary cirrhosis
- Alcoholic hepatitis
- Influenza vaccination
- Hepatitis B vaccination
- Passively transferred antibodies
- Antibodies to class II leukocytes
- Renal transplantation
- Chronic renal failure
- Stevens-Johnson syndrome
- Positive rapid plasma reagent test

ELISA, enzyme-linked immunosorbent assay.

*Data from Libman and Witzburg,<sup>4</sup> Proffitt and Yen-Lieberman,<sup>5</sup> Schlech et al,<sup>6</sup> Challakere and Rapaport,<sup>8</sup> Hsia,<sup>10</sup> Schieupner,<sup>13</sup> and Jindal et al.<sup>19</sup>*

human globulins conjugated to an enzyme or a radioactive probe. The spectrum of bands present is used as the interpretive criterion for the test (figure 2).

However, interpretation of the Western blot for HIV-1 has not been universally established. The majority of laboratories have accepted the recommendations of the Association of State and Territorial Public Health Laboratory Directors and the Centers for Disease Control.<sup>5</sup> These criteria

**Table 2. Causes of false-negative results for HIV on ELISA**

- Incubation "window" period before antibody development
- Immunosuppressive therapy
- Replacement transfusion
- Malignant disorders
- B-cell dysfunction
- Bone marrow transplantation
- Kits that detect primarily antibody to p24
- Starch powder from laboratory gloves

ELISA, enzyme-linked immunosorbent assay.

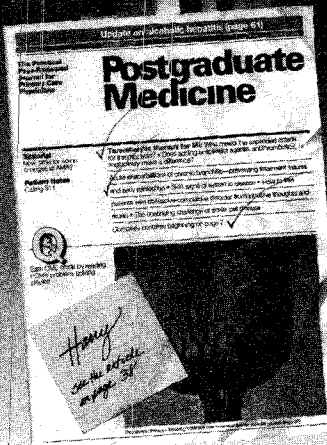
*Data from Libman and Witzburg,<sup>4</sup> Proffitt and Yen-Lieberman,<sup>5</sup> Lampe et al,<sup>12</sup> and Schieupner.<sup>13</sup>*

require two of three key bands to be present for a positive result. When assessing Western blot results, physicians should be aware of the criteria used by the laboratory performing the test (table 3).

Specimens from high-risk patients that are repeatedly reactive on ELISA are usually unequivocally positive on Western blot. However, false-positive and indeterminate results can occur because of cross-reactivity with other proteins (table 4).

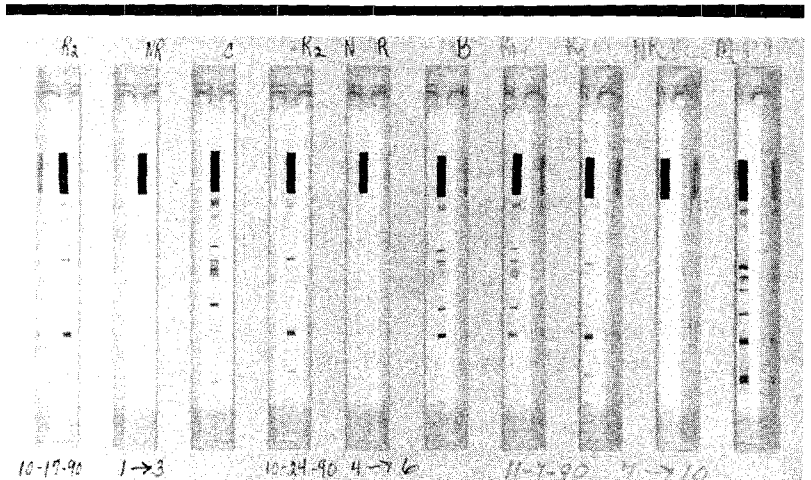
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**Evidence suggests  
that low-risk patients  
with consistently  
indeterminate Western  
blot results do not  
have HIV infection.**



**Figure 2.** Typical Western blot assay for HIV showing separation of viral protein antigens according to molecular weight.

### Interpretation of test results

Differences in laboratory criteria and variations among results of different Western blot kits may cause a patient's serum to test positive in one laboratory and negative in another. Thus, incidences of inaccurate results vary from a false-positive rate of 1 in 20,000 tests to indeterminate results in 20% to 40% of cases in which the ELISA test was seronegative.<sup>5</sup> The incidence of indeterminate results is one reason Western blot is not used as a screening test.

In patients with known risk factors for HIV, an indeterminate result on the Western blot test fol-

lowing a positive ELISA result may represent early infection; seroconversion is incomplete in patients with early HIV infection. Also, patients presenting in the late stage of infection may have severe immunoincompetence, causing an indeterminate result. Such patients would be expected to be clinically symptomatic, however, which usually indicates a poor prognosis. In patients with no identifiable risk factors who have an indeterminate result, follow-up testing may resolve the uncertainty.

Evidence suggests that low-risk patients with consistently indeterminate Western blot results do

*continued on page 185*

**Obtaining accurate information about patients' HIV risk factors can be difficult because of physicians' reluctance to take a detailed sexual history and patients' denial of high-risk behaviors.**

not have HIV infection.<sup>14</sup> The recommendations of the Centers for Disease Control<sup>15</sup> state, "A person whose Western blot results continue to be consistently indeterminate for at least 6 months in the absence of any known risk factors, clinical symptoms, or other findings may be considered negative for antibodies to HIV-1."

In low-risk persons concerned about HIV infection, a negative ELISA is a good indicator that HIV antibody is not present. For persons with known exposure to the virus, the combination of a negative ELISA and the absence of all bands on Western blot at 3, 6, 9, and 18 months after exposure provides good evidence that HIV antibody is not present.<sup>16</sup>

Strict guidelines for interpreting results and use of reliable laboratories are helpful, particularly for evaluation in a low-risk population. The screening program used by the US military for civilian recruits is an example. In one study,<sup>17</sup> the use of conservative criteria for interpretation of test results and verification of positive results with testing of a second, newly drawn serum sample resulted in a false-positive rate of 1 in 135,187 persons tested.

Interpretation of test results should take into account the pa-

**Table 3. Criteria for interpreting Western blot results for HIV**

**ASTPHLD/CDC**

Negative: no bands

Positive: at least two of key bands p23, gp41, and gp120/160

Indeterminate: single band or combination of bands that does not fit a positive result

**American Red Cross**

Positive: three or more bands, one from each of gene groups *gag*, *pol*, and *env*

ASTPHLD/CDC, Association of State and Territorial Public Health Laboratory Directors and Centers for Disease Control.

**Table 4. Causes of false-positive and indeterminate Western blot results for HIV**

- Normal human ribonucleoproteins
- Other human retroviruses
- Antibodies to mitochondrial, nuclear, and T-cell leukocyte antigens
- Globulins produced during polyclonal gammopathy
- Proteins on filter paper
- Anticarbhydrate antibodies
- Heat-inactive serum
- High concentration of bilirubin in serum
- Passively acquired antibodies

Data from Libman and Witzburg,<sup>4</sup> Schlech et al,<sup>5</sup> Schlepner,<sup>13</sup> Jindal et al,<sup>13</sup> Migali et al,<sup>20</sup> Healey and Bolton,<sup>21</sup> and Ascher and Roberts.<sup>22</sup>

tient's history and known risk factors for HIV. However, obtaining accurate information about patients' HIV risk factors can be difficult because of physicians' reluctance to take a detailed sexual history and patients' denial of

high-risk behaviors. It has been found that 85% of seropositive individuals subsequently admit to high-risk behaviors after denying such behaviors when initially tested.<sup>18</sup>

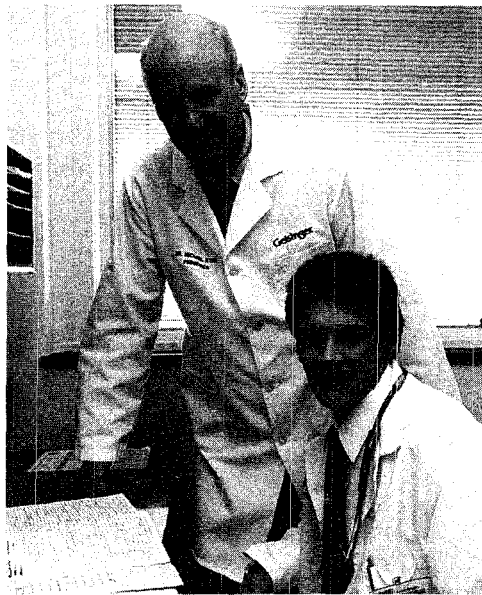
Another option to help inter-

*continued*

## Polymerase chain reaction could potentially replace Western blot as a confirmatory test for HIV.

**Robert J. Cordes, DO**  
**Michael E. Ryan, DO**

Dr Cordes (right) is a pediatric resident and Dr Ryan (left) is vice chairman, department of pediatric subspecialties, Geisinger Medical Center, Danville, Pennsylvania.



pret the significance of indeterminate results is to perform additional confirmatory tests. The immunofluorescent assay, which uses uninfected cells and HIV-infected cells as a substrate, can help differentiate specimens with antibodies reactive to cellular rather than HIV proteins.<sup>5</sup> Polymerase chain reaction is a method of gene amplification that allows detection of proviral DNA. Although results from laboratories inexperienced in the process can be unreliable, the test could potentially replace Western blot as a confirmatory test.<sup>5</sup> HIV antigen

ELISA can be useful for detecting antigen during the early and late stages of infection, when HIV antibody levels may be low.<sup>5</sup> Finally, HIV can be cultured from lymphocytes separated from anticoagulated blood. However, the test is time-consuming, and 30 days may be needed to produce a result.

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### Summary

**Enzyme-linked immunosorbent assay (ELISA) and Western blot assay are the most**

commonly used laboratory tests for HIV infection. Both detect antibodies to HIV. ELISA results are based on detection of antigen-antibody complexes by using antibodies labeled with an enzyme that produces a color change in the presence of a specific substrate. Currently licensed ELISA tests have greater than 98% sensitivity and specificity for HIV. Western blot analysis detects antibodies to specific HIV antigens and is best used as a confirmatory test.

In spite of the high sensitivity and specificity of both tests, false-positive and false-negative results do occur. Physicians should be aware of specific causes of inaccurate results. In individual cases, knowledge of the patient's history and the criteria used by the laboratory performing the test is important. **FGM**

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# Pitfalls in HIV testing

Application and limitations of current tests

Robert J. Cordes, DO Michael E. Ryan, DO

## Preview

**Inaccurate results on HIV tests are rare, but they do occur. Therefore, it is important that primary care physicians understand the laboratory techniques involved. Which assays are used for detection of the virus? How are results of such tests interpreted? What factors cause false-positive and false-negative results? Drs Cordes and Ryan answer these questions and clarify the uncertainty surrounding HIV testing.**

The diagnosis of HIV infection is based on serologic evidence of antibodies to the virus, detection of an antigenic portion of the virus, or culture of the virus. The enzyme-linked immunosorbent assay (ELISA) is the initial screening test, and the Western blot assay is most commonly used to confirm results. Other confirmatory tests include the polymerase chain reaction, HIV antigen ELISA, and HIV blood culture.

It is important for physicians to understand the application and limitations of serologic tests for HIV. Results of these tests should be interpreted in conjunction with the patient's history and clinical status.

## Immunology of HIV

Infection with HIV leads to a detectable immune response, generally within 1 week to 3 months. After that, a decline in viremia occurs, while viral replication continues in the lymphatic system.<sup>1</sup>

IgG antibody to HIV is usually detectable indefinitely once the immune response occurs, although it may fall to undetectable levels late in the course of the disease.<sup>2</sup>

The core protein p24 is the first detectable protein encoded for by the *gag* (group-specific antigen) gene.<sup>3</sup> This protein is a marker for viremia. Its appearance is followed by the development of antibodies to p24 and to envelope glycoprotein gp41. As antibody response occurs, plasma viremia declines. Figure 1 shows the pattern of immune response to HIV infection.

Antibodies to HIV are usually detectable by ELISA within 4 to 12 weeks after infection. In advanced disease, antibody levels decrease and viremia increases (figure 1). During this stage, a test for p24 antigen may be helpful.

## ELISA

The ELISA is performed as follows: The patient's serum sam-

ple is placed into wells of microtiter plates that have been coated with recombinant HIV antigens. The serum is incubated, and then the wells are washed, leaving complexed antibodies to HIV, if present. Enzyme-labeled anti-human antibody is added, followed by a substrate. If a reaction occurs between complexed HIV antibodies and the substrate, a color change takes place. The absorbance of the color change is read with a spectrophotometer and is proportionate to the amount of complexed HIV antibody. The absorbance reading of each sample is compared with that of HIV-positive and HIV-negative control specimens, which are run with each test.

If the sample is reactive, at least one more ELISA is performed. A sample must be repeatedly reactive to be considered positive. If the sample is nonreactive, the result of the ELISA is reported as nonreactive and the test is not repeated. In cases in which the ELISA is repeatedly reactive, a confirmatory test should be performed.

The sensitivity and specificity of currently licensed ELISA tests are greater than 98% and may approach 100%.<sup>4,5</sup> The predictive value of a positive test result depends on the prevalence of HIV

*continued*