HTLV-I Antibody Testing (11/29/88)

Date: November 29, 1988

From: Director, Center for Biologics Evaluation and Research

Subject: HTLV-I Antibody Testing

To: All Registered Blood Establishments

Three manufacturers, Abbott Laboratories, Inc., Cellular Products Inc., and E. I. DuPont De Nemours and Co., received licenses from the Food and Drug Administration on November 29, 1988 to manufacture and distribute Human T-Lymphotropic Virus Type I (HTLV-I) enzyme immunosorbent assay (EIA) test kits designed to detect antibodies to HTLV-I in human serum or plasma. The Food and Drug Administration (FDA) recommends testing donations of whole blood and cellular components for transfusion for antibodies to HTLV-I with licensed EIA tests. The FDA does not recommend that donations of source plasma intended for use in further manufacturing be tested. The basis for FDA's recommendations includes a consensus in favor of blood screening with HTLV-I EIA tests that has developed among members of the Blood and Blood Products Advisory Committee, during an open workshop on HTLV-I testing sponsored by the Center for Biologics Evaluation and Research and held at the National Institutes of Health on September 15, 1988, and among the major agencies representing blood collection establishments such as the American Association of Blood Banks, the Council of Community Blood Centers, and the American Red Cross.

The intent of blood screening is to prevent transmission of HTLV-I infection to recipients of blood components. HTLV-I is a retrovirus only distantly related to human immunodeficiency virus (HIV) and does not cause AIDS. In a recent study of 39,898 random blood donors in eight U.S. cities, 10 (0.025%) were found to have antibodies against HTLV-I (1)[Williams AE, Fang CT, Slamon DJ, et al. Seroprevalence and epidemiological correlates of HTLV-I infection in U. S. blood donors. Science 1988;240:643-646.]. It is well established that HTLV-I infection may be transmitted to recipients by the transfusion of cellular blood products from infected donors (2)[Okochi K, Sato H, Hinuma Y. A retrospective study on transmission of adult T-cell leukemia virus by blood transfusion: seroconversion in recipients. Vox Sang 1984;46:245-253.]. The plasma fraction of blood from donors infected with HTLV-I has not been found to transmit infection to transfusion recipients. An in-depth presentation of medical and biological aspects of HTLV-I can be found in the accompanying reprint from the December 9, 1988, Volume 37, Number 48 edition of Morbidity Mortality Weekly Report (MMWR), and in a brief summary of background information which is provided as an enclosure (enclosures 1 and 2).

The package insert of licensed HTLV-I EIA test kits states that serum or plasma specimens that are reactive on initial EIA

testing are to be tested again in duplicate. If either or both repeat tests is reactive, the specimen is considered repeatably reactive, and is interpreted as positive by the screening EIA. Additional, more specific tests (such as Western immunoblot (WIB) and/or radioimmunoprecipitation assay (RIPA)) are necessary to validate whether any repeatably reactive serum or plasma specimen contains antibodies to HTLV-I. There are currently no FDA-licensed additional, more specific tests for antibodies to HTLV-I. Guidelines for the use of additional, more specific tests are presented in detail in the included MMWR reprint.

The following items are FDA recommendations for the use of HTLV-I EIA Test kits and are also summarized in tabular form in Table 1.

SUMMARY OF RECOMMENDED ACTIONS (enclosure 3).

RECOMMENDATIONS FOR HANDLING OF DONATIONS WITH REPEATABLY REACTIVE EIA TEST RESULTS:

- Whole blood and blood components that test repeatably reactive by FDA-licensed HTLV-I EIA antibody screening tests are not recommended for transfusion and should be quarantined and destroyed unless labeled with two cautionary statements as follows:

  "Positive by a test for HTLV-I antibodies. The risk of transmission of HTLV-I is present." and

  "For further manufacture into in-vitro diagnostic reagents for which there are no alternative sources." or

  "For laboratory research use only."

RECOMMENDATIONS FOR DONOR DEFERRAL:

- Donors with repeatably reactive donations should be permanently deferred whenever additional, more specific tests confirm that the donor has antibodies to HTLV-I or HTLV-II.

- Donors should be indefinitely deferred whenever their donations have repeatably reactive screening tests for HTLV-I antibodies on more than one separate donation. (Additional, more specific tests may be negative or indeterminate.)

RECOMMENDATIONS FOR DONOR NOTIFICATION AND COUNSELING:

- Notification and counseling of donors with repeatably reactive screening tests for antibodies to HTLV-I without the results of additional, more specific tests is not recommended.

- Guidelines for notification and counseling are provided in detail by the Public Health Service in the enclosed MMWR article and are summarized below:

    1. Donor notification and counseling should be delayed until completion of additional more specific testing of EIA repeatably reactive blood.

    2. Additional, more specific testing may consist of WIB and/or RIPA which should be interpreted according to the guidelines published by the Public Health Service in the enclosed MMWR article.

    3. All deferred donors should be notified. If additional, more specific testing is indeterminate or negative on the first EIA repeatably reactive donation, the donor remains eligible for future donation and need not be notified although the presently donated unit should not be used for transfusion. If on any subsequent donation there is a second repeatably reactive EIA, the donor should be indefinitely deferred and notified.

    4. All donors who are notified of test results should receive medical counseling by appropriately trained medical personnel.

Because neither additional, more specific serologic tests nor FDA-licensed EIA screening tests can reliably distinguish between antibodies to HTLV-I and HTLV-II, and because the health implications of positive test results are poorly defined, the following counseling approach is suggested:

- If additional, more specific testing is positive, the donor should be advised that he/she is infected by either HTLV-I or HTLV-II. The modes of transmission of these viruses and disease associations should be explained (for reference see enclosed MMWR reprint). In addition, the donor should be advised by appropriately trained medical personnel that:

1. His/her name has been put on a list of permanently deferred donors and he/she should not donate blood for transfusion.

2. The positive HTLV-I antibody test is not a test for AIDS and does not mean the person has

excess risk of AIDS or HIV-I infection.

3. The donor should not share needles used for percutaneous injection.

4. Breast feeding of infants by infected woman should be discouraged.

5. Because there is a risk of transmission of HTLV-I infection to sexual partners, appropriate preventative measures should be discussed.

- If additional, more specific testing is indeterminate or negative but EIA testing is repeatably reactive on more than one occasion, the donor should be advised that:

1. A blood test for HTLV-I antibodies has yielded inconclusive results which do not necessarily imply infection with HTLV-I or HTLV-II.

2. At present, licensed diagnostic tools are not available to allow a resolution of the blood test.

3. The inconclusive test is not a test for AIDS and does not mean the person has excess risk of AIDS or HIV-I infection.

4. His/her name has been placed on the list of indefinitely deferred donors until such time as more specific tests become available and a resolution of inconclusive test results is possible.

RECOMMENDATIONS FOR BLOOD PRODUCT LABELING:

- The HTLV-I antibody test results do not have to appear on the product container label.

- The package insert should indicate that all products have been tested for antibodies to HTLV-I.

RECOMMENDATIONS FOR AMENDING EDUCATIONAL BROCHURES AND INFORMED CONSENT:

- Amendment of educational brochures and informed consent statements should provide information on "other retroviruses" and may include topics outlined in the enclosed MMWR reprint.

- In addition to other donor exclusion criteria, the test for HTLV-I antibodies will help guarantee the

safety of the blood supply.

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BACKGROUND INFORMATION ON HTLV-I AND HTLV-II

HTLV-I and HTLV-II are closely related human type C retroviruses. HTLV-I is endemic primarily in the Caribbean, Southwestern Japan, and possibly in some areas of Africa. Other possible endemic areas such as islands of Micronesia and parts of South America have been less well studied. In the United States, HTLV-I has been identified in patients with adult T-cell leukemia (ATL), intravenous drug abusers, and healthy individuals. Among random U.S. blood donors, seroprevalence rates vary from less that 0.01% to 0.1%. Among random U.S. plasma donations, between 0.4 and 0.7% have been found positive for HTLV-I antibodies. Proven cases of HTLV-II infection have been rare. Although associated with selected populations of intravenous drug abusers, its epidemiology in the US remains largely unknown.

Transmission of viral infection occurs parenterally. The virus preferentially infects circulating lymphocytes bearing the CD-4 receptor (T-helper lymphocytes) where viral genes may persist indefinitely. Transmission of HTLV-I infection to transfusion recipients of infected cellular blood products is well documented and may result in seroconversion between several weeks to several months after transfusion. Other known modes of transmission include breast milk, sexual contact, and sharing of contaminated needles and syringes. Perinatal transmission is suspected but remains unproven.

HTLV-I and HTLV-II do not cause acquired immunodeficiency syndrome (AIDS) and no cross-reactivity with antibodies to HIV has been demonstrated for HTLV-I serologic assays. However, HTLV-I has been etiologically associated with two distinct diseases: adult T-cell leukemia and tropical spastic paraparesis.

Adult T-cell leukemia (ATL) is a malignant proliferation of T-lymphocytes accompanied by cutaneous manifestations resembling Sezary syndrome and mycosis fungoides as well as hypercalcemia and lytic bone lesions. It occurs equally in males and females with a mean age of between 40-60 years. There is geographic clustering where populations exhibit increased prevalence of HTLV-I antibodies. The lifetime risk of ATL, regardless of age at infection has been estimated to be 2% for a Japanese population. The lifetime risk of ATL among persons infected before the age of 20 years was estimated to be 3-5% in a study in Jamaica. Japanese investigators estimate that infection with HTLV-I may lie dormant for over 20 years before onset of ATL;

however, firm estimates of disease latency are not yet available. Preliminary reports of antibodies to HTLV-I/II which are associated with chronic lymphocytic leukemias of the B or T cell type have appeared. Immune perturbations resulting in immunodeficiency have also been noted in association with HTLV-I antibodies.

Tropical spastic paraparesis (TSP) is a demyelinating neurologic disorder first noted in association with HTLV-I antibodies in the Caribbean in the early 1980s. The same syndrome has been observed in Japan where it is termed HTLV-I Associated Myelopathy (HAM). TSP is a progressive, bilateral symmetrical impairment of posterior column and pyramidal tract neurologic function, mainly at the lumbar level. Thus lower extremity weakness and autonomic dysfunction such as incontinence can occur. The lifetime risk of TSP given HTLV-I infection is unknown. In a series of 460 patients with HAM reported in Japan, 26% gave a history of blood transfusion; the mean interval between transfusion and onset of neurologic symptoms was estimated to be 4 years. However, firm estimates of the disease incubation period are unavailable.

HTLV-II infection has been associated with only two cases of disease. In both cases the patient had a rare form of lymphocytic leukemia termed hairy cell leukemia. Reliable disease associations for this virus are unclear and nothing is known regarding lifetime risk of disease among individuals infected with HTLV-II.

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Current Trends

Licensure of Screening Tests for Antibody to Human T-Lymphotropic Virus Type 1

Screening tests for antibody to human T-lymphotropic virus type I (HTLV-I), the first-recognized human retrovirus, have been licensed by the Food and Drug Administration (FDA), These tests have been recommended by the FDA for screening of blood and cellular components donated for transfusion. They have also been approved as diagnostic tests, which may be useful in evaluating patients with clinical diagnoses of adult T-cell leukemia/lymphoma (ATL) and tropical spastic paraparesis (TSP)/HTLV-l-associated myelopathy (HAM), both of which have been associated with HTLV-I infection. Because licensure will probably result in widespread use of these tests, the information presented below is provided for physicians and public health officials who may need to interpret HTLV-I test results and to

counsel persons whose serum specimens are reactive in these tests. Users of the new HTLV-I screening tests are cautioned that additional, more specific tests are necessary to confirm that serum specimens that are repeatably reactive in these screening tests are truly positive for HTLV-I antibody. Users should also be aware that neither the screening tests nor more specific tests can distinguish between antibody to HTLV-I and antibody to the closely related human retrovirus human T-lymphotropic virus type II (HTLV-II).

HTLV-I does not cause AIDS, and the finding of HTLV-I antibody in human blood does not imply infection with human immunodeficiency virus (HIV) or a risk of developing acquired immunodeficiency syndrome (Aids).

BACKGROUND:HTLV-I

HTLV-I was isolated in 1978 and first reported in 1980 (1) [Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC, Detention and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc Natl Acad Sci USA 1980; 77:7415-9.]. Although a member of the family of retroviruses, HTLV-I is not closely related to HIV, the virus that causes AIDS, HTLV-I does not cause depletion of T-helper lymphocytes, and it is not generally associated with immunosuppression.

HTLV-I differs from HIV in its morphologic and genetic structure and in that HTLV-I antigens should not cross-react with the antigens of HIV. The HTLV-I genome contains four major genes: gag, which encodes core proteins of 15,000 (p15), 19,000 (p19), and 24,000 (p24) daltons; pol, which encodes a polymerase (reverse transcriptase) protein of 96,000 daltons; env, which encodes envelope glycoproteins of 21,000 (gp2l) and 46,000 (gp46) daltons; and tax, which encodes a transactivator protein of 40,000 daltons (p4Ox).

Seroprevalence

HTLV-I infection is endemic primarily in southwestern Japan. the Caribbean, and some areas of Africa (2)[Blattner WA, Retroviruses. In: AS Evans, ed. Viral infections of humans: epidemiology and control 3rd ed. New York: Plenum, 1989(in press).]. Seroprevalence in well-characterized areas appears to increase with patient age, with rates in females markedly higher than those in males beginning in the 20-30-year age range. Seroprevalence as high as 15% in the general population and 30% in older age groups have been reported in some areas of Japan (3). [Hinuma Y, Komoda H. Chosa T, et al. Antibodies to adult T-cell leukemia-virus-associated antigen (ATLA) in sera from patients with ATL and controls in Japan: a nation-wide sero-epidemiologic study. Int J Cancer 1982;29:631-5.]. In the Caribbean islands, rates may be as high as 5% in the general population and 15% in older age groups (4)[Clark JW, Saxinger C. Gibbs WN, et al. Seroepidemiologic studies of human T-cell

leukemia/lymphoma virus type I in Jamaica. Int J Cancer 1985;36:37-41.].

ln the United States, HTLV-I infection has been identified mainly in intravenous-drug users (IVDUs), with seroprevalence rates ranging from 7% to 49%, (5,6). Elevated rates have also been reported in female prostitutes (in whom IV-drug use is a major risk factor) (7)[Khabbaz RF, Darrow WW, Lairmore M, et al. Prevalence of antibody to HTLV-I among 1415 female prostitutes in the United States [Abstract].IV International Conference on AIDS. Book 1. Stockholm, June 12-16, 1988:270.] and in recipients of multiple blood transfusions (8) [Minamoto GY, Gold JWM, Scheinberg DA, et al. Infection with human T-cell leukemia virus type I in patients with leukemia N Engl J Med 1988;318:219-22.]. Seropositivity is rare among homosexual men and among patients in sexually transmitted disease clinics (9,10) (9)[Manns A. Obrams I, Detels R, et al. Seroprevalence of human T-cell lymphotropic virus type I among homosexual men in the United States N. Engl J Med 1988;319 516-7.] (10)[Wiktor S. Cannon RO, Atkinson WA, Quinn TH. Parenteral drug use is associated with HTLV-I and HIV infection among patients attending sexually transmitted disease (STD) clinics [abstract]. IV International Conference on AIDS Book 2. Stockholm, June 12-16, 1988:191.], and it appears to be nonexistent hemophilic men without other risk factors (11) [Jason JM, Lairmore M, Hartley T, Evatt BL. Absence of HTLV-I coinfection in HIV-infected hemophilic men [Abstract] 28th Interscience Conference on Antimicrobial Agents and Chemotherapy, Los Angeles, California, October 23-26. 1988:302.]. Systematic determination of HTLV-1 seroprevalence in the general population of the United States has not been undertaken. However,in a study of 39,898 random blood donors in eight U. S. cities, 10 (0.025%) were seropositive for HTLV-I (12)[Williams AE, Fang CT, Slamon DJ, et al. Seroprevalence and epidemiological correlates of HTLV-I infection in U.S. blood donors. Science 1988:240:643-6.].

Transmission

Transmission of HTLV-I infection by blood transfusion is well documented in Japan, with a seroconversion rate of 63% in recipients of the cellular components of contaminated units (whole blood, red blood cells, and platelets) (13)[Okochi K, Sato H. Hinuma Y. A retrospective study on transmission of adult T cell leukemia virus by blood transfusion; seroconversion in recipients, Vox Sang 1984;46:245-53]. Transmission by the plasma fraction of contaminated units has not resulted in infection; this finding has been attributed to the fact that HTLV-I is highly cell-associated. Transmission among IVDUs is presumed to occur by sharing of needles and syringes contaminated with infectious blood.

Transmission from mother to child occurs through breastfeeding; breastfed infants of seropositive mothers have an approximately 25% probability of becoming infected (14)[Sugiyama H. Doi H, Yamaguchi K, Tsuji Y, Miyamoto T. Hino S. Significance of

postnatal mother-to-child transmission of human T-lymphotropic virus type-I on the development of adult T-cell leukemia/lymphoma. J Med Virol 1986;20:253-60.]. However, infection has also occurred in infants who are not breastfed, suggesting that intrauterine and/or perinatal transmission may occur.

Sexual transmission of HTLV-I appears to be relatively inefficient (15)[Bartholomew C, Saxinger WC, Clark JW, et al. Transmission of HTLV-I and HIV among homosexual men in Trinidad. JAMA 1987;257:2604-8]. Transmission from male to female, however, appears to be more efficient than from female to male (16)[Kajiyama W, Kashiwagi S, Ikematsu H, Hayashi J. Nomura H, Okochi K. Intrafamilial transmission of adult T cell leukemia virus. J Infect Dis 1986;154:851-7.].

Disease Associations

HTLV-I has been etiologically associated with adult T-cell leukemia/lymphoma(ATL) a malignancy of mature T-lymphocytes characterized by skin lesions, visceral involvement, circulating abnormal lymphocytes, hypercalcemia and lytic bone lesions (17) [Kuefler PR, Bunn PA Jr. Adult T cell leukaemia/lymphoma, Clin Haematol 1986;15:695-726]. ATL has been recognized in Japan, the Caribbean, and Africa. No systematic attempt has been made to record cases of ATL in the United States, but 74 cases were reported to the National Institutes of Health between 1980 and 1987 (18)[Levine PH, Jaffe ES, Manns A, Murphy El. Clark J. Blattner WA. Human T-cell lymphotropic virus type I and adult T-cell leukemia/lymphoma outside of Japan and the Caribbean basin, Yale J Biol Med (in press).]. Approximately half of these cases occurred in persons of Japanese or Caribbean ancestry; most of the remainder were in blacks from the southeastern United States. ATL tends to occur equally in men and women with peak occurrence in persons 40-60 years of age.

It is thought that a person must be infected with HTLV-I for years to decades before ATL develops. The lifetime risk of ATL among HTLV-I-infected persons has been estimated to be approximately 2% in two studies in Japan (19,20) (19)[Tajima K Kuroishi T. Estimation of rate of incidence of ATL among ATLV (HTLV-I) carriers in Kyushu, Japan. Jpn J Clin Oncol 1985;15:423-430.] (20)[Kondo T. Nonaka H. Miyamoto N. et al. Incidence of adult T-cell leukemia-lymphoma and its familial clustering Int J Cancer 1985;35:749-51]. In Jamaica, the lifetime risk of ATL among persons infected before the age of 20 years was estimated to be 4% (21)[Murphy El, Hanchard B, Figueroa JP, et al. Modeling the risk of adult T-cell leukemia/lymphoma (ATL) in persons infected with human T-lymphotropic virus type I. Int J Cancer (in press)].

Because of the long latent period of ATL; the risk of this disease among persons infected by blood transfusion (many of whom are elderly and may not survive their underlying disease) is not

thought to be substantial. In fact no cases of ATL associated with blood transfusion have been reported.

HTLV-I has also been associated with a degenerative neurologic disease known as tropical spastic paraparesis (TSP) in the Caribbean and as HTLV-I-associated myelopathy (HAM) in Japan (22,23) (22)[Gessain A. Barin F. Vernant JC, et al. Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. Lancet 1985;2:407-10.] (23)[Osame M. Usuku K. Izumo S, et al. HTLV-I associated myelopathy, a new clinical entity Lancet 1986;1:1031-2.]. TSP/HAM is characterized by progressive difficulty in walking, lower extremity weakness, sensory disturbances, and urinary incontinence. Although most cases have been reported from countries in which HTLV-I is endemic, a few cases have occurred in the United States (24)[Bhagavati S. Ehrlich G. Kula RW, et al. Detection of human T-cell lymphoma/leukemia virus type I DNA and antigen in spinal fluid and blood of patients with chronic progressive myelopathy. N Engl J Med 1988;318:1141-7.]. TSP/HAM occurs in persons of all age groups, with peak occurrence in ages 40-49 years. Rates are higher in females than in males. The lifetime risk of TSP/HAM among persons infected with HTLV-I is unknown but appears to be very low. The latent period for this disease appears to be less than for ATL, and the disease probably can be caused by blood transfusion. Of 420 Japanese patients with HAM from whom information was available, 109 (26%) gave a history of blood transfusion; the mean interval between transfusion and onset of neurologic symptoms was estimated to be 4 years (M. Osame, unpublished data).

HTLV-I does not cause Aids, and the finding of HTLV-I antibody in human blood does not imply infection with HIV or a risk of developing AIDS.

BACKGROUND: HTLV-II

HTLV-II is closely related to HTLV-II. The genome of HTLV-II encodes viral proteins that are similar to those of HTLV-I, and there is extensive serologic cross-reactivity among proteins from HTLV-I and HTLV-II.

No specific information is available regarding the seroepidemiology of the modes of transmission of HTLV-II. There is some evidence that some of the HTLV-I seropositivity in the United States, especially in IVDUs, may be caused by HTLV-II (5)[Robert-Guroff M, Weiss SH, Giron JA, et al. Prevalence of antibodies to HTLV-I, -II, and -III in intravenous drug abusers from an AIDS endemic region. JAMA 1986;255":3133-7.].

Two cases of disease have been associated with HTLV-II infection. HTLV-II was first isolated from a patient with a rare T-lymphocytic hairy cell leukemia (25)[Kalyanaraman VS, Sarngadharan MG, Robert-Guroff M, et al. A new subtype of human T-cell leukemia virus (HTLV-II) associated with a T-cell variant of hairy cell leukemia Science 1982;218:571-3.]. In the second

case, HTLV-II was isolated from a patient who had the more common B-lymphocytic form of hairy cell leukemia and who also suffered from a T-suppressor lymphoproliferative disease (26)[Rosenblatt JD, Golde DW, Washsman W, et al. A second isolate of HTLV-II associated with atypical hairy-cell leukemia. N Engl J Med 1986, 315:372-7.]. No serologic evidence of HTLV-II infection has been found in 21 additional cases of hairy cell leukemia (27)[Rosenblatt, JD Gasson JC, Glaspy J, et al. Relationship between human T-cell leukemia virus-II and atypical hairy cell leukemia: a serologic study of hairy cell leukemia patients Leukemia 1987;1:397-401.]. Thus the disease associations of HTLV-II are unclear and nothing is known regarding lifetime risk of disease among infected persons.

SEROLOGIC TESTS FOR HTLV-I

Interpretation

The screening test that have been licensed by the FDA are enzyme immunoassays (EIA) to detect HTLV-I antibody in human serum or plasma. Specimens with absorbance values greater than or equal to the cutoff value determined by the manufacturer are defined as initially reactive. Initially reactive specimens must be retested in duplicate to minimize the chance that reactivity is due to technical error. Specimens that are reactive in either of the duplicate tests are considered repeatably reactive. Specimens that do react in either of the duplicate repeat tests are considered nonreactive. Additional, more specific serologic tests are necessary to confirm that serum specimens repeatably reactive in the screening tests are positive for HTLV-I antibody. Users of the screening tests must have available to them additional, more specific tests to properly interpret repeatably reactive screening tests. Such tests are available in research institutions, industry, and some diagnostic laboratories. No such tests have been licensed by the FDA.

Tests used to confirm HTLV-I seropositivity must be inherently capable of identifying antibody to the core (gag) and envelope (env) proteins of HTLV-I. (The immunoreactivities of the polymerase [pol] and transactivator [tax] proteins of HTLV-I have not been well-defined in current test systems.) Specific tests include Western immunoblot (WIB) and radioimmunoprecipitation assay (RIPA). Indirect fluorescent antibody (IFA) testing for HTLV-I has been used in some laboratories, but IFA does not detect antibody to specific HTLV-I gene products.

WIB appears to be the most sensitive of the more specific tests for antibody to gag protein products p19, p24, and (gag-derived) p28, whereas RIPA appears to be most sensitive for antibody to the env glycoproteins gp46 and(env precursor) gp61/68. Based on experience with these tests in several laboratories, the following confirmatory criteria for HTLV-I seropositivity have been adopted by the Public Health Service Working Group: a specimen must demonstrate immunoreactivity to the gag gene product p24 and to an env gene product (gp46 and/or gp6l/68)to be

considered "positive." Serum specimens not satisfying these criteria but having immunoreactivities to at least one suspected HTLV-I gene product (such as p19 only, p19 and p28, or p19 and env) are designated "indeterminate." Serum specimens with no immuno-reactivity to any HTLV-I gene products in additional, more specific tests are designated "negative." Both WIB and RIPA may be required to determine whether a serum specimen is positive, indeterminate, or negative.

Although additional more specific tests have been somewhat standardized, the quantities and the molecular weights of HTLV-I proteins produced by various cell lines vary considerably. Hence, the cell of origin for HTLV-I antigens used in either WIB or RIPA, as well as the method of antigen preparation, may markedly influence test sensitivity and interpretation of immunoreactivity against individual HTLV-I proteins. Laboratories performing these tests, however, should be able to detect antibody to the gag and env gene products of HTLV-I in WIB and/or RIPA.

Sensitivity, Specificity, and Predictive Value

Using the WIB and RIPA available in research laboratories and the confirmatory criteria described above to define the presence of HTLV-I antibody, the sensitivities of the three EIAs that have been licensed by the FDA have been estimated from the performance of the tests on a reference panel of 137 antibody-positive serum specimens. All three EIAs were repeatably reactive for 137 of 137 panel serum specimens, yielding an estimated sensitivity of 97.3%-100% by the binomial distribution at 95% confidence. Specificity* of the EIAs was estimated for each test from screening of at least 5000 normal U.S. blood donors in nonendemic areas. [*Specificity was calculated as follows:(total donations screened minus total number repeatably reactive in EIA) divided by (total donations screened minus number confirmed as positive by additional testing).] Estimated specificities ranged from 99.3% to 99.9% by the binomial distribution at 95% confidence. However, a specificity >99% but <100% may still yield a low positive predictive value when the screening test is used in low-prevalence population. For example, in the study of U.S. blood donors cited above, 68 donors were repeat reactors in the screening test, but only 10 (15%) were determined to be HTLV-I-seropositive in more specific testing. This relatively low positive predictive value emphasizes the need for additional, more specific testing of specimens repeatably reactive in the EIA.

Neither the EIAs nor the additional, more specific tests can distinguish between antibodies to HTLV-I and HTLV-II. More sophisticated techniques, such as virus isolation and gene amplification (polymerase chain reaction [PCR]) are required to differentiate HTLV-l from HTLV-II infection.

USE OF HTLV-I SCREENING TESTS IN BLOOD BANKS

The FDA recommends that whole blood and cellular components donated for transfusion be screened for HTLV-I antibody using a licensed EIA screening tents. The FDA further recommends that units that are repeatably reactive by EIA be quarantined, then destroyed, unless otherwise stipulated by the FDA. Source plasma (obtained from plasma donors) intended for use in further manufacturing need not be screened for HTLV-I antibody.

DONOR DEFERRAL AND NOTIFICATION

FDA recommends permanent deferral of donors whose sera are repeatably reactive in EIA and confirmed as positive for HTLV-I antibody by additional, more specific testing. Such donors should be notified and counseled accordingly.

Donors whose serum specimens are repeatably reactive in the EIA but not confirmed as positive for HTLV-I antibody need not be notified on the first occasion. Although the donated units must be destroyed, the donors remain eligible for future donation. If, however, the donors test repeatably reactive in the EIA on a subsequent donation, they should be deferred indefinitely as donors and notified and counseled accordingly.

GUIDELINES FOR COUNSELING

Counseling should be considered a routine adjunct depending on the results of HTLV-I testing. Given some of the uncertainties related to testing, e.g., the inability to distinguish between antibodies to HTLV-I and HTLV-II, and the low probability that disease will occur in seropositive persons, every effort should be made to minimize the anxiety provoked by a repeatably reactive screening test, particularly one that is not confirmed as HTLV-I-seropositive by additional testing.

Persons confirmed as seropositive for HTLV-I should be notified that they have antibody to HTLV-I and are likely infected with HTLV-I or HTLV-II. They should be given information concerning disease associations and possible modes of transmission. ln addition, they should be advised that they have been permanently deferred as blood donors and should neither give blood for transfusion nor share needles that have been used for percutaneous injection or infusions with other persons. Breastfeeding of infants should be discouraged. The paucity of data concerning sexual transmission of HTLV-I/HTLV-II does not permit a firm recommendation concerning sex practices; specific recommendations, such as the use of condoms to reduce the potential risk of sexual transmission, should be developed in consultation with a health-care professional.

Persons whose serum specimens are repeatably reactive on more than one occasion in the EIA but not confirmed as positive for HTLV-I antibody in more specific testing should be informed that they have inconclusive test results that do not necessarily imply infection with HTLV-I or HTLV-II. Nevertheless, they should be notified that they have been deferred indefinitely as donors and

should not donate blood for transfusion. Periodic follow-up of such donors with EIA, more specific serologic tests, and possibly sophisticated techniques such as virus isolation and/or PCR may provide more reliable information regarding the presence of viral infection.

Date : February 1, 1989

From : Director, Center for Biologics Evaluation and Research

Subject: Use of the Recombigen HIV-1 LA Test

To: All Register Blood and Plasma Establishment

On December 13, 1988, the Food and Drug Administration licensed Cambridge Bioscience Corporation, Worcester, MA to manufacture and distribute a rapid latex agglutination screening test for antibodies to HIV-1 called "Recombigen" HIV-1 LA Test." The test is labeled for use "by properly trained personnel as a screening test in hospital laboratories, medical clinics, physicians' offices, and emergency care situations, and in blood banks or other settings where enzyme immunoassays are not practical or available." The purpose of this letter is to clarify FDA's concept of the appropriate use of this test in blood and plasma establishments.

The latex agglutination test can be performed in as little as five minutes with a sample of venous or capillary whole blood, serum or plasma which is diluted on a card and then mixed-with a suspension of microscopic latex particles which have been coated with a recombinant DNA-derived protein related to the virus envelope. The test endpoint is determined by visual inspection for an agglutination reaction in comparison with a negative control. As documented in the package insert, this test, when properly performed, is at least as sensitive as the licensed EIA screening tests.

Several clinical studies have shown that the Recombigen test may frequently give rise to false positive results. The primary cause of this problem is that the agglutination reaction can be easily overinterpreted by inexperienced operators. For this reason, the manufacturer requires that users familiarize themselves with the test by the use of a training panel and an "Interpretation Guide." In addition, the test can be falsely positive in a variety of medical conditions including common viral infections and the presence of certain abnormal serum immune globulins. Also, the format of the test, which lacks automated procedures and objective read-out, may dispose to errors inherent with tests that utilize subjective reading and interpretation.

The particle agglutination test is not recommended for routine use in registered blood and plasma collection establishments because of the procedural difficulties and hence errors that

would be likely to arise in handling large numbers of samples and because of the likelihood of false positive tests. Users of the test should be aware that donors with repeatably reactive tests by this procedure should be counseled and permanently excluded from donating blood or plasma unless they can be requalified by the reentry algorithm defined in a letter of April 29, 1987. In the event that a donor is deferred on the basis of the latex agglutination test, the latex test should be read in place of the initial EIA or "iEIA" mentioned in the document on reentry. Since the latex test uses a purified and genetically engineered polypeptide related to the viral envelope protein, the different screening test referred to in the algorithm as "dEIA" may be a test based on virus cultured in either the H-9 or the CEM cell line.

As is the case for other licensed screening tests for antibodies to HIV, it is recommended that additional more specific tests such as Western blot be performed prior to notification of donors who have been deferred. It is not appropriate to "validate" the results of the latex agglutination test with other licensed screening tests prior to notification. Also, it is not appropriate to "re-screen" donors previously positive by the latex agglutination test with licensed EIA tests prior to distributing units for transfusion or further manufacture. Routine "pre-screening" of donors such as on mobile collection facilities would not be a valid use since that practice would be likely to generate a large number of false-positive tests leading to excessive donor exclusion and further testing. Medical discretion should be used to decide whether the latex agglutination test is indicated in a particular instance because screening by the EIA is impractical or unavailable. Emergency situations involving donations of rare blood types, or urgent management of disasters might be examples. It is expected that the test will be used as the primary blood screen in some developing countries. It should be noted by such users that the manufacturer has made no claim for test sensitivity for detection of antibodies to HIV-2.

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