
Subspecialty Clinics: Infectious Diseases

Serologic Testing for Human Immunodeficiency Virus Antibodies

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Familiarity with available serologic tests for antibodies to human immunodeficiency virus (HIV) has become increasingly important in a wide variety of clinical settings. Enzyme-linked immunosorbent assay (ELISA) commercial kits are most often used as screening tests, and Western blot techniques are used for confirmation of positive results. ELISA specificity and sensitivity exceed 98%; the predictive value of a positive test varies from 2% for a weakly positive test in a low-prevalence population to 99% for a strongly positive test in a high-risk group. Confirmatory Western blot testing identifies antibodies with affinity for specific HIV antigens. Indeterminate Western blot antibody patterns necessitate subsequent testing or alternative methods for interpretation. A "window" period of up to 3 or more months follows acute HIV infection before seropositivity occurs.

Since 1985 when the Food and Drug Administration licensed the first enzyme-linked immunosorbent assay (ELISA) commercial kits for the detection of antibodies to human immunodeficiency virus (HIV), use of these tests has become almost universal for screening blood and organ donors, in accordance with the recommendations from the Centers for Disease Control.¹ In the United States, use of the ELISA test has also been proposed or mandated for applications other than ensuring a safe blood supply, including screening programs for the military, federal inmates, and immigrants and the voluntary testing of certain transfusion recipients, surgical candidates, pregnant women, patients at clinics for

sexually transmitted diseases or drug abuse, and premarital examinees.² The circumstances in which laboratory evidence of HIV infection, such as serologic reactivity, is necessary—to fulfill the case definition of acquired immunodeficiency syndrome (AIDS) established by the Centers for Disease Control³—are outlined in Table 1. Serologic evidence of infection is also useful in the assessment of patients with HIV-compatible symptom complexes and in the context of a history of possible exposure to AIDS.

As the number of persons screened for HIV increases, so will the number of patients with reactive test results who will be referred to private physicians for counseling and further testing. Most patients (68 to 89%) from low-risk groups (prevalence of 0.1% or less) who show reactivity on screening tests will have false-positive results.^{4,5} Additionally, an estimated 2 million persons in the United States alone will become infected with HIV (and will thus be seropositive) by the year 1991.

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In theory, definitive assessment of HIV infection and infectivity is based on recovery of the virus from body tissue or fluids.⁶⁻⁹ Current techniques for HIV culture, however, are cumbersome, time-consuming, expensive, and of low sensitivity,^{9,10} and such testing is primarily available only at research facilities. An alternative approach is the direct detection of HIV antigens; although methods to accomplish direct detection show clinical promise, these techniques also are not widely available at present.^{10,11} The detection of antibody to HIV currently remains the cornerstone of screening for HIV infection by blood banks and clinical laboratories. Like other viruses, the detection of antibody to HIV provides evidence of past exposure to the virus. Unlike many other viruses, however, the appearance of specific anti-HIV antibodies does not presage clearing of the viremia, loss of infectivity, or clinical recovery. Indeed, anti-HIV seropositive patients, whether asymptomatic or symptomatic, can be assumed to be viremic, and current evidence as

well as analogy with the biologic features of other retroviruses suggests that most, if not all, such patients will remain so indefinitely.^{12,13} Although patients have been described who are culture positive but seronegative for HIV,^{14,15} this situation is rare except during a "window" period of 3 or more months after acute HIV infection before antibodies develop. These observations establish the practical utility and importance of serologic testing for HIV antibodies.

Several laboratory techniques are available for the detection of antibody to HIV. Currently, ELISA and Western blot techniques are used most often for screening and confirmatory tests, respectively. Other techniques that have proved useful in the research setting are radioimmuno-precipitation and membrane or cytoplasmic indirect immunofluorescence assays. These tests vary in complexity, cost, time to perform, specificity, sensitivity (defined both in epidemiologic terms and in terms of the antibody concentration necessary for positivity as assessed by serial dilution), antibody specificities detected, and time course for seroconversion after initial infection. All require HIV antigen, but the source and the method of preparation of HIV antigen vary, as does the means by which antibody-antigen binding is detected.

HIV ANTIGENS

The antigenic source material for commercially available ELISA and Western blot tests (as well as most other methods) has been HIV grown in human T-cell lines, most often the H9 cell line (a cloned lymphoid leukemia cell line that is immortalized by HIV infection and thereby allows continuous production of virus).⁶ Figure 1 schematically illustrates the HIV genome and the major antigens that have been characterized. Antigens are labeled by their estimated size in kilodaltons and are proteins (p) or glycoproteins (gp).

The *gag* gene codes for a precursor polyprotein p55, present in infected cells but not in purified extracellular virus. It is cleaved to form smaller structural proteins including p17 and the antigenically important core protein p24, present in both extracellular virus and homogenized, HIV-infected cells.

The *env* gene codes for a highly immunogenic precursor glycoprotein gp160, again present in infected cells but not in purified extracellular virus, which is cleaved to form the envelop

Table 1.—Role of Serologic Testing in Diagnosing AIDS, Based on the Case Definition Established by the Centers for Disease Control*

Laboratory evidence of HIV infection not required
Esophageal or pulmonary candidiasis
Extrapulmonary cryptococcosis
Chronic symptomatic cryptosporidiosis
Disseminated cytomegalovirus infection
Pulmonary, esophageal, or chronic mucocutaneous herpes simplex infection
Kaposi's sarcoma or primary brain lymphoma in patients <60 years old
Lymphocytic interstitial pneumonitis in patients <13 years old
Disseminated <i>Mycobacterium avium</i> or <i>kansasii</i> infection
<i>Pneumocystis carinii</i> pneumonia
Progressive multifocal leukoencephalopathy
Toxoplasmosis of the central nervous system
Laboratory evidence of HIV infection required
Any of the aforementioned diseases, if patient is immunodeficient from another cause
Recurrent serious bacterial infections in patients <13 years old
Disseminated histoplasmosis or coccidioidomycosis
Chronic symptomatic isosporiasis
Kaposi's sarcoma or primary brain lymphoma in patients >60 years old
Small, noncleaved B-cell lymphoma or immunoblastic sarcoma
Extrapulmonary tuberculosis
Any disseminated atypical mycobacterial infection
Recurrent <i>Salmonella</i> septicemia
HIV wasting syndrome
HIV encephalopathy

*AIDS = acquired immunodeficiency syndrome; HIV = human immunodeficiency virus.

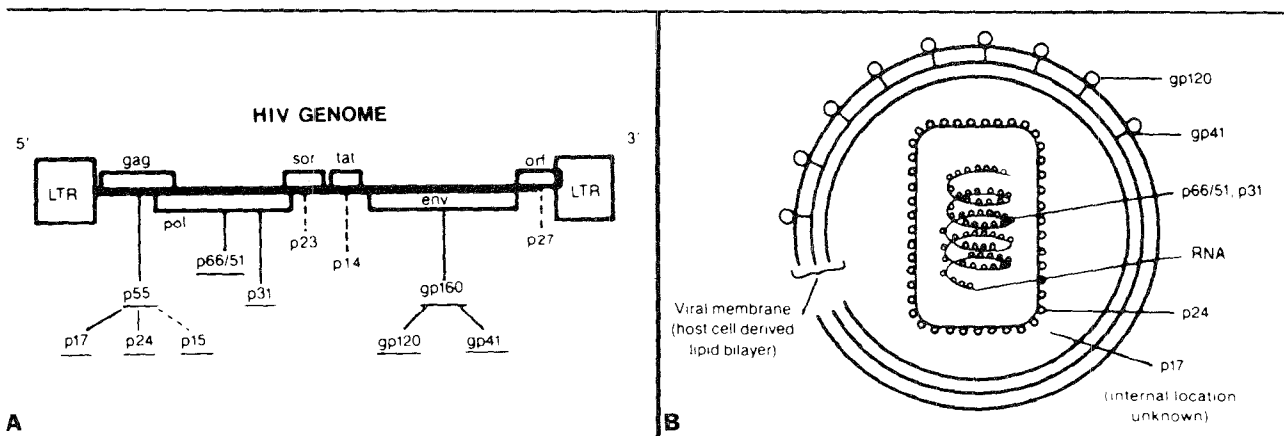


Fig. 1. Schematic representation of human immunodeficiency virus (HIV) genome and antigens, which are labeled by their estimated size in kilodaltons. *gp* = glycoproteins; *p* = proteins. See text for discussion. (From Human Immunodeficiency Virus [HIV] Biotech/Du Pont HIV Western Blot Kit package insert, Du Pont Company, Wilmington, Delaware.)

attachment glycoprotein gp120 (highly immunogenic) and the transmembrane glycoprotein gp41 (moderately immunogenic). Both of these glycoproteins are present in extracellular virus and in infected cell homogenates.

The *pol* gene codes for the viral reverse transcriptase (p66/p51) and the endonuclease protein p31.

Methods that use concentrated extracellular virus as a source of antigen (ELISA and Western blot commercial kits) are rich in p17 and p24 and will best detect anti-p24 and anti-p17 antibodies. In contrast, the high-molecular-weight antigens gp160, gp120, and, to a lesser extent, gp41 are generally more immunogenic. Antibodies directed to epitopes of these glycoproteins are present in high concentrations in serum specimens. Unfortunately, these antigens are poorly preserved in the preparation of current-generation commercial test kits; thus, these kits are relatively insensitive for detection of antibodies directed against these important antigens.¹⁶ Improvements in the purity and composition of antigens used in serologic tests could be expected to enhance their performance substantially. One important approach with good preliminary results bypasses HIV culture and uses recombinant DNA techniques to express cloned HIV antigenic polypeptides in bacteria.¹⁷⁻¹⁹

ENZYME-LINKED IMMUNOSORBENT ASSAYS

In the "generic" ELISA, test serum is incubated with HIV antigens (as described in the foregoing

material) immobilized on beads or wells; non-specific antibody in the serum is removed by washing, but anti-HIV antibodies with affinity for the antigens present in the system remain bound. The presence of bound anti-HIV is detected by an anti-human IgG linked to an enzyme such as horseradish peroxidase; when appropriate substrate is added, spectrophotometrically detectable color is produced, the optical density of which is proportional to the amount of anti-HIV bound. The cutoff optical density is established by the manufacturer and affects sensitivity and specificity of the assay inversely. A sample that is initially reactive is retested in duplicate and deemed positive only if "repeatedly positive"—that is, at least one of the subsequent two tests is also reactive. Estimates of sensitivity have ranged from 82% in early studies²⁰ to more than 99% more recently,²¹ and the estimated specificity is 93 to more than 99%.²⁰ Currently licensed tests properly performed likely have sensitivity and specificity for HIV infection that exceeds 98 to 99%.³ Because of the lack of a readily available definitive test, these estimates are based on the assumptions that all patients with AIDS or AIDS-related complex have anti-HIV antibodies and that all low-risk random donors are antibody negative.

The degree of reactivity and the risk factors of the patient both influence the clinical interpretation of a positive ELISA test. In screening programs of low-risk persons such as blood donors, the positive predictive value of a weakly

reactive ELISA is only 2%, in comparison with a predictive value of 87% for a strongly positive test in the same population.²² In contrast, a strongly reactive ELISA in a high-risk population has a positive predictive value of 99%.^{10,22,23} The degree of reactivity, however, may decline late in the course of AIDS.²⁴⁻²⁷

False-positive ELISA reactivity has been noted with low frequency in panels of serum specimens from patients with other immunologic abnormalities including hematologic malignant lesions (0 to 7%), acute DNA viral infections (5%), or serum samples positive for rheumatoid factor, antinuclear antibody, and other autoantibodies (17%).^{20,28,29} A recent retrospective study in which an enzyme immunoassay kit was used found high false-positivity rates in patients with multiple myeloma (21%), primary biliary cirrhosis (20%), and primary sclerosing cholangitis (12%);²⁹ in addition, false-positive results have been reported in 13% of 95 patients with alcoholic hepatitis.³⁰ One notable association with false-positive ELISA reactivity in some commercial preparations has been patients with anti-HLA-DR4 antibodies, most often multiparous or multiply transfused patients.^{28,31} This reactivity has apparently been due to H9 cell line-derived class II HLA antigens present in the assay system. An exclusionary assay is available that includes a control well prepared from uninfected H9 cells; serum samples that are nonspecifically reactive with HLA antigens will have comparable reactivity in both the HIV antigen-containing well and the non-antigen-containing well. With use of this system, however, confirmatory testing of positive serum specimens with an alternative method is still recommended. Assays that use antigen produced in the CEM cell line (an alternative "immortalized" neoplastic human T-cell line), such as the lymphadenopathy-associated virus (LAV) enzyme immunoassay (Genetic Systems Corp., Seattle, Washington), also lack class II HLA cross-reactivity,³² as do techniques that use recombinant DNA antigen.

WESTERN BLOT METHOD

Procedure.—Western blot techniques have been the qualitative test most widely available and used for confirmation of ELISA-reactive serum samples, although other methods, particularly immunofluorescence assays,^{33,34} may also be applicable. Like the ELISA test, Western blot

techniques use a mixture of HIV antigens traditionally derived from extracellular, partially purified, disrupted whole virus. In the Western blot test, however, the HIV antigens are electrophoretically separated into discrete bands that are transferred ("blotted") onto nitrocellulose paper. On incubation of nitrocellulose test strips with patient serum specimens, anti-HIV immunoglobulins bind with specific antigenic bands and thus facilitate separation and identification of the antibody specificities present (Fig. 2). Traditionally performed in reference laboratories, the Western blot method lacks standardization, is cumbersome, and is subjective in interpretation of banding patterns. Some laboratories purchase commercially prepared nitrocellulose test strips loaded with antigen, whereas others prepare their own test strips. Antigen composition and quality

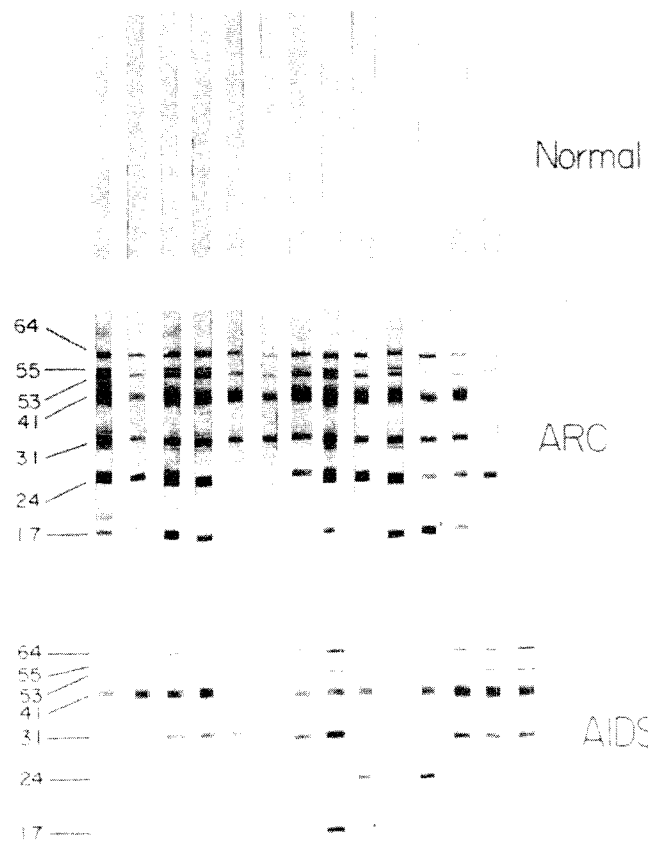


Fig. 2. Typical Western blot banding patterns. AIDS = acquired immunodeficiency syndrome; ARC = AIDS-related complex. (From Burke and associates.³⁵ By permission of the American Society for Microbiology.)

banding may vary considerably, depending on the source of antigen and the method of preparation. Antigenic components present on test strips have also undergone changes during the past several years; glycoproteins and high-molecular-weight proteins (more than 100 kilodaltons) are more difficult to preserve on prepared test strips, but with the increasing recognition of the importance of these antigens, several test systems now contain such antigens, particularly gp120. Variations in methods and reagents among laboratories and over time make the overall sensitivity and specificity difficult to assess, but the Western blot technique is generally thought to be more specific and less sensitive than ELISA.⁴

Indeterminate Results.—The definition of positivity for the Western blot method has been somewhat controversial. Test strips showing no bands are negative; strips showing bands at both p24 and gp41 are considered positive. On the basis of recommendations from the Centers for Disease Control, p24 alone is also positive, but many laboratories find this pattern nonspecific,³⁶ particularly in the context of screening programs (low-risk subjects). Single bands other than p24 and multiple band patterns lacking p24 are also indeterminate. For a recently licensed commercial Western blot kit (Biotech/Du Pont), the presence of multiple bands—namely, p24, p31, and either gp41 or gp160—is necessary for a positive interpretation, according to the manufacturer's recommendations. Any other banding pattern with this system is considered indeterminate.

Clinical interpretation of indeterminate results necessitates correlation with the patient's clinical status and medical evaluation in conjunction with subsequent Western blot testing or retesting with alternative methods. A few patients with indeterminate results have early HIV infection and an incomplete serologic response; in such patients, subsequent testing will disclose seroconversion. An incomplete (indeterminate) pattern may also be seen in advanced AIDS as immunologic responsiveness wanes, particularly with loss of anti-p24 antibody. This situation is usually clinically apparent. Screening programs can, however, detect normal persons with anti-p24 alone or an indeterminate multiple band pattern who are at low risk for HIV infection, on follow-up fail to show evolution to seropositivity, and remain without evidence of HIV infection.^{36,37}

SEROCONVERSION AFTER ACUTE INFECTION

A special problem in the interpretation of HIV serologic tests arises in the presence of possible recent primary infection. Clearly, time must elapse before antibody production and seroconversion occur. Positive HIV cultures have been substantiated during this "window" period.³⁸ This phenomenon is of concern in screening programs for blood, sperm, and organ donors and also needs to be considered in the assessment and counseling of patients with possible infectious exposure to HIV or symptoms consistent with primary HIV infection.

Information about the time course of antibody responses after primary HIV infection in humans is available from only a small number of case reports of patients whose infecting event and date of infection are known and whose serologic status was assessed prospectively at frequent intervals. In some reports, the date of infection is not known but seroconversion with a characteristic sequence of antibody responses has occurred during or shortly after a well-described acute, self-limited, symptomatic illness attributed to primary HIV infection. Case studies have included health-care workers,^{39,40} transfusion and blood product recipients,⁴¹⁻⁴⁴ intravenous drug users,³⁸ and homosexual men.^{38,44-47}

Symptoms associated with seroconversion range from none to a symptom complex beginning 3 to 8 weeks after infection and resolving spontaneously within 3 weeks in most patients. This syndrome includes fever, severe fatigue, myalgias, arthralgias, nausea, vomiting, diarrhea, loss of weight, headache, and photophobia. Findings have included adenopathy and a pruritic, maculopapular, or urticarial rash. An associated lymphocytic meningoencephalitis and peripheral neuropathy have been reported.^{38,44,47}

Seroconversion^{19,38,41,46,48-50} has generally been detected (by any method) within 2 to 3 months after the initial infection or during to shortly after a symptomatic illness in most reported cases in which serologic testing was performed at least monthly. A consistent sequence of antibody responses to specific viral components, based on the ability of Western blot techniques and radioimmunoprecipitation to distinguish antibody specificities, then occurs (Fig. 3). The earliest antibodies to appear are directed against gp160, gp120, p24, and p17; 2 to 4 weeks later,

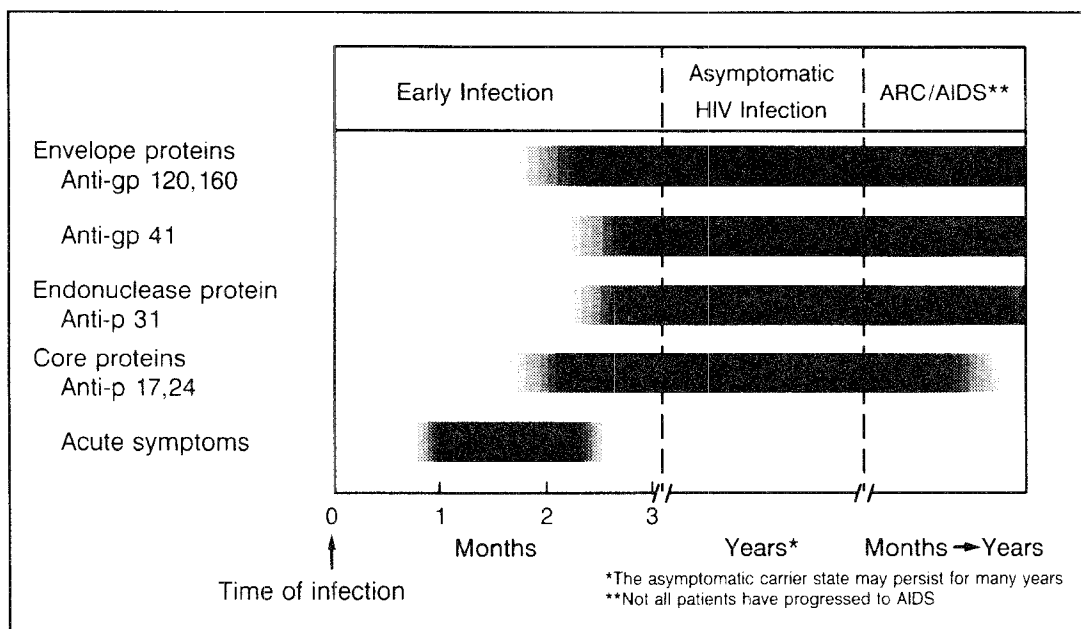


Fig. 3. Time course of serologic responses in patients with human immunodeficiency virus (HIV) infection. AIDS = acquired immunodeficiency syndrome; ARC = AIDS-related complex; gp = glycoproteins; p = proteins.

antibodies with affinity for gp41 and antigens in the 50- to 65-kilodalton range become detectable.^{19,38,41,46,48,51,52} Anti-p31 appears 3 to 6 weeks after the earliest antibodies.^{19,46,52} Antibody titers continue to increase during a period of several months and eventually stabilize in asymptomatic carriers. In patients who have progression to AIDS, antibody to p24 declines or disappears, and antibodies to *env*-derived antigens gp41, gp120, and gp160 become the most consistent findings.^{19,24,27,35,52} Antibody to the *pol*-derived antigen p31 diminishes in AIDS but not to the same extent as anti-p24.³⁵

Methods most sensitive for the detection of antibody to p24 or higher molecular weight antigens would be expected to be the first to confirm seroconversion in early HIV infection. As noted by Burke and associates,³⁵ sensitivities of commercial ELISA kits for detection of antibodies to individual viral proteins differ substantially; in particular, the Abbott ELISA kit optical density values most closely reflect antibody to gp41 and p31, whereas three other ELISA kits correlate more closely with p24, especially the kit manufactured by Du Pont. Therefore, the time to sero-

conversion will differ with use of various ELISA kits. Such a tendency was shown by Cooper and colleagues,⁴⁶ who found that the longest time period for seroconversion occurred with the Abbott assay; in comparison with other ELISA assays tested, however, the difference was not statistically significant.

CONCLUSION

As a screening test for HIV infection, the ELISA is highly sensitive and specific. The predictive value of a positive ELISA varies from 2 to 99%, depending on the degree of ELISA reactivity and the presence of risk factors for HIV infection. False-positive results may occur in patients with other conditions, including chronic liver disease, myeloma, and autoimmune disease.

Positive ELISA results should be confirmed with an alternative method; most often, Western blot techniques are used. In some cases, Western blot results are indeterminate, and subsequent testing or alternative serologic methods may be necessary for clinical interpretation. In patients with acute HIV infection, Western blot bands appear in a characteristic sequence, usually

within 3 months after infection. In patients with AIDS, reactivity with core-related antigens such as p24 may decline with progression of the course of the disease.

Serologic tests for HIV antibodies and alternative diagnostic strategies are dynamic and continue to undergo refinement. Improved laboratory techniques (such as the use of recombinant DNA antigens) will likely enhance test results in the future.

REFERENCES

- Centers for Disease Control: Provisional Public Health Service inter-agency recommendations for screening donated blood and plasma for antibody to the virus causing acquired immunodeficiency syndrome. *MMWR* 34:1-5, 1985
- AMA AIDS testing policy. *Am Med News*, July 3-10, 1987, p 1
- Centers for Disease Control: Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. *MMWR* 36 (Suppl 1S):1S-15S, 1987
- Council on Scientific Affairs: Status report on the acquired immunodeficiency syndrome: human T-cell lymphotropic virus type III testing. *JAMA* 254:1342-1345, 1985
- Petricciani JC: Licensed tests for antibody to human T-lymphotropic virus type III: sensitivity and specificity. *Ann Intern Med* 103:726-729, 1985
- Popovic M, Sarngadharan MG, Reed E, Gallo RC: Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science* 224:497-500, 1984
- Gallo RC, Salahuddin SZ, Popovic M, Shearer GM, Kaplan M, Haynes BF, Palker TJ, Redfield R, Oleske J, Safai B, White G, Foster P, Markham PD: Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science* 224:500-503, 1984
- Salahuddin SZ, Markham PD, Popovic M, Sarngadharan MG, Orndorff S, Fladager A, Patel A, Gold J, Gallo RC: Isolation of infectious human T-cell leukemia/lymphotropic virus type III (HTLV-III) from patients with acquired immunodeficiency syndrome (AIDS) or AIDS-related complex (ARC) and from healthy carriers: a study of risk groups and tissue sources. *Proc Natl Acad Sci USA* 82:5530-5534, 1985
- Markham PD, Salahuddin SZ, Popovic M, Patel A, Veren K, Fladager A, Orndorff S, Gallo RC: Advances in the isolation of HTLV-III from patients with AIDS and AIDS-related complex and from donors at risk. *Cancer Res* 45 (Suppl):4588S-4591S, 1985
- Goedert JJ: Testing for human immunodeficiency virus (editorial). *Ann Intern Med* 105:609-610, 1986
- Goudsmit J, de Wolf F, Paul DA, Epstein LG, Lange JMA, Krone WJA, Speelman H, Wolters EC, Van Der Noordaa J, Oleske JM, Van Der Helm HJ, Coutinho RA: Expression of human immunodeficiency virus antigen (HIV-Ag) in serum and cerebrospinal fluid during acute and chronic infection. *Lancet* 2:177-180, 1986
- Broder S, Gallo RC: A pathogenic retrovirus (HTLV-III) linked to AIDS. *N Engl J Med* 311:1292-1297, 1984
- Feorino PM, Jaffe HW, Palmer E, Peterman TA, Francis DP, Kalyanaraman VS, Weinstein RA, Stoneburner RL, Alexander WJ, Raevsky C, Getchell JP, Warfield D, Haverkos HW, Kilbourne BW, Nicholson JKA, Curran JW: Transfusion-associated acquired immunodeficiency syndrome: evidence for persistent infection in blood donors. *N Engl J Med* 312:1293-1296, 1985
- Salahuddin SZ, Groopman JE, Markham PD, Sarngadharan MG, Redfield RR, McLane MF, Essex M, Sliski A, Gallo RC: HTLV-III in symptom-free seronegative persons. *Lancet* 2:1418-1420, 1984
- Mayer KH, Stoddard AM, McCusker J, Ayotte D, Ferriani R, Groopman JE: Human T-lymphotropic virus type III in high-risk, antibody-negative homosexual men. *Ann Intern Med* 104:194-196, 1986
- Essex M, Allan J, Kanki P, McLane MF, Malone G, Kitchen L, Lee TH: Antigens of human T-lymphotropic virus type III/lymphadenopathy associated virus. *Ann Intern Med* 103:700-703, 1985
- Schulz TF, Aschauer JM, Hengster P, Larcher C, Wachter H, Fleckenstein B, Dierich MP: Envelope gene-derived recombinant peptide in the serodiagnosis of human immunodeficiency virus infection (letter to the editor). *Lancet* 2:111-112, 1986
- Thorn RM, Beltz GA, Hung C, Fallis BF, Winkle S, Cheng K-L, Marciani DJ: Enzyme immunoassay using a novel recombinant polypeptide to detect human immunodeficiency virus *env* antibody. *J Clin Microbiol* 25:1207-1212, 1987
- Burke DS, Brandt BL, Redfield RR, Lee TH, Thorn RM, Beltz GA, Hung CH: Diagnosis of human immunodeficiency virus infection by immunoassay using a molecularly cloned and expressed virus envelope polypeptide: comparison to Western blot on 2707 consecutive serum samples. *Ann Intern Med* 106:671-676, 1987
- Weiss SH, Goedert JJ, Sarngadharan MG, Bodner AJ, AIDS Seroepidemiology Collaborative Working Group, Gallo RC, Blattner WA: Screening test for HTLV-III (AIDS agent) antibodies: specificity, sensitivity, and applications. *JAMA* 253:221-225, 1985
- Groopman JE, Chen FW, Hope JA, Andrews JM, Swift RL, Benton CV, Sullivan JL, Volberding PA, Sites DP, Landesman S, Gold J, Baker L, Craven D, Boches FS: Serological characterization of HTLV-III infection in AIDS and related disorders. *J Infect Dis* 153:736-742, 1986
- Ward JW, Grindon AJ, Feorino PM, Schable C, Parvin M, Allen JR: Laboratory and epidemiologic evaluation of an enzyme immunoassay for antibodies to HTLV-III. *JAMA* 256:357-361, 1986
- Sivak SL, Wormser GP: Predictive value of a screening test for antibodies to HTLV-III. *Am J Clin Pathol* 85:700-703, 1986
- Schüpbach J, Haller O, Vogt M, Lüthy R, Joller H, Oelz O, Popovic M, Sarngadharan MG, Gallo RC: Antibodies to HTLV-III in Swiss patients with AIDS and pre-AIDS, and in groups at risk for AIDS. *N Engl J Med* 312:265-270, 1985
- Sarngadharan MG, Popovic M, Bruch L, Schüpbach J, Gallo RC: Antibodies reactive with human T-lymphotropic retroviruses (HTLV-III) in the serum of patients with AIDS. *Science* 224:506-508, 1984
- Schüpbach J, Popovic M, Gilden RV, Gonda MA, Sarngadharan MG, Gallo RC: Serological analysis of a subgroup of human T-lymphotropic retroviruses (HTLV-III) associated with AIDS. *Science* 224:503-505, 1984
- Goudsmit J, Lange JMA, Paul DA, Dawson GJ: Antigenemia and antibody titers to core and envelope antigens in AIDS, AIDS-related complex, and subclinical

- human immunodeficiency virus infection. *J Infect Dis* 155:558-560, 1987
28. Mortimer PP, Parry JV, Mortimer JY: Which anti-HTLV III/LAV assays for screening and confirmatory testing? *Lancet* 2:873-877, 1985
 29. Cockerill FR III, Edson RS, Chase RC, Katzmann JA, Taswell HF: Acquired immune deficiency syndrome (AIDS) "false-positive" antibodies to human immunodeficiency virus (HIV) detected by an enzyme-linked immunosorbent assay (ELISA) in low-risk patients (submitted for publication)
 30. Mendenhall CL, Roselle GA, Grossman CJ, Rouster SD, Weesner RE, Dumaswala U: False positive tests for HTLV-III antibodies in alcoholic patients with hepatitis (letter to the editor). *N Engl J Med* 314:921-922, 1986
 31. Kühnl P, Seidl S, Holzberger G: HLA DR4 antibodies cause positive HTLV-III antibody ELISA results. *Lancet* 1:1222-1223, 1985
 32. Handsfield HH, Wandell M, Goldstein L, Shriver K, Cooperative Study Group: Screening and diagnostic performance of enzyme immunoassay for antibody to lymphadenopathy-associated virus. *J Clin Microbiol* 25:879-884, 1987
 33. Imrie AA, Kehrer S, Smith GW, Penny R, Cooper DA: Seroimmunology of AIDS retrovirus infection. I. Use of immunofluorescence assay to confirm sera with ELISA reactivity. *Pathology* 18:438-443, 1986
 34. Lennette ET, Karparkin S, Levy JA: Indirect immunofluorescence assay for antibodies to human immunodeficiency virus. *J Clin Microbiol* 25:199-202, 1987
 35. Burke DS, Redfield RR, Putman P, Alexander SS: Variations in Western blot banding patterns of human T-cell lymphotropic virus type III/lymphadenopathy-associated virus. *J Clin Microbiol* 25:81-84, 1987
 36. Josephson SL, Swack NS, Hausler WJ Jr: Studies of "p24 only" immunoblot reactivity to human immunodeficiency virus (abstract MP.129). Program and abstracts of the Third International Conference on Acquired Immunodeficiency Syndrome (AIDS), Washington, DC, 1987
 37. Burke DS, Redfield RR: False-positive Western blot tests for antibodies to HTLV-III (letter to the editor). *JAMA* 256:347, 1986
 38. Ho DD, Sarngadharan MG, Resnick L, diMarzo-Veronese F, Rota TR, Hirsch MS: Primary human T-lymphotropic virus type III infection. *Ann Intern Med* 103:880-883, 1985
 39. Needlestick transmission of HTLV-III from a patient infected in Africa. *Lancet* 2:1376-1377, 1984
 40. Centers for Disease Control: Update: Human immunodeficiency virus infections in health-care workers exposed to blood of infected patients. *MMWR* 36:285-289, 1987
 41. Esteban JI, Shih JW-K, Tai C-C, Bodner AJ, Kay JWD, Alter HJ: Importance of Western blot analysis in predicting infectivity of anti-HTLV-III/LAV positive blood. *Lancet* 2:1083-1086, 1985
 42. Boiteux F, Vilmer E, Girot R, Muller J-Y, Rouzioux C, Chamaret S, Montagnier L: Lymphadenopathy syndrome in two thalassemic patients after LAV contamination by blood transfusion (letter to the editor). *N Engl J Med* 312:648-649, 1985
 43. Tucker J, Ludlam CA, Craig A, Philp I, Steel CM, Tedder RS, Cheingsong-Popov R, Macnicol MF, McClelland DBL, Boulton FE: HTLV-III infection associated with glandular-fever-like illness in a haemophiliac (letter to the editor). *Lancet* 1:585, 1985
 44. Carne CA, Tedder RS, Smith A, Sutherland S, Elkington SG, Daly HM, Preston FE, Craske J: Acute encephalopathy coincident with seroconversion for anti-HTLV-III. *Lancet* 2:1206-1208, 1985
 45. Cooper DA, Gold J, Maclean P, Donovan B, Finlayson R, Barnes TG, Michelmore HM, Brooke P, Penny R: Acute AIDS retrovirus infection: definition of a clinical illness associated with seroconversion. *Lancet* 1:537-540, 1985
 46. Cooper DA, Imrie AA, Penny R: Antibody response to human immunodeficiency virus after primary infection. *J Infect Dis* 155:1113-1118, 1987
 47. Piette AM, Tusseau F, Vignon D, Chapman A, Parrot G, Leibowitch J, Montagnier L: Acute neuropathy coincident with seroconversion for anti-LAV/HTLV-III (letter to the editor). *Lancet* 1:852, 1986
 48. Matheron S, Dormont D, Rey MA, Brun-Vezinet F, Bousin F, Saimot AG: Kinetics of HIV infection after IV exposure to blood from an AIDS patient (abstract TP.27). Program and abstracts of the Third International Conference on Acquired Immunodeficiency Syndrome (AIDS), Washington, DC, 1987
 49. Noel L, Retrovirus Group of the French Society of Blood Transfusion: Western-blot in HIV seroconversion: the importance of detecting anti gp 110/120, the earliest envelope antibodies (abstract TP.80). Program and abstracts of the Third International Conference on Acquired Immunodeficiency Syndrome (AIDS), Washington, DC, 1987
 50. Marlink RG, Allan JS, McLane MF, Essex M, Anderson KC, Groopman JE: Low sensitivity of ELISA testing in early HIV infection (letter to the editor). *N Engl J Med* 315:1549, 1986
 51. Taylor PE, Stevens CE, Rubinstein P: Western blot assay patterns of early antibody response to the human immunodeficiency virus (abstract TP.12). Program and abstracts of the Third International Conference on Acquired Immunodeficiency Syndrome (AIDS), Washington, DC, 1987
 52. Lange JMA, Coutinho RA, Krone WJA, Verdonck LF, Danner SA, Van Der Noordaa J, Goudsmit J: Distinct IgG recognition patterns during progression of subclinical and clinical infection with lymphadenopathy associated virus/human T lymphotropic virus. *Br Med J [Clin Res]* 292:228-230, 1986