Human immunodeficiency virus testing: Update

WALTER C. HOOK, MT(ASCP), MS
JOHN J. FERNANDES, DO

Human immunodeficiency virus (HIV) types 1 and 2 have been associated with the acquired immunodeficiency syndrome (AIDS). The detection of HIV infections is based on the screening of serum samples for the presence of antibodies to HIV proteins. Serum samples that test positive on screening must be assayed by a confirmatory test to provide a definitive report on the presence of HIV infection. This article reviews the currently available screening and confirmatory testing procedures and their limitations.

(Key words: Human immunodeficiency virus, laboratory tests, screening tests, acquired immunodeficiency syndrome)

The human immunodeficiency virus (HIV) (*Figure*) selectively infects lymphocytes and macrophages, thereby causing immunosuppression. The immunosuppression allows the development of secondary opportunistic infections that are ultimately fatal to the host. During infection, gp120, a glycoprotein on the viral envelope, binds to the CD4 phenotypic marker found primarily on helper/inducer T lymphocytes (T4 lymphocytes). The specific binding of CD4 receptors by the gp120 glycoprotein accounts for the selective infectivity of this cell class, although other cells, such as macrophages, also possess the CD4 marker. The viral transmembrane glycoprotein gp41 is required for viral entry into the host cell.

Owing to the individual variability of hosts' abilities to produce antibodies, and because the cells of hosts' immune systems are specifically targeted, there is considerable variability in the time frame (window) in which HIV infections may be detected. During that window of time, the infected individual may be unwittingly spreading the virus.

Molecular biology of the HIV

The HIV is an RNA-containing retrovirus, of the *Lentivirinae* subfamily, that requires a living host for its survival. The HIV core carries a reverse transcriptase that catalyzes synthesis of complementary DNA (cDNA) from the viral RNA. The cDNA then integrates into the chromosomal DNA of the infected cell.

During early infection with the HIV, very few cells contain the proviral DNA. This paucity makes detecting the infection difficult when in vitro tissue culture methods are used. Successful detection of the HIV from viral culture ranges from 23% for plasma and up to 97% for peripheral blood mononuclear cells.[1]

From the Department of Pathology and Laboratory Medicine, Philadelphia College of Osteopathic Medicine, Philadelphia, Pa, where Dr Fernandes was formerly chairman. Mr Hook is currently at the Pathology Department, Suburban General Hospital, Norristown, Pa, and Dr Fernandes is currently at the Department of Pathology, Olympia Fields Osteopathic Hospital and Medical Center, Olympia Fields, Ill.

The authors have no commercial or proprietary interest in the assays discussed nor do they have any financial interest (as consultant, reviewer, or evaluator) in the assays. Furthermore, specific identification of assay manufacturers does not constitute endorsement by the authors.

Reprint requests to Mr Walter C. Hook, Pathology Department, Suburban General Hospital, 2701 DeKalb Pike, Norristown, PA 19401.

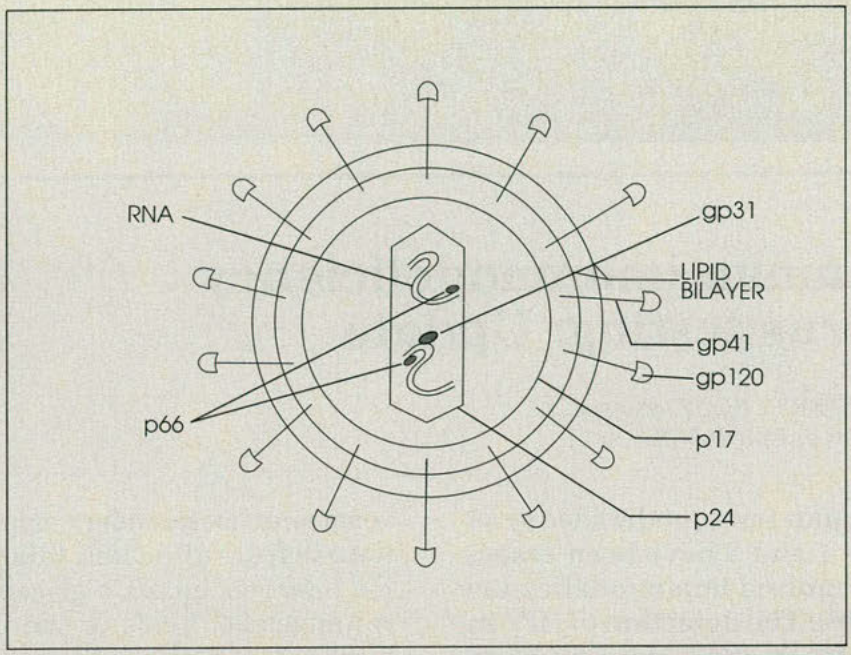

Figure. *Human immunodeficiency virus.*

Because of the difficulty of using tissue culture techniques to isolate the virus, HIV infection is currently detected by using immunochemical methods that detect specific host antibodies directed against viral proteins.

The HIV virus has a cylindric core surrounded by a lipid envelope derived mainly from the cell membrane of the infected cell. However, the envelope also has projections composed of two glycoproteins designated gp120 and gp41. The virus core consists of two strands of RNA and several proteins. The core proteins are identified by number based on their molecular weights. The core protein designated p24 is significant in that it is unique to the HIV. This protein induces antibody production in the host during early stages of infection and is therefore valuable in identifying HIV infection. The core also contains reverse transcriptase, which allows the viral RNA to produce proviral DNA to be incorporated into the host genome.

The HIV types 1 (HIV-1) and 2 (HIV-2) exhibit approximately 60% homology for the DNA sequences found in the *gag* and *pol* genes, and approximately 40% homology for other genes. Therefore, assays developed to detect HIV infections in a population infected primarily with HIV-1 may not produce conclusive test results with individuals infected with the HIV-2.

A 1990 review by Smith[2] summarizes the pathobiology of HIV infection.

HIV tests

Cultural assays for the HIV in which the virus is grown in tissue culture are not currently feasible for use in the clinical laboratory. The common clinical laboratory tests for HIV can be classified into two groups: screening tests and confirmatory tests.

Screening tests

Screening tests are basically enzyme-linked immunosorbent assays (ELISAs) that use an enzyme-labeled immunoreactant (a mixture of HIV antigens in this case) and a solid support (beads, microtiter plate, test tube).

The ELISAs are currently widely used as screening tests to detect antibodies to the HIV. The specificity of most ELISAs exceeds 99.8%. However, the tests are used to screen populations that have a very low prevalence of HIV infection. Under these conditions, the positive predictive value of ELISA may be 10% or less.[3] Therefore, a positive ELISA does not conclusively prove the presence of the HIV, and a negative ELISA does not rule out HIV expo-

(continued on page 496)

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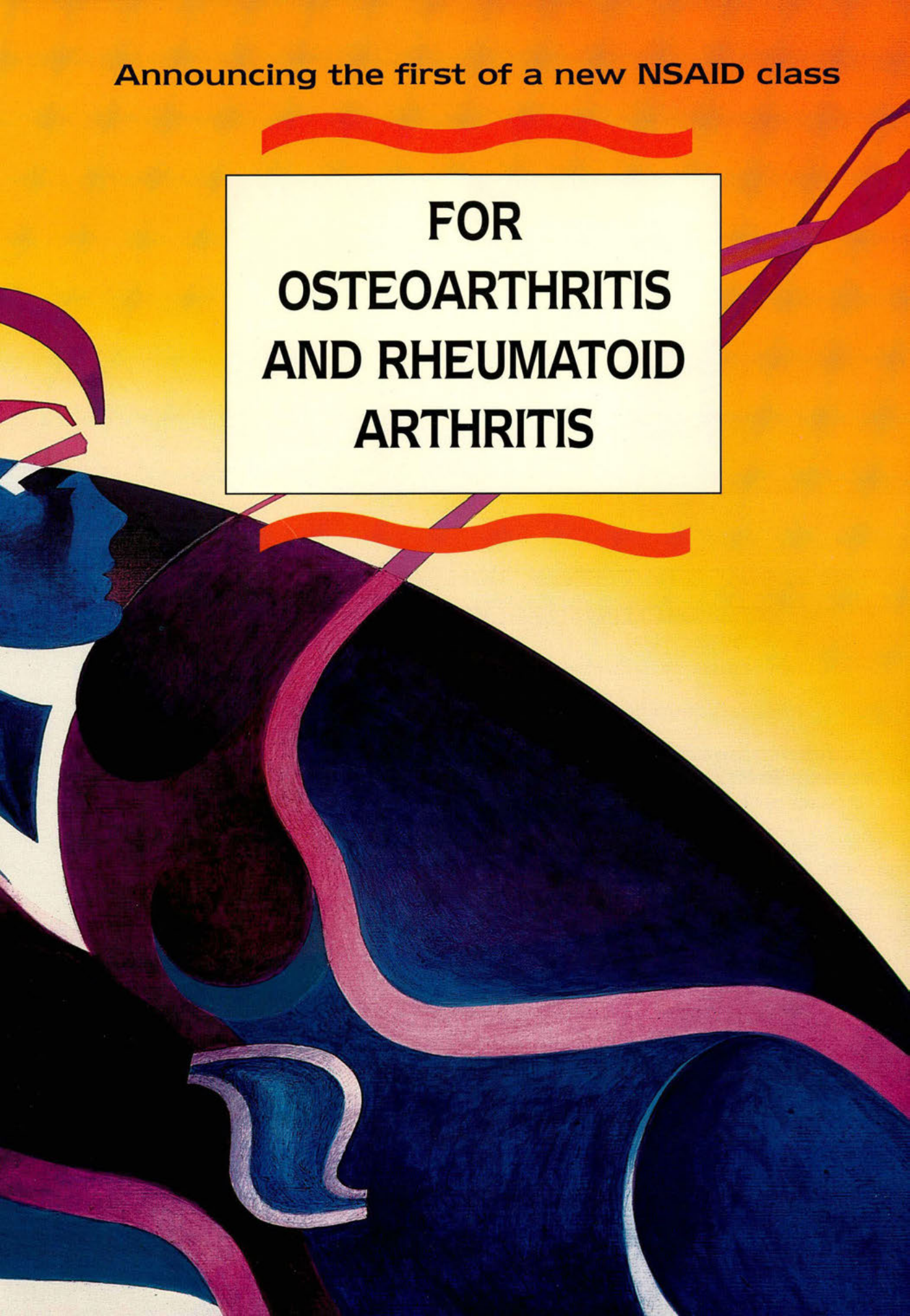

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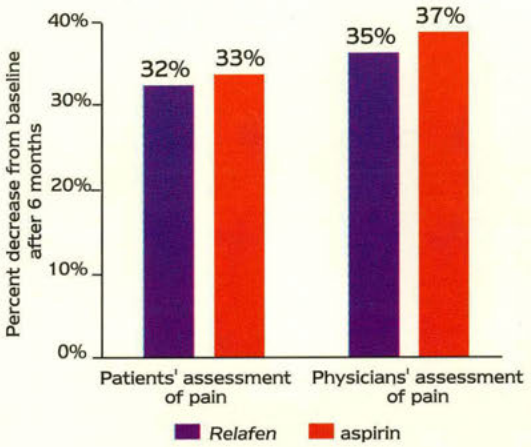

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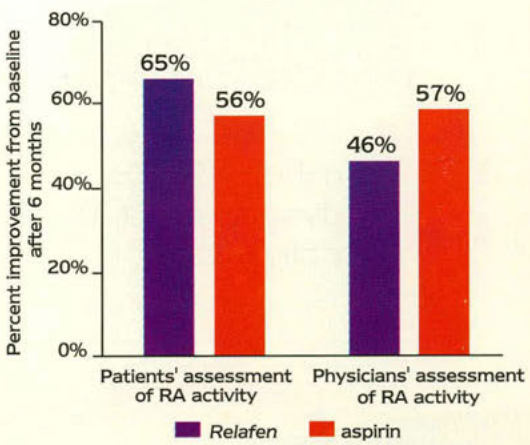

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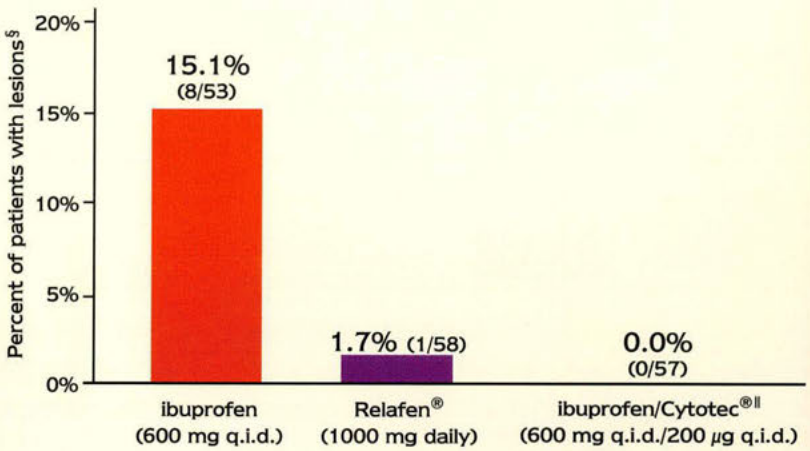

†The trials were not designed to correlate symptoms and endoscopy scores. The clinical relevance of these endoscopy findings, i.e., either G.I. symptoms or serious G.I. events, is not known.

‡ $P=0.013$.
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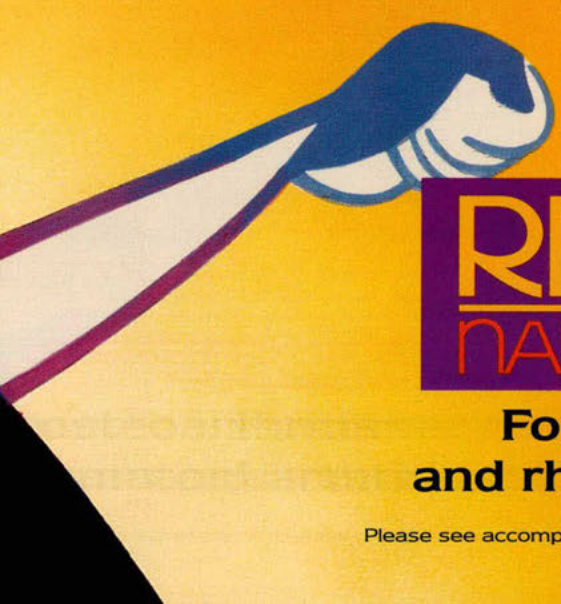

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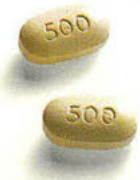

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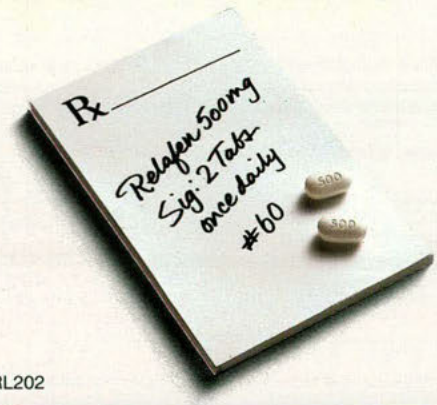
- Starting dose 1000 mg/day given as two 500 mg tablets
- Can be titrated up to 2000 mg/day



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The May issue also will feature an article on optical devices designed for patients with low vision. In addition, the issue will provide coverage of the annual convention of the American College of General Practitioners in Osteopathic Medicine and Surgery and the midwinter conference of the Colorado Society of Osteopathic Medicine.

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sure. Consequently, it is necessary for attending physicians to be sensitive to the patient's anxieties and present the patient with both pretest counseling, as well as posttest counseling. It is suggested that a positive ELISA test report not be given to the patient until a positive result has been obtained by a confirmatory test.

Similarly, negative test results should be followed by posttest counseling because screening tests have a limited potential for yielding false-negative results. Persons with negative results may be retested, based on physician judgment. Individuals who have indeterminate test results (either after screening or on confirmatory testing) should be retested within 3 to 6 months. Furthermore, the difficulty in detecting HIV in infected people is the fact that the virus infects a select cell population. Once the cells are infected, there are few proviral elements per cell; and, when the viral genome is incorporated into the host cell, there is a lengthy latency period. An individual who has consistently indeterminate confirmatory test results during a 6-month period and who has no known risk factors may be considered to be negative for HIV antibodies.

Confirmatory tests

The Western blot technique is most commonly used to verify the presence of HIV antibodies. The first stage of this procedure involves the growth and purification of the HIV. The virus is lysed, and the lysate is electrophoresed. After the proteins are separated by electrophoresis, the proteins, as bands, are transferred to nitrocellulose by placing the nitrocellulose over the electrophoresed proteins. Thereby, the proteins are transferred to the nitrocellulose in a pattern identical to their electrophoretic separation. The desired bands can then be cut from the nitrocellulose and can be used to detect the presence of antibodies to the particular HIV proteins. (This portion of the Western blot test is currently produced for users by commercial diagnostics manufacturers such as Du Pont, Wilmington, Del, and Electro-Nucleonics, Columbia, Md).

In the common clinical laboratory use of the Western blot technique, serum samples are incubated with the nitrocellulose strips coated with HIV proteins. The strips are washed to remove nonadherent antibody. The strips are then incubated with horseradish peroxidase conjugated anti-IgG. After this incubation, the strips are washed again, and a substrate is added to allow visualization of the bands where antibody has attached to bound HIV proteins. The viral proteins that are detected by this method include the viral core *gag* proteins (p17, p24, p55), polymerase *pol* proteins (p31, p51, p66), and envelope *env* proteins (gp41, gp120, and gp160). This method is very sensitive and very specific for detecting antibodies to HIV. However, there is no consensus regarding the interpretation of results.

Western blot interpretation

Four major organizations have established similar, but different, criteria for the interpretation of Western blot test results *(Table)*.[4] Although agencies and organizations such as the Food and Drug Administration, the American Red Cross, and the Centers for Disease Control agree that the absence of bands on the blot indicates a negative test result, there is significant divergence of opinions as to what bands (complexes of antibody and viral protein) must be present for the test to be interpreted as positive. According to a report by O'Gorman and coworkers,[5] interpretation of Western blot assay results using each organization's criteria, in addition to criteria developed by the University of North Carolina Hospitals—Chapel Hill, resulted in variable numbers of positive, negative, and indeterminate results.

Unusual Western blot patterns have been observed when non–HIV-1 retrovirus infections yield *gag* and *pol* gene products. In cases in which test results are indeterminate, every effort should be made to qualify the test results. Recommendations for resolution should include retesting the patient's serum sample within 6 weeks to 2 months.

HIVAGEN, a proprietary, second-generation, ELISA-based confirmatory assay for HIV antibodies is available from SmithKline Beecham Clinical Laboratories (SKBL). It is the contention of SKBL that because the HIV

Table
Criteria for Interpretation of Western Blot Assays[3*]

Organization	Interpretive criteria		
	Positive	Negative	Indeterminate
American Red Cross	Minimum of 3 bands: 1 each of *env, gag, pol*	No bands	Failure to meet positive test criteria
Centers for Disease Control	Minimum of 2 of the p24, gp41, or gp160/120	No bands	Failure to meet positive test criteria
Food and Drug Administration	p24, p31, and gp41 and/or gp120/160	No bands	Failure to meet positive test criteria
Consortium for Retrovirus Serology Standardization	p24 or p31 and gp41 or gp160/120	No bands	Failure to meet positive test criteria

*Adapted from Centers for Disease Control: Interpretation and use of the Western blot assay for serodiagnosis of human immunodeficiency virus type 1 infections. *MMWR* 1989; 38(suppl 7):1-7.

used in ELISA screening tests and Western blot procedures is grown in human tissue cultures, the virus extracts contain significant amounts of human cell material. Furthermore, because ELISA-based screening assays have been adjusted to maximum sensitivity, specificity is decreased and false-positive reactions can occur. SmithKline Beecham Clinical Laboratories further notes that false-positive reactions are caused when non-HIV antibodies react with the contaminating human cell material or cross-react with HIV protein. The Western blot assay, according to some estimates based on proficiency testing, may have a false-negative rate of up to 10% and a false-positive rate of up to 5%.[6] Also, SKBL contends that the Western blot assay has other limitations, including:

- Many laboratories do not use standardized reagents or procedures when performing the Western blot technique.
- Variable reporting and interpretive data are used for positive, negative, and indeterminate test results.
- Technical accuracy is dependent on the skill of the person performing the test.
- Interpretation of test strip reactions is subjective.

HIVAGEN consists of a six-panel ELISA assay. The HIV antigens used to bind patient antibodies are purified peptides produced by recombinant DNA technology. This assay uses two recombinant antigens (Kp24 and Kp55) to detect antibodies to the viral core, or *gag* gene product; three recombinant antigens (Kp41, Kp120N, Kp120CC) to detect antibodies to envelope, or *env*, products; and one antigen (Kp66/31) to detect antibodies to *pol* polymerase/enolase gene products. According to information provided by SKBL, HIVAGEN has not shown any false-positive or false-negative results. However, according to the same data, HIVAGEN demonstrated a 4% indeterminate rate, albeit significantly lower than that demonstrated by Western blot technique.

HIV detection by use of polymerase chain reaction

The HIV infects a select cell population, providing a limited number of proviral particles, with a lengthy latency before the expression of viral proteins. Investigators[1,7] have developed an in vitro DNA amplification technique called *polymerase chain reaction* (PCR) in an attempt to improve diagnosis by eliminating false-negative or indeterminate test results.

The technique uses two synthetic oligonucleotide primers that are complementary to DNA sequences that border a section of viral cDNA of interest. A DNA probe, complementary to the viral cDNA under investigation, is incorporated with the oligonucleotides. One

oligonucleotide is complementary to the negative DNA strand, while the other is complementary to the positive DNA strand. By lysing the infected cell, viral cDNA may be opened (the complementary strands of DNA separate), and the primers anneal to the proviral DNA. A polymerase extends the length of primers in the presence of deoxynucleoside triphosphates. Polymerase cycling amplifies the proviral sequence under investigation to the point that millions of copies of the DNA fragment may be produced. By amplification, the possibility for detecting specific HIV cDNA is significantly enhanced. The PCR is a difficult procedure to perform and, to date, there have been no significant developments in reagents or instrumentation that make wide-scale use feasible.

Comment

Despite the rapid technologic advances in the detection and identification of HIV infections, there is currently no single assay with a 100% predictive value that is inexpensive and easy to perform. For the time being, it appears that the testing sequence for HIV diagnosis will continue to rely on screening with subsequent confirmatory testing. Because of the nature of the infection, even negative test results cannot be considered to correctly indicate that the individual is not infected. Therefore, physicians must be judicious in the interpretation of test results.

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