VIRUSMYTH HOMEPAGE

CONCERNS ABOUT HIV/AIDS TESTING AND MEASUREMENT

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HIV-positive people are some of the most poked, prodded, tested and measured in the world. Yet, surprisingly enough, most of the tests and measurements are not nearly as accurate as is generally stated.

HIV Testing

HIV testing is generally considered to be extraordinarily accurate. In fact, it has to be this way, because if people were told that there was a 90% chance that they had a fatal disease and had to take extraordinarily toxic therapy, many might decide that they were in the 10% of false test results. Only by claiming that HIV tests are virtually always right can people be terrorized into taking their medications. Examine these quotes and consider whether this is really true.

From these quotes you will learn that 20 million HIV tests resulted in over 20,000 positive tests - but only 112 were true positives! You will learn that mice and human placentas can be HIV-positive - without exposure to HIV! You will learn that there is no 'Gold Standard' against which the tests can be tested. Validation is performed by comparing each test against the others, like a tiger chasing its tail, with no obvious beginning or end.

The tests listed below represent all those used to diagnose or monitor HIV status. If they are not sound, there is no reliable way to diagnose HIV, and thus no point even discussing how to treat the conditions that it supposedly causes.

Surrogate Markers

Surrogate markers are measurements that are used not to determine whether someone has a condition, such as an HIV infection, but to find out how serious their condition is. CD4 immune cell counts, sometimes in conjunction with CD8 cell counts, were the first surrogate marker used instead of a direct measurement of the health of HIV-positive people, particularly those who do not have any AIDS-defining conditions. Increasingly, viral load is being used, based on claims that it can accurately count the number of HIV particles in a sample of blood. However, surrogate markers have significant limitations. Scientists have often noted that surrogate markers are never totally reliable and may result in incorrect treatment decisions, and that changing the value of a surrogate marker is not guaranteed to improve the health of the patient.

The quotes are categorized as:

Antibody Tests (ELISA, Western Blot)

Antibody tests are the most commonly performed. Usually an ELISA test is performed, and then repeated if positive. Following that, for positive ELISAs only, a Western Blot (WB) is performed. ELISA is not a Yes/No test, it is only a continuum of color change that is interpreted in this way because of an arbitrary cutoff point. Western Blot has the purported HIV proteins separated on a strip, with various methods used for interpretation (varying from country to country, and from organization to organization). Both types of tests measure antibodies, which in many diseases are considered a sign of immunity (particularly in the absence of symptoms). Why are antibody tests considered a sign of fatal disease in HIV/AIDS? Why are two of the same type of test used to validate each other?

"Antibodies directed to the env-encoded surface glyco-protein gp160 were detected in the cervicovaginal secretions of a small proportion of HIV-seronegative sex workers in Abidjan. In 2.9 to 12.3% of these women, depending on the test used, the anti-HIV antibodies were present in vaginal fluids that were free of contaminating semen. Since there is no established gold standard test, it is unclear which of these two proportions is the best estimate of the real prevalence rate of cervicovaginal anti-HIV antibodies in the absence of contaminating semen in HIV seronegative sex workers...The 25 HIV-1-seronegative sex workers with anti- HIV antibodies in their semen-free cervicovaginal secretions by both in-house ELISA and Seradyn Sentinel HIV-1 Urine EIA [ELISA] had no evidence of HIV-1 RNA in plasma. It is therefore unlikely that these antibodies are part of a primary HIV infection, although these women were not followed up...In the present study, increased sexual exposure was not associated with the presence of HIV-antibodies in cervicovaginal secretions, as measured by either of the two tests. Similarly, Mazzoli et al. did not find an association between the duration of unprotected sex and the presence of HIV-specific antibodies in the urine or cervicovaginal secretions of HIV-seronegative female partners of HIV-seropositive males in sero- discordant couples [11]."

> Ghys PD et al. Cervicovaginal anti-HIV antibodies in HIV-seronegative female sex workers in Abidjan, Cote d'Ivoire. AIDS. 2000;14:2603-8.

"The serological diagnosis of HIV infection is usually made on the basis of the detection of circulating antibodies specific for viral antigens gp41, gp120 and gp160. Despite using recombinant immunogenic oligopeptides, which improved the sensitivity and specificity of immunological tests, a number of both false-positive and false-negative reactions have been reported. Although the emergence of new viral serotypes or recent infection could be responsible, at least partly, for the low sensitivity of serological assays in detecting early antibody responses, false-positive results could be explained by crossreactions with unrelated antigens. Spehar and Strand recently demonstrated the cross- reactivity of anti-gp41 murine monoclonal antibodies with the human cytoskeletal protein alpha-actinin, and antibodies reacting with both the immunodominant region of HIV gp41 and alpha-actinin have been found in the sera of HIV-infected individuals...Only three out of 108 sera with IgG anti-Tricomonas vaginalis, and one out of 32 with IgM, tested positive for HIV using both kits."

> Fiori PL, Rappelli P. Do anti-Tricomonas vaginalis antibodies recognize HIV gp41?. AIDS. 2000;14(13):2057.

"A confirmed positive test [i.e. one or two ELISA tests, followed by a Western Blot] indicates that a person has been exposed to the virus and has mounted an immunologic response (serum antibodies). However, this test does not indicate whether the person currently harbors the virus"

> Zhang Z-Q et al. Sexual Transmission and Propagation of SIV and HIV in Resting and Activated CD4+ T Cells. Science. 1999 Nov 12;286(5443):1353-7.

"[Table shows that of 1,326,030 HIV tests 1,072 had reactive EIA/ELISA tests that were followed by negative or indeterminate Western Blot. Only 276 were confirmed positive]...Some of the EIA repeat reactive [but] Wb [Western Blot] negative or ID [indeterminate] donors were tested on multiple occasions. No donor in...the HIV-1...neg or ID group became Wb positive...The Wb neg or ID donors outnumbered the confirmed reactives 4 to 1 in HIV-1 testing"

> Haley NR et al. Abstract S110: Comparisons of confirmed and unconfirmed HIV-1 and HTLV-I positive donors to the donor base. Transfusion. 1992;32(suppl):30S.

"In blood donor studies in the developed world, about 20% of sera referred to confirmatory laboratories give indeterminate western blot results, almost all of which are on presumed negative specimens."

> Mortimer PP. The fallibility of HIV Western blot. Lancet. 1991 Feb 2;337:286-7.

"100 ELISA-negative donors...were tested by WB [note that normally a negative ELISA will not result in a Western Blot 'confirmatory' test]. 20 were WBi, with p24 being the predominant (70%) and generally the only band. Among recipients of WBi blood, 36% were WBi in their 6 month post-transfusion sample, but so were 42% of a control population that had received only WB-negative blood. When serial samples from recipients with a WB pattern were tested on two occasions, only 35% of results were reproducible. No recipients of WBi blood became ELISA positive, true positive for WB, positive for HIV-1 antigen, or positive for ELISA reactivity against recombinant p24 or gp41...Thus WBi patterns are exceedingly common in randomly selected donors and recipients"

> Genesca J et al. What do Western Blot indeterminate patterns for Human Immunodeficiency Virus mean in EIA-negative blood donors?. Lancet. 1989 Oct 28;II:1023-5.

"Natural antibodies capable of neutralizing HTLV-III [HIV] infection of H9 cells were detected in 60% of adult AIDS patients and in 80% of adults with ARC, but in 0% of normal healthy heterosexual controls. Geometric mean antibody titers were two-fold higher in ARC patients compared to AIDS patients and were even higher in 2 antibody positive healthy homosexuals. This finding suggest that virus neutralizing antibodies may exert some in vivo protect effect"

> Robert-Guroff M, Gallo RC. Method for detecting HTLV-III neutralizing antibodies in sera. US Patent Office. 1988 Jul 5;4,755,457.

"The strength of ELISA reactivity...was predictive of positivity on immunoblot [Western Blot] testing. The immunoblot was positive in all 21 specimens with ratios higher than 4.0, in 5 of 7 specimens with ratios of 2.0 to 3.9, and in only 1 of 16 specimens with ratios between 1.0 and 1.9 [this illustrates that the HIV test only became a Black and White test through an arbitrary cutoff value]"

> Hoff R et al. Seroprevalence of Human Immunodeficiency Virus among Childbearing Women. NEJM. 1988 Mar 3;318(9):525-30.

"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…The predictive value of a positive ELISA varies

from 2 to 99%…the Western blot method lacks standardization, is cumbersome, and is subjective in interpretation of banding patterns."

> Steckelberg JM, Cockerill F. Serologic testing for human immunodeficiency virus antibodies. Mayo Clin Proc. 1988;63:373-9.

"approximately 1 percent of all initial screening ELISAs were reactive, 50 percent of repeat ELISAs were reactive, and 30 to 40 percent of first Western blot assays were reactive and diagnostic."

> Burke DS et al. Measurement of the false positive rate in a screening program for human immunodeficiency virus infections. NEJM. 1988;319(15):961-4.

"We selected the 20 most strongly [indeterminate or atypical Western Blot] reactive samples for further evaluation...Atypical WB [Western Blot] paterns in 19 of 20 of our donors remained substantially the same over time...our data show that the presence of p24 alone in WB should not be regarded as a false positive without subsequent testing of the individual...All study donors had normal immune status...[2] donors were multiparous females [multiple children], and one other probably had received a blood transfusion...we observed a large proportion of individuals who had either lived or worked on dairy farms (6/16) and frequently drank unpasteurized cows' milk (7/16)..undefined autoimmune phenomena [such as multiple pregnancies], bovine exposure, or corss-reactivity with other human retroviruses could be possible causes for consistently reactive HIV immunologic assays"

> Dock NL et al. Evaluation of atypical human immunodeficiency immunoblot reactivity in blood donors. Transfusion. 1988;28:412.

"Our data support the use of category I as a positive result [positive WB if 3 of 4 "key" bands are positive - this was the method used by FDA at the time]; however, if this were the only criterion that was finally established as unequivocal positive, more than 50% of patients with AIDS would be put into a "probable" positive or indeterminate category. Our data support the inclusion of categories I, IIa and IIb as unequivocal positives [basically you need 2 of 4 key bands]. Applying these criteria would increase the percent positive for patients with AIDS to 79% without diminishing specificity"

> Lundberg GD. Serological diagnosis of Human Immunodeficiency Virus infection by Western Blot testing. JAMA. 1988;260:674-9.

"Reactivity with [HIV proteins] p24 and/or gp41 has been suggested as a minimum requirement for HIV seropositivity by WB [Western Blot]. While testing ELISA positive serum from Swedish blood donors we detected 3 sera with false-positive WB reactions to p24 and p55...The 3...had no risk factors for HIV infection"

> Biberfeld G et al. Blood donor sera with false-positive western blot reactions to human immunodeficiency virus. Lancet. 1986 Aug 2;2:289-90.

"The sera [from 6720 blood donors] were examined by various enzyme-linked immunoassay (ELISA) screening tests and, usually, by one of three types of confirmatory assay. 45 samples (0.21%) were confirmed as positive. Only 2 were positive in all three confirmatory tests."

> Hunsmann G. HTLV-III antibody Positive Blood Donors. Lancet. 1985 May 25;1:1223.

"When the ELISA is used to screen populations in whom the prevalence of HTLV-III infections is low, the proportion of positive results that are falsely positive will be high"

Provisional Public Health Service Inter-Agency Recommendations for Screening Donated Blood and Plasma for Antibody to the Virus Causing Acquired Immunodeficiency Syndrome. MMWR. 1985 Jan 11;34(1):1-5.

"Of 96 patients with AIDS or AIDS-related complex and healthy individuals at risk for AIDS, 4 had no detectable antibodies to viral proteins, though [HIV] was isolated from their lymphocytes. 3 of these subjects were symptom-free and 1 had lymphadenopathy. All 4 were sexual partners of patients with AIDS or AIDS-related complex...none of the patients studied here had evidence of impaired production of antibody other viruses"

Salahuddin SZ et al. HTLV-III in symptom free seronegative persons. Lancet. 1984 Dec 22/29;2:1418-20.

"The results provide evidence for the involvement of LAV [HIV] in AIDS...Specific antibodies against [HIV] have been detected in approximately 70 percent of patients with persistent lymphadenopathy and 40 percent of AIDS patients studied."

Klatzmann D et al. Selective tropism of lymphadenopathy associated virus (LAV) for helper-inducer T lymphocytes. Science. 1984 Jul 6;225:59-63.

"Antibody to LAV [HIV] p25 [p24] was found in the serum of 51 of 125 AIDS patients, 81 of 113 patients with lymphadenopathy syndrome, 0 of 70 workers at the Centers for Disease Control (some of whom had handled specimens from AIDS patients), and 0 of 189 random blood donors."

Kalyanaraman VS et al. Antibodies to the core protein of lymphadenopathy-associated virus (LAV) in patients with AIDS. Science. 1984 Jul 20;225(4659):321-3.

"A positive [antibody] test for most individuals in populations at greater risk of acquring AIDS will probably mean that the individual has been infected at some time with [HIV]. Whether the person is currently infected or immune is not known, based on the serologic [antibody] test alone - [HIV] has been isolated in both the presence and absence of antibody "

[e-version incomplete] et al. Antibodies to a retrovirus etiologically associated with acquired immunodeficiency syndrome (AIDS) in populations with increased incidences of the syndrome. MMWR. 1984 Jul 13;33(37):377-9.

Antigen Tests (p24)

Antigen tests are the opposite of antibody tests. An antibody is a protein produced by an immune system in response to a foreign protein (the antigen), such as a virus coat. Antigen tests (usually with the p24/p25 protein) have not been spectacularly successful, because may people who are antibody positive do not have HIV antigen. One would think that this would mean that these people have only been exposed to HIV (hence the antibodies) but not infected (hence no antigens). Yet, in the topsy-turvy world of AIDS, the opposite interpretation is taken.

"p24 antigen was detected in 6 patients of group P [positive] and 2 patients of group N [HIV-negative]. "

Urano H et al. HIV isolation may not correlate with clinical state or immunological function of respective HIV infected patients. Int Conf AIDS. 1994 Aug;10(2):255.

"At the time of delivery, HIV-1 p24 antigen was detected in serum from 16 of 108 [HIV+] women (15%)"

Blanche S. Relation Of The Course Of HIV Infection In Children To The Severity Of The Disease In Their Mothers At Delivery. NEJM. 1994 Feb 3;330(5):308-12.

"Of 61 infected children tested 46 (75%) had at least one positive test for antigen"

> Kind C et al. Epidemiology of vertically transmitted HIV-1 infection in Switzerland: results of a nationwide prospective study. Eur J Pediatr. 1992;151:442-8.

"Alloimmune mice...were shown to make antibodies against gp120 and p24 of human immunodeficiency virus (HIV), and mice of [two] autoimmune strains...made antibodies against gp120. This is surprising because the mice were not exposed to HIV. [i.e. HIV proteins are found in uninfected mice!!]"

> Kion TA, Hoffmann GW. Anti-HIV and anti-anti-MHC antibodies in alloimmune and autoimmune mice. Science. 1991 Sep 6;253:1138-40.

"Baseline serum p24 antigen levels were measured in 71 patients. At entry, 37 (52%) were positive for the antigen...and 34 (48%) were negative [yet all were positive for HIV antibodies]"

> McKinney RE et al. A multicenter trial of oral zidovudine in children with advanced human immunodeficiency virus disease. NEJM. 1991 Apr 11;324(15):1018-25.

"Cryostat sections of human normal term placentae were …examined for HIV protein antigens gp120, p17, p24, and gp41. No evidence for gp41 was found. Antigens gp120 and p17 were identified in normal chorionic villi in vimentin-positive fibroblast-like cells and in endothelium, respectively. Antigen p24 was localized to HLA-DR positive cells that morphologically resembled macrophages in areas of villitis. [i.e. HIV proteins are found in uninfected human placentas!!]"

> Faulk WP, Labarrere CA. HIV proteins in normal human placentae. American Journal of Reproductive Immunology. 1991;25:99-104.

"HIV-1 p24 is the HIV-1 protein most prone to "false-positive" reactions"

> Ng V. Serological diagnosis with recombinant peptides/proteins. Clin Chem. 1991;37(10):1667-8.

"there were 16 sera from 30 viraemic patients which did not have detectable p24 antigen"

> Semple M et al. Direct measurement of viraemia in patients infected with HIV-1 and its relationship to disease progression and zidovudine therapy. J Med Virol. 1991;35:38-45.

"there were 16 sera from 30 viraemic patients which did not have detectable p24 antigen (<5 pg/ml, Fig. 2). As a consequence, p24 antigen concentration and HIV-1 RNA did not correlate well."

> Semple M et al. Direct measurement of viraemia in patients infected with HIV-1 and its relationship to disease progression and zidovudine therapy. J Med Virol. 1991;35:38-45.

"205 subjects (of 406 tested (50%)) had detectable serum levels of HIV antigen before treatment [i.e. 50% were negative for HIV antigen, although positive for HIV antibodies]"

> Fischl MA et al. A randomized controlled trial of a reduced daily dose of Zidovudine in patients with the Acquired Immunodeficiency Syndrome. NEJM. 1990;323(15):1009-14.

"In only 45 percent of persons from whom we isolated plasma-associated HIV [by co-culture techniques] was p24 antigen detected in plasma or serum"

> Coombs RW et al. Plasma viremia in human immunodeficiency virus infection. NEJM. 1989 Dec 14;321(24):1626-31.

"In only 45 percent of persons from whom we isolated plasma-associated HIV was p24 antigen detected in plasma or serum"

> Coombs RW et al. Plasma viremia in human immunodeficiency virus infection. NEJM. 1989 Dec 14;321(24):1626-31.

"no infants, including those who later had AIDS, were positive for serum antigen [p24] during the neonatal period"

> Rogers MF et al. Use of the polymerase chain reaction for early detection of the proviral sequences of human immunodeficiency virus in infants born to seropositive mothers. NEJM. 1989 Jun 22;320(25):1649-54.

"Whether the production of HIV antigen [p24 or p25] accurately reflects complete viral replication with the production of infectious virions is still to be investigated"

> Lange JMA et al. Persistent HIV antigenaemia and decline of HIV core antibodies associated with transition to AIDS. BMJ. 1986;293:1459-62.

"antibodies to the structural proteins of HTLV, notably p24 and p19 are not detectable in most AIDS patients"

> SchŸpbach J et al. Serological Analysis of a Subgroup of Human T-Lymphotropic Retroviruses (HTLV-III) Associated with AIDS. Science. 1984 May 4;224:503-505.

Co-culture Tests

Co-culture is often claimed to result in 'isolation' of HIV. However, co-culture is a very complicated procedure, with lots of opportunities for misinterpretation of data, and with very vague outcomes. Co-cultures are a combination of an immortal line of cancerous cells, a sample of cells or fluids from a potentially HIV-infected person and stimulating chemicals. Detection of one of a number of non-specific phenomena (e.g. reverse transcriptase activity, p24 antigen, production of particles about the size and shape of a retrovirus) in this witches brew is assumed to be proof of the presence of HIV in the original sample. However, none of these phenomena are specific to a retrovirus, let alone HIV.

"the expression of HERVs [Human Endogenous Retroviruses] has been linked with disruption of immune function, particularly associated with autoimmune disease. Reports of retrovirus-like particles in Sjogren's syndrome and sequencesw in Graves disease and multiple sclerosis suggest that their expression may have considerable impact on the immune system...We detected a 6 kb mRNA and lower levels of a 4.5 kb RNA...in PHA-stimulated T cells...the transcripts are absent or undetectable in unstimulated T cells [which, by extension, could mean that HIV is an endogenous (built-in) retrovirus that is inactive until stimulated by PHA using co-culture techniques]"

> Kelleher CA et al. Expression of novel-transposon-containing mRNAs in human T cells. J Gen Virol. 1996;77:1101-10.

"Overall, plasma viremia [as measured by culture] developed during follow-up in 17 of 27 patients (63 percent) who initially did not have viremia, and was sustained in 35 (88 percent) of the 40 patients who initially had viremia"

> Coombs RW et al. Plasma viremia in human immunodeficiency virus infection. NEJM. 1989 Dec 14;321(24):1626-31.

"#879 claims that this shows that ÒHIV culture-positive rate [is] 20% in high-risk, antibody-negative peopleÓ"

Imagawa DT et al. Human immunodeficiency virus type I infection in homosexual men who remain seronegative for prolonged periods. NEJM. 1989 Jun 1;320(22):1458-62.

"The main difference in the [retroviral and retrotransposon life] cycles is the presence of infectious, extracellular, membrane-enveloped particles in the retroviruses [i.e. the presence of reverse transcription cannot be taken to indicate the presence of any retrovirus, let alone a specific retrovirus]"

Boeke JD, Corces VG. Transcription and reverse transcription of retrotransposons. Ann Rev Microbiol. 1989;43:403-34.

"The protein fraction [of HIV] is obtained by disruption of sucrose gradient purified HTLV-III [HIV] [without any proof that the material obtained represents a retrovirus, let alone HIV]"

Saxinger WC, Gallo RC. Competitive ELISA for the detection of HTLV-III antibodies. US Patent Office. 1987 Apr 28;4,661,445.

"Continuous production of HTLV-III is obtained after repeated exposure of parental HT cells to concentrated [not purified] culture fluids containing HTLV-III harvested from short term cultured T-cells (grown with TCGF [T-Cell Growth Factor]) which originated from patients with pre-AIDS or AIDS. The concentrated fluids were first shown to contain particle-associated reverse transcriptase (RT) [i.e. no direct evidence of the presence of a retrovirus]...Samples exhibiting more than one of the following were considered positive [for an HTLV-family virus]: repeated detection of a Mg++ dependent reverse transcriptase activity in supernatant fluids [but reverse transcription occurs in cells even without retroviruses present]; virus observed by electron microscopy [but merely looking at particles cannot prove that they are retrovirus particles]; intracellular expression of virus-related antigens detected with antibodies from sero-positive donors or with hyperimmune serum [but, without purification of HIV, it is not possible to say with certainty what virus-related antigens are, and even if it was, antibody cross-reactions are still possible]; or transmission of particles, detected by reverse transcriptase assays or by electron microscopic observation, to fresh human cord blood, bone marrow, or peripheral blood T-lymphocytes [but the ability to stimulate a cellculture to produce particles is not proof that an infectious agent is present, and certainly not that it is a specific retrovirus]. All isolates not classified as either HTLV-I or HTLV-II by immunological or nucleic acide analysis were classified as HTLV-III."

Gallo RC, Popovic M. Method of continuous production of retroviruses (HTLV-III) from patients with AIDS and pre-AIDS using permissive cells. US Patent Office. 1987 Mar 24;4,652,599.

"The study population consisted of...patients with AIDS and patients with AIDS-related complex...The criteria for eligibility also included...an absolute number of [CD4 cells] less than 500 per cubic millimeter..., serum positive for antibody to HIV...HIV was isolated [by co-culture] at entry in 57 percent of the AZT group and 58 percent of the placebo group [i.e. almost half of patients with AIDS and pre-AIDS were negative by HIV by culturing techniques]"

Fischl MA et al. The Efficacy of Azidothymidine (AZT) in the Treatment of Patients with AIDS and AIDS-Related Complex. NEJM. 1987;317:185-191.

"In an early set of experiments, HTLV-III [HIV] was cultured from 48 subjects, including 18 or 21 patients with ARC [AIDS-related Complex, also known as pre-AIDS], 3 of 4 clinically normal mothers of children with AIDS, and [only] 26 of 72 adults and children with AIDS...Of interest is the finding, that while antibody to HTLV-III was more often associated with advanced disease, HTLV-III itself was more frequently culture in ARC or newly diagnosed AIDS patients"

Layon J, Warzynski M, Idris A. Acquired immunodeficiency syndrome in the United States: a selective review. Critical Care Medicine. 1986;14(9):819-27.

"In the last few years, the view that reverse transcription is solely a retroviral mechanism has been disproven [even though this assumption is the basis of much co-culture evidence]"

Baltimore D. Retroviruses and retrotransposons: the role of reverse transcription in shaping the eukaryotic genome. Cell. 1985 Mar;40(3):481-2.

"Evidence for the presence of HTLV-III included: (i) viral reverse transcriptase (RT) activity in supernatant fluids; (ii) transmission of virus by coculturing T cells…; (iii) observation of virus by electron microscopy; and (iv) the expression of viral antigens…using serum from a patient positive for antibodies to HTLV-III…or antisera prepared against purified, whole disrupted HTLV-III"

Gallo RC et al. Frequent Detection and Isolation of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and at Risk for AIDS. Science. 1984 May 4;224:500-3.

"…a growing constellation of eukaryotic genetic elements Ð various pseudogenes and repetitive sequences Ð appear to depend upon reverse transcriptases of unknown provenance for their existence or amplification [yet, in an HIV-coculture, the presence of reverse transcription is considered unambiguous evidence for the existence of HIV]"

Varmus HE. A growing role for reverse transcription. Nature. 1982 Sep 16;299(5880):204-5.

Viral Load (PCR; Polymerase Chain Reaction)

Viral load tests are often claimed to detect the genetic material of HIV, either the DNA embedded in infected cells or the RNA in viral particles circulating in the body. Yet these tests search for only a small fraction of the HIV genome, and HIV has never been properly isolated to allow the genome to be unambiguosly determined. Furthermore, the viral load test cannot distinguish between infectious and defective virus, and in some studies only 1 out of 60,000 viral particles was estimated to be infectious (see section on discordance).

"That a clinical benefit may not have been achieved with multi-drug rescue therapy calls into question the current wisdom of deeming an undetectable viral load the goal of therrapy in the heavily pre-treated population. Even if it can be accepted that an undetectable viral load is an appropriate surrogate marker for clinically relevant outcomes in treatment-inexperienced patients who are initiating combination therapy, it cannot necessarily be accepted without proof that it is a useful surrogate in heavily pre-treated patients...Therefore, until controlled trials are able to prove the utility of an undetectable viral load as a surrogate marker for clinically relevant outcomes in heavily pre-treated patients, we bleieve that clinicians should show caution before striving for complete viral suppression at any cost"

Deeks SG, Martin JN. Editorial Comment: Reassessing the goal of antiretroviral therapy in the heavily pre-treated HIV-infected patient. AIDS. 2001;15(1):117-9.

"Because of the RNA assay's 1.9% to 3.0% false-positive rate, results must be carefully interpreted and compared to HIV-1 viral load levels seen during proven HIV-1 seroconversion. We report the case of a sexually active woman with symptoms suggestive of ARS who had a false-positive HIV-1 RNA assay result."

More D et al. Utility of an HIV-1 RNA assay in the diagnosis of acute retroviral syndrome. S Med J. 2000 Oct;93(10):1004-6.

"Negative results (<50 copies/mL), were obtained in 30/32 (94%) [bDNA 3.0] assays [meaning that false-positive results were obtained in 2/32 or 6% of cases]"

> Erice A et al. Performance characteristics of the bDNA 3.0 assy for quantitation of HIV-1 RNA in plasma [abstract]. 7th Conf. Retroviruses and Opp Infections. 2000 Jan 30-Feb 2.

"In contrast to previous reports...the viral load in the majority of the [long-term survivors] tested was detectable and, in some [long-term survivors], quite high...and variable over time."

> Betts MR et al. AIDS Res Hum Retro. 1999 Oct;15:1219-28.

"The results obtained for patients with a broad range of plasma viral loads before and after antiretroviral therapy reveal a constant mean viral (v)RNA copy number (3.6 log10 copies) per infected cell, regardless of plasma virus load or treatment status."

> Hockett RD et al. Constant Mean Viral Copy Number per Infected Cell in Tissues Regardless of High, Low, or Undetectable Plasma HIV RNA. Journal of Experimental Medicine. 1999 May 17;189(10):1545-54.

"Plasma viral load tests for HIV-1 were neither developed nor evaluated for the diagnosis of HIV-1 infection; therefore their diagnostic specificity is not well delineated when applied to persons who are negative for HIV antibody. We report two cases of false-positive results obtained by using branched-chain DNA assay...and one case...by using HIV reverse transcriptase polymerase chain reaction (RT-PCR)...These three cases illustrate the potential problems of using HIV-1 plasma viral load tests for diagnosis of HIV infection...Only patients who have a high pre-test probability of a positive result should be evaluated for primary infection by using plasma viral load testing [i.e. preconceptions about risk groups such as gay men makes a positive test more likely]...Their performance in patients who are not infected with HIV is unknown"

> Rich JD et al. Misdiagnosis of HIV Infection by HIV-1 Plasma Viral Load Testing: A Case Series. Ann Int Med. 1999 Jan 5;130:37-9.

"We observed that clade A strains [of HIV] were not detected by RT-PCR [Reverse Transcriptase-Polymerase Chain Reaction] and that clade G-strains were not detected by NASBA [Nucleic Acid Sequence-Based Amplification]. However, the copy number detected by RT-PCR in one clade E (CM235) and in one clade F (163.3070) was much lower than the copy number detected by bDNA [branched DNA] and NASBA...for clades B and D (UG270), the HIV-1 RNA levels measured by bDNA were lower than those obtained by RT-PCR and NASBA"

> Coste J et al. Effect of HIV-1 genetic diversity on HIV-1 RNA quantification in plasma: comparative evaluation of three commercial assays. JAIDS. 1997;15:174.

"The AMPLICOR HIV-1 MONITOR Test is an in vitro nucleic acid amplification test for the quantitation of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma...[It] is not intended to be used as a screening test for HIV or as a diagnostic test to confirm the presence of HIV infection...Quantitative [co-]culture has limited utility for monitoring virus levels in infected individuals since only a small fraction of virus particles is infectious in vitro. Infectious virus is often undetectable in asymptomatic individuals...The clinical specificity of the...test was determined by analysis of 495 anti-HIV-1 negative blood donors. None of these specimens was reactive...Assuming a zero prevalence of HIV-1 infection in the seronegative blood donors, the specificity of the test was 100%"

> Amplicor HIV-1 Monitor Test. Roche. 1996.

"the high level of plasma virus observed by Piatak et al, was about 99.9 per cent non-culturable, suggesting that it was either neutralized or defective. Therefore, rather than supporting a cytopathic model, this observation actually may help explain the relatively slow dissemination of the infected cell burden and thus the relative ineffectiveness of therapy with nucleoside analogues which target this process."

> Sheppard HW, Ascher MS, Krowka JF. Viral burden and HIV disease. Nature. 1993 Jul 22;364(6435):291-2.

"Circulating levels of plasma virus determined by QC-PCR also correlated with, but exceeded by an average of nearly 60,000-fold..., titers [amounts] of infectious HIV-1 determined by quantitative endpoint dilution culture of identical portions of plasma."

> Piatak M Jr et al. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. Science. 1993 Mar 19;259:1749-54.

"Our study of PBMC [Peripheral Blood Mononuclear Cells] from 56 HIV-1-seropositive patients, using in situ hybridization alone, also [as did other studies] revealed only 1 in 5000 to 1 in 100,000 cells positive for HIV-1-specific nucleic acids [DNA]...Our finding with the use of in situ PCR that large numbers [if you consider 0.1% to 13.5% of cells a 'large number'] of PBMC from HIV-1-seropositive patients contain the provirus suggests that direct cytopathic [cell-killing] effects of the virus may be an important but not necessarily the sole cause of depletion of CD4-positive lymphocytes. Our data also argue strongly against the theory that HIV-1 is not the primary etiologic [causative] agent of AIDS [huh?]...we were able to...show a relation between viral load and the stage of HIV-1 clinical infection. Patients in Stage II [HIV+, but no symptoms] had a significantly lower percentage of HIV-1-positive PBMC than those in Stage III and Stages IV-A to IV-C [but the authors neglect to mention that this is only true of the average value, there was considerable overlap between individual values. More importantly, they also do not mention that the average value at Stage III (lymph gland enlargement) was higher than at Stage IV-A to C (AIDS) and much higher than at Stage IV-D (Kaposi's Sarcoma)]. Patients in Stage IV-D (who had Kaposi's sarcoma only) had relatively low numbers of HIV-1-infected cells."

> Bagasra O et al. Detection of human immunodeficiency virus type 1 provirus in mononuclear cells by in situ polymerase chain reaction. NEJM. 1992;326(21):1385-91.

"This proficiency study of PCR detection of HIV-1 DNA in serum identified a disturbingly high rate of nonspecific positivity with a widely employed gag primer pair system. In fact, the overall rate of positivity was not significantly different for serum specimens from serpositive patients and seronegative control donors (26% versus 18%) [some would say that HIV DNA should not be found in serum, which means that these are all false positive reactions]"

> Busch MP et al. Poor sensitivity, specificity, and reproducibility of detection of HIV-1 DNA in serum by polymerase chain reaction (PCR). Journal of Acquired Immune Deficiency Syndrome . 1992;5(9):872-879.

"We found that in infected but asymptomatic patients with HIV-1, [an average of] 1 in 50,000 PBMC harbored the virus. When such a patient's condition progressed to AIDS-related complex or AIDS, the viral titer increased significantly, to approximately 1 in 400 PBMC...It is unclear from this study, however, what percentage of the infected cells carry HIV-1 latently and what percentage of the cells express the virus actively. If 1 in 10,000 PBMC...express viral messenger RNA...then 99.6% of the infected monuclear cells harbor the virus latently and the remaining 0.4 % express it actively [i.e. 1 in 100,000 cells have active virus!]...this information on the quantitation of HIV-1 [Òhigh levels of HIV-1 viremiaÓ] should reduce residual doubts about whether HIV-1 is the true etiologic agent of AIDS"

Ho DD et al. Quantitation of Human Immunodeficiency Virus type 1 in the blood of infected persons. NEJM. 1989;321(24):1621-5.

"we analyzed samples from individuals konwn to be at especially high risk of HIV infection-seronegative sexual partners of seropositive individuals...Of 16 seronegative partners tested, 5 were unequivocally positive for HIV DNA. The clinical records of these 5 subjects confirmed that they were seronegative by enzyme-linked immunsorbent assay and western blot and negative for the p24 antigen at the time the blood samples were taken for the DNA assay...the same 5 samples were found to be positive with a second HIV-specific oligonucleotide...The serological [antibody] status was confirmed in each case and each of the 5 individuals was negative for anti-HIV antibodies and p24 antigen 2 and 3 months after the initial detection of HIV DNA"

Loche M, Mach B. Identification of HIV-infected seronegative individuals by a direct diagnostic based on hybridization to amplified viral DNA. Lancet. 1988;ii:418-21.

"A reanalysis is presented of 43 cases of apparent transient viremina [one or more positive culture or PCR assays for HIV-1 and the subsequent inability to detect HIV-1 in the speciments on multiple occasions or seroreversion, or both]. 41 cases occurred among 1561 infants in five studies of mother-to-infant HIV-1 transmission...Our negative studies of 43 cases of suspected transient infection indicate that the phenomenon...remains to be proven and that most cases suggestive of transient HIV-1 infection are cases of mislabeling of specimens or their contamination in the laboratory"

Frenkel LM et al. Genetic Evaluation of Suspected Cases of Transient HIV-1 Infection of Infants. Science. 15 May 1998;280:1073-1077.

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Frenkel LM et al. Genetic Evaluation of Suspected Cases of Transient HIV-1 Infection of Infants. Science. 15 May 1998;280:1073-1077.

Discordance between HIV Tests

There are many examples of inconsistency between different HIV tests. This may mean that all but one of the tests are giving false results. But, which one? And, how do we know that any of the tests are giving valid results (see section on validation)?

"[conditions associated with false positive ELISA are] autoimmune disease, renal failure, cystic fibrosis, multiple pregnancies, blood transfusions, liver diseases, parenteral substance abuse, hemodialysis, or vaccinations for hepatitis B, rabies, or influenza...Causes of indeterminate WB [Western Blot] results include...nonspecific antibody reactions (eg, due to lymphoma, multiple sclerosis, injection drug use, liver disease, or autoimmune disorders). Also, there appear to be healthy individuals with antibodies that cross-react with specific HIV-1 peptides or recombinant antigens...The Association of Public Health Laboratories now recommends that patients who have minimal positive results on WB, eg, p24 and gp160 only, or gp41 and gp160 only, be told that these patterns have been seen in persons who are not infected with HIV and that follow-up testing is required to determine actual infective status. The clinician must judge the test results within the context of other epidemiological and clinical information [i.e. gay men and IV drug users are likely to be defined as positive based on this prejudice in the presence of ambiguous test results]. In the

appropriate clinical setting, positive ELISA and WB test results in patients with a normal CD4 + count and CD4/ CD8 ratio and undetectable HIV-1 RNA should be questioned, repeated, or confirmed with supplemented testing. A false-positive serological test result may be supported by normal CD4 + count and CD4/CD8 ratio and undetectable HIV-1 RNA, but is ultimately established by subsequent serological testing and, especially, close follow-up. [i.e. there is no test that can be absolutely relied on]"

> Mylonakis E et al. Report of a False-Positive HIV Test Result and the Potential Use of Additional Tests in Establishing HIV Serostatus. Arch Intern Med. 2000 Aug 14/28;160:2386-8.

"LTNP [long-term non-progressor (to AIDS)] status was defined as asymptomatic HIV-1 infection for at least 8 years with stable CD4+ cell counts and no antiretroviral therapy...A wide range of plasma viral loads was observed among the LTNPs with HIV-1 RNA levels ranging from < 20 up to 860,000 RNA copies/ml plasma and a similar range was observed for the controls [Median: 40,000; Range: 2,200 up to 1,860,000] (Table I)...Among the 47 LTNPs with plasma viral load higher than 800 copies/ml, 30 had a viral load higher than 10,000 copies/ml and 3 had a viral load higher than 500,000 copies/ml despite fulfilling the inclusion criteria."

> Candotti D et al. Status of long-term asymptomatic HIV-1 infection correlates with viral load but not with virus replication properties and cell tropism. J Med Virol. 1999 July;58(3):256-63.

"a peripheral blood sample was positive for HIV-1 by culture and a second sample from a separate blood draw was positive by either culture or HIV-1 DNA polymerase chain reaction (PCR) testing. Uninfected infants had at least two peripheral blood samples that were negative for HIV-1 by both culture and DNA PCR, with 1 or the 2 samples obtained at no earlier than 14 weeks of age. We did HIV-1 antibody testing on the infants at 12 and 18 months of age to confirm their HIV-1 infection status. We defined infants with a confirmed infection as having an early infection if a peripheral blood sample drawn within 24h of birth was positive for HIV-1 by culture or DNA PCR testing. Likewise, infected infants were defined as having a late infection if a peripheral blood sample drawn within 24h of birth was negative by culture or DNA PCR testing. Infected infants who did not have a blood sample obtained within the first 24h after birth were not further classified. Results from cord blood samples were not used for the determination of infection status nor for the timing of infection...Twelve infants were positive by both tests at [the study visit at which each of the 19 infected infants first had a positive virologic test], 5 were positive only by PBMC culture, and 2 were positive only by DNA PCR. Nine infected infants had plasma cultures done at the first positive visit, and 5 (56%) were positive. Likewise, 11 had quantitative RNA PCR testing done, and all were positive."

> Van Dyke RB et al. The Ariel Project: A Prospective Cohort Study of Maternal-Child Transmission of Human Immunodeficiency Virus Type 1 in the Era of Maternal Antiretroviral Therapy. JID. 1999 Feb;179(2):319-28.

"This report describes the field and laboratory investigation of eight patients who had clinical evidence of HIV infection, but repeatedly negative HIV-1 antibody screening results in the course of their clinical care. In all patients, HIV infection was proven [sic] by other diagnostic methods [PCR/viral load, p24 antigen and co-culture techniques]...Patient 1...had 3 negative HIV EIA [ELISA antibody test] results in the 2 years before admission, and 5 other document negative EIA tests in the 8 years before that. On one occasion, 9 years before admission, one reactive HIV EIA test result was obtained, but the confirmatory Western blot result was negative...After the diagnosis of HIV infection was confirmed by HIV RNA PCR, the patient was prescribed zidovudine and lamivudine. Two weeks after initiation of therapy, serum from the patient was strongly reactive with all HIV EIA"

> Sullivan PS, Schable C. Persistently negative HIV-1 antibody enzyme immunoassay screening results for patients with HIV-1 infection and AIDS: serologic, clinical, and virologic results. AIDS. 1999 Jan 14;13(1):89-96, www.aidsonline.org.

"This report describes the field and laboratory investigation of eight patients who had clinical evidence of HIV infection, but repeatedly negative HIV-1 antibody screening results in the course of their clinical care. In all patients, HIV infection was proven [sic] by other diagnostic methods [PCR/viral load, p24 antigen and co-culture techniques]...Patient 2...HIV EIA result was negative during admission, but HIV infection was identified by HIV p24 antigen testing and DNA PCR...His wife was tested for HIV infection by HIV EIA and DNA PCR; the results of both tests were negative "

> Sullivan PS, Schable C. Persistently negative HIV-1 antibody enzyme immunoassay screening results for patients with HIV-1 infection and AIDS: serologic, clinical, and virologic results. AIDS. 1999 Jan 14;13(1):89-96, www.aidsonline.org.

"This report describes the field and laboratory investigation of eight patients who had clinical evidence of HIV infection, but repeatedly negative HIV-1 antibody screening results in the course of their clinical care. In all patients, HIV infection was proven [sic] by other diagnostic methods [PCR/viral load, p24 antigen and co-culture techniques]...Patient 3...HIV-1 EIA and an HIV-1/2 combination test administered 1 month [after hospital admission] were negative...HIV-1 p24 antigen tests were positive...The diagnosis of HIV infection was confirmed by HIV-1 DNA PCR. During the following 27 months, the patient had eight negative HIV EIA results; 3 HIV-1 DNA PCR tests and 3 HIV-1 RT PCR tests were positive"

> Sullivan PS, Schable C. Persistently negative HIV-1 antibody enzyme immunoassay screening results for patients with HIV-1 infection and AIDS: serologic, clinical, and virologic results. AIDS. 1999 Jan 14;13(1):89-96, www.aidsonline.org.

"This report describes the field and laboratory investigation of eight patients who had clinical evidence of HIV infection, but repeatedly negative HIV-1 antibody screening results in the course of their clinical care. In all patients, HIV infection was proven [sic] by other diagnostic methods [PCR/viral load, p24 antigen and co-culture techniques]...Patient 4...first HIV EIA, performed at the time of diagnosis of oral thrush 4 months [after persistent high fever], was negative...[8 months later, after worsening health problems] an HIV EIA result was negative...[but] specimens were positive by DNA PCR and p24 antigen tests...In the 11 months following the positive PCR and antigen tests at CDC, the patient had 3 negative HIV EIA results"

> Sullivan PS, Schable C. Persistently negative HIV-1 antibody enzyme immunoassay screening results for patients with HIV-1 infection and AIDS: serologic, clinical, and virologic results. AIDS. 1999 Jan 14;13(1):89-96, www.aidsonline.org.

"This report describes the field and laboratory investigation of eight patients who had clinical evidence of HIV infection, but repeatedly negative HIV-1 antibody screening results in the course of their clinical care. In all patients, HIV infection was proven [sic] by other diagnostic methods [PCR/viral load, p24 antigen and co-culture techniques]...Patient 5...results of two HIV EIA performed during the initial evaluation [for acute respiratory distress] were negative, although two quantitative RT-PCR tests were positive...Viral [co-]culture was positive; however, a later blood sample...was negative by HIV EIA and positive by p24 antigen EIA...The patient had 4 children...All were tested by HIV EIA, p24 antigen EIA, and RNA PCR with negative results"

> Sullivan PS, Schable C. Persistently negative HIV-1 antibody enzyme immunoassay screening results for patients with HIV-1 infection and AIDS: serologic, clinical, and virologic results. AIDS. 1999 Jan 14;13(1):89-96, www.aidsonline.org.

"This report describes the field and laboratory investigation of eight patients who had clinical evidence of HIV infection, but repeatedly negative HIV-1 antibody screening results in the course of their clinical care. In all patients, HIV infection was proven [sic] by other diagnostic methods [PCR/viral load, p24 antigen and co-culture techniques]...Patient 6...became acutely ill after vaccination for measles, mumps and rubella...[she had a] negative HIV EIA on 2 occasions, a positive HIV-1 p24 antigen result, and a positive HIV-1 DNA PCR result. Prior HIV EIA results were negative 2 years, 1 year and 2 weeks before hospitalization...Of her 17 lifetime sexual partners, four were tested at CDC by HIV EIA and HIV-1 DNA PCR; all test results were negative"

> Sullivan PS, Schable C. Persistently negative HIV-1 antibody enzyme immunoassay screening results for patients with HIV-1 infection and AIDS: serologic, clinical, and virologic results. AIDS. 1999 Jan 14;13(1):89-96, www.aidsonline.org.

"there is approximately 15% probability that an HIV-negative sample will evidence nonspecific reactions to p24 on WB [Western Blot]...samples with strong reactivity to gag antigens...including p17, p24, p32, p46...and p55...can be misinterpreted as p17, p24, p31, gp41 and p55 bands, and this results in an overall positive interpretation...The 4 donors we studied all lacked HIV risk factors and were proven by HIV PCR and, in two cases, culture and p24 antigen analyses not to be infected"

> Sayre KR et al. False positive HIV-1 Western Blot tests in noninfected blood donors. Transfusion. 1996;36:45-52.

"HIV was isolated from 32 patients (54%) of 59 [HIV+] patients examined. In the group with positive blood culture (group P), CD4+ cell count and CD4/8 were significantly lower than those in the group with negative blood culture (group N). p24 antigen was detected in 6 patients of group P and 2 patients of group N. There was no difference in beta 2-m and cytokine levels between the two groups. HIV isolation had no influence on the subsequent changes in the clinical state and immunological data."

> Urano H et al. HIV isolation may not correlate with clinical state or immunological function of respective HIV infected patients. Int Conf AIDS. 1994 Aug;10(2):255.

"Culturable virus in plasma was reduced to undetectable levels coincident with seroconversion in five of six patients, and was substantially reduced in the sixth. Circulating p24 antigen also decreased with seroconversion, even by use of immune complex dissociation tests. However, despite decreases in total plasma virus levels by QC-PCR of up to 236-fold that closly paralleled declines in culturable virus, plasma virion-associated RNA remained readily detectable throughout the full follow-up in all six patients."

> Piatak M et al. Viral dynamics in primary HIV-1 infection. Lancet. 1993 Apr 24;341:1099.

"Circulating levels of plasma virus determined by QC-PCR also correlated with, but exceeded by an average of nearly 60,000-fold..., titers [amounts] of infectious HIV-1 determined by quantitative endpoint dilution culture of identical portions of plasma."

> Piatak M Jr et al. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. Science. 1993 Mar 19;259:1749-54.

"concordance [of viral load] with serology [ELISA/Western Blot antibody tests] varied from 40 to 100%."

> Defer C et al. Multicenter quality control of polymerase chain reaction for detection of HIV DNA. AIDS. 1992;6:659-63.

"we identified a group of 6 subjects who had been infected [with HIV] through a single common [blood] donor...Throughout follow-up (range 6.8-10.1 years after infection), 5 of the [HIV antibody positive] recipients and the donor...remained clinically free of symptoms, with normal CD4 cell counts and no p24 antigenaemia. HIV-1 was isolated [via co-culture, which is not really isolation] from only 1 recipient [in other words, the only evidence of HIV was antibodies, all other measures indicated no HIV and no AIDS]"

> Learmont J et al. Long-term symptomless HIV-1 infection in recipients of blood products from a single donor. Lancet. 1992;340:863-7.

"In blood donor studies in the developed world, about 20% of sera referred to confirmatory laboratories give indeterminate western blot results, almost all of which are on presumed negative specimens."

> Mortimer PP. The fallibility of HIV Western blot. Lancet. 1991 Feb 2;337:286-7.

"there were 16 sera from 30 viraemic patients which did not have detectable p24 antigen (<5 pg/ml, Fig. 2). As a consequence, p24 antigen concentration and HIV-1 RNA did not correlate well."

> Semple M et al. Direct measurement of viraemia in patients infected with HIV-1 and its relationship to disease progression and zidovudine therapy. J Med Virol. 1991;35:38-45.

"HIV was isolated [using co-culture] from only 36% of plasma samples, and the isolation rate was closely related to CD4 cell counts, increasing gradually from 0% in subjects with >800 [million] CD4 cells [per liter] to 88% in those with < 100 [million] CD4 cells [per liter]...The comparison of p24 antigenaemia with plasma viral cultures was not clear-cut. Concordant data were found in 62 subjects...while discordant data was observed in 37"

> Venet A et al. Correlation between CD4 cell counts and cellular and plasma viral load in HIV-1-seropositive individuals. AIDS. 1991;5:283-8.

"Five patients who did not yield virus isolates [via co-culture] in a total of 14 attempts all had virus present in 1 per 10,000 or more cells, while five from whom virus was isolated in 11 of a total of 16 attempts all had virus present in 1 per 3,000 or fewer cells."

> Simmonds P et al. Human Immunodeficiency Virus-infected individuals contain provirus in small numbers of peripheral mononuclear cells and at low copy numbers. J Virol. 1990 Feb;64(2):864-72.

"the specificity and sensitivity of PCR for detection of HIV DNA were 100% (225/225 seronegative, low-risk individuals tested negative) and 94% (67/71 seropositive individuals tested positive), respectively. In a second study ... 7/474 (1.5%) antibody-negative specimens were found to be positive [for HIV DNA], 149/151 (99%) antibody-positive specimens were positive [for DNA], and 12/13 (92%) antibody-indeterminate specimens were negative for HIV DNA"

> Young KKY et al. Detection of HIV DNA in peripheral blood by the polymerase chain reaction: a study of clinical applicability and performance. AIDS. 1990;4:389-91.

"Overall, plasma viremia [as measured by culture] developed during follow-up in 17 of 27 patients (63 percent) who initially did not have viremia, and was sustained in 35 (88 percent) of the 40 patients who initially had viremia...In only 45 percent of persons from whom we isolated plasma-associated HIV was p24 antigen detected in plasma or serum"

Coombs RW et al. Plasma viremia in human immunodeficiency virus infection. NEJM. 1989 Dec 14;321(24):1626-31.

"100 ELISA-negative donors...were tested by WB [note that normally a negative ELISA will not result in a Western Blot 'confirmatory' test]. 20 were WBi, with p24 being the predominant (70%) and generally the only band. Among recipients of WBi blood, 36% were WBi in their 6 month post-transfusion sample, but so were 42% of a control population that had received only WB-negative blood. When serial samples from recipients with a WB pattern were tested on two occasions, only 35% of results were reproducible. No recipients of WBi blood became ELISA positive, true positive for WB, positive for HIV-1 antigen, or positive for ELISA reactivity against recombinant p24 or gp41. [PCR] was negative for gag and env HIV-1 sequences in all donors and recipients. Thus WBi patterns are exceedingly common in randomly selected donors and recipients"

Genesca J et al. What do Western Blot indeterminate patterns for Human Immunodeficiency Virus mean in EIA-negative blood donors?. Lancet. 1989 Oct 28;II:1023-5.

"The eight persons at risk who were positive for anti-nef protein antibodies were also positive for HIV DNA; five of the eight remained anti-nef antibody positive and HIV seronegative (by ELISA and Western blotting) and p24/25 antigen negative for eight months (one person...) and four months (four people), respectively, after the detection of HIV DNA"

Ameisen JC et al. Persistent antibody response to HIV-1-infected seronegative persons. NEJM. 1989 Jan 26;320(4):251-2.

"23 of 25 biopsies from HIV seropositive individuals were positive for HIV DNA...An average of 0.0001 to 0.01 HIV DNA copies per cell was estimated to be present in biopsies with follicular hyperplasia or involution. The positive lymphoma biopsies contained approximately tenfold fewer HIV DNA. In contrast, 19 of 20 biopsies from seronegative or low risk individuals were negative for HIV DNA."

Shibota D et al. Human immunodeficiency viral DNA is readily found in lymph node biopsies from seropositive individuals. Am J Pathol. 1989;135:697.

"approximately 1 percent of all initial screening ELISAs were reactive, 50 percent of repeat ELISAs were reactive, and 30 to 40 percent of first Western blot assays were reactive and diagnostic."

Burke DS et al. Measurement of the false positive rate in a screening program for human immunodeficiency virus infections. NEJM. 1988;319(15):961-4.

"Infectious virus was recovered from the serum of 20(25.6%) of [78 randomly selected, HIV+ individuals, or whom about 30% were asymptomatic] and was generally present in low titers. Only undiluted serum (not a tenfold dilution) yielded infectious virus...In one serum sample, 25,000 infectious particles per milliliter were detected as measured by end dilution of the serum. This sample came from a clinically healthy individual with very low levels of antibody to HIV[!]. Nine of the positive serum samples came from 39 individuals whose PMCs [peripheral blood mononuclear cells] were also tested. Virus was isolated [sic] from the PMCs of approximately 50% of these individuals and one third also yielded infectious virus in their serum. Three serum samples contained infectious HIV without any virus being recovered from the individuals' PMCs...These studies demonstrate further that not all seropositive individuals have virus recoveraable from their PMCs and that isolation from serum is not a common event"

Michaelis BA, Levy JA. Recovery of human immunodeficiency virus from serum (letter). JAMA. 1987 Mar 13;257(10):1327.

"The sera [from 6720 blood donors] were examined by various enzyme-linked immunoassay (ELISA) screening tests and, usually, by one of three types of confirmatory assay. 45 samples (0.21%) were confirmed as positive. Only 2 were positive in all three confirmatory tests."

Hunsmann G. HTLV-III antibody Positive Blood Donors. Lancet. 1985 May 25;1:1223.

False Positives

Although it is often denied, there is a lot of evidence that HIV tests can generate false positives. In fact, with no proper validation of HIV tests, it is not clear whether or not all positive HIV tests are false positives.

"Kaushalya greets visitors with a warm welcome, hot tea and biscuits, but her big dark eyes look sad. In the last three years, the 29-year-old woman has lost her husband and social life. "They broke our lives four years ago," she says softly while other members of the family nod. The local hospital said that Kaushalya's husband died of AIDS. Doctors at the medical college in Rohtak city later explained that Kaushalya and her daughter too had tested HIV-positive. The doctors pressured Kaushalya, who was in her sixth month of pregnancy, to get an abortion because of her HIV-infection. "By telling me a lie they made me lose my only son," the young widow mourns. But late last year, a second test [probably a Western Blot or a second ELISA] showed that neither Kaushalya nor her daughter had the AIDS virus. Other members of the family too have since been tested negative for HIV. Like most Indian hospitals, the Rohtak hospital carried out only a single HIV test [ELISA] on her husband. In most other nations, at least three tests with similar results [two ELISA and one Western Blot] are required before a patient is confirmed HIV-positive. However, her family is still well known as 'the family of Chochi's first AIDS case. "For years we lived with the trauma that the whole family was finished. But it all was a big fraud," says Kaushalya's father-in- law Mangaram Joon. "

Oberhuber N. India:Village Still to Recover from AIDS 'Stigma'. Terraviva Europe Daily Journal. 2001 Jan 15, http://www.ips.org/index.htm.

"Between January 1, 1989 and July 31, 1995, voluntary preoperative screening tests for human immunodeficiency virus (HIV) infection, using an enzyme-linked immunosorbant assay, were completed on 2,727 patients who underwent elective orthopedic surgical procedures. There were 2,719 (99.7%) negative, 4 (0.15%) positive, and 3 (0.11%) false-positive results; 1 test was indeterminate (0.04%)"

LaPorte DM et al. Human immunodeficiency virus testing for elective orthopedic procedures: results in a community-based hospital. Orthopedics. 2001 Jan;24(1):52-5.

"Because of the RNA assay's 1.9% to 3.0% false-positive rate, results must be carefully interpreted and compared to HIV-1 viral load levels seen during proven HIV-1 seroconversion. We report the case of a sexually active woman with symptoms suggestive of ARS who had a false-positive HIV-1 RNA assay result."

More D et al. Utility of an HIV-1 RNA assay in the diagnosis of acute retroviral syndrome. S Med J. 2000 Oct;93(10):1004-6.

"Negative results (<50 copies/mL), were obtained in 30/32 (94%) [bDNA 3.0] assays [meaning that false-positive results were obtained in 2/32 or 6% of cases]"

Erice A et al. Performance characteristics of the bDNA 3.0 assy for quantitation of HIV-1 RNA in plasma [abstract]. 7th Conf. Retroviruses and Opp Infections. 2000 Jan 30-Feb 2.

"In 1984, I was informed that test results showed that I had an 'HIV' infection and I had at best five years to live...I am now 50 years old and have taken back control of my own health. For the past eight years, I've had no need for doctors, and have been on a diet of pure water, organic food and a positive belief system."

Anderson A. Letter. Alive. 2000.

"False positive and false negative results are to be expected [in HIV antibody testing] as with all screening tests"

Zhang Z-Q et al. Sexual Transmission and Propagation of SIV and HIV in Resting and Activated CD4+ T Cells. Science. 1999 Nov 12;286(5443):1353-7.

"We describe here a case of heterophile antibodies that are cross-reactive with bovine and caprine proteins occurring in a 22-month-old child, causing false-positive immunoassay results to human immunodeficiency virus type 1 (HIV-1) and a number of other infectious serology tests...we believe the positive test results observed in this patient were due to heterophile antibodies reactive with BSA and caprine proteins. All of the positive tests observed used BSA [bovine serum albumin] as a blocking agent for the preparation of the microELISA reaction wells."

Willman JH et al. Heterophile Antibodies to Bovine and Caprine Proteins Causing False-Positive Human Immunodeficiency Virus Type 1 and Other Enzyme-Linked Immunosorbent Assay Results. Clinical and Diagnostic Laboratory Immunology. 1999 Jul;6(4):615-6.

"18 subjects had 1 or 2 positive results with v.2.0 and an undetectable confirmatory test for a false positive rate of 4.4%. The rate is similar at baseline (9/183 subjects = 4.9%), wk. 4 (7/162= 4.3%) and wk. 26 (2/44 = 4.5%). Of the 18 pos. specimens, 9 tested pos. once and 9 twice. With version 3.0, 11 of 67 samples tested were pos. (16.4%). 6 were pos. once and 5 twice. The range of false pos. rates was 9.1% at wk. 4 (total of 22 specimens) to 26.7% at wk. 26 (total of 15 specimens). A week 4 sample with two values of 8,000 copies/ml on v.2.0 was neg. by DNA PCR, p24 antigen and Western Blot. Follow-up testing of this subject at wk. 26 was negative for HIV antibody and RNA. Discussion: The emotional impact of a false positive screening RNA test in a recently exposed person is significant. With the high false positive rate, we do not advocate the routine use of HIV RNA tests to screen asymptomatic people. The high rate of repeat false positive tests in a given sample (50%) suggests a possible biologic mechanism."

Roland ME et al. Pitfalls of HIV RNA testing in the San Francisco post-exposure prevention (PEP) project. Conf Retroviruses Opportunistic Infect. 1999 Jan 31-Feb 4;6(101):Abstract no. 179.

"The Centers for Disease Control and Prevention (CDC) states that the two tests used to identify HIV - the ELISA and the Western blot (WB) - used in combination, have a better than 99% accuracy rate, but only if they are performed repeatedly. (The exact rate is unknown and the CDC states that it has no data on just how many false positives versus false negatives occur!)…The CDC estimates that 0.6% of Americans are HIV-positive…Using the CDC estimate that 0.6% of Americans are HIV-positive, in a population of 10,000, 60 Americans would test positive! This 60 must include all the false positives, 30, leaving only 30 people actually infected. This leads to the following conclusion: using a 99% accuracy, one finds as many false positives as true positives. Even if the results of both AIDS tests, the ELISA and WB, are positive, the chances are only 50-50 that the individual is infected."

Stine GJ. Testing for Human Immunodeficiency. AIDS Update 1999. 1999;357-371.

"Results - Of 421 donors who were positive for HIV-1 by Western blot, 39 (9.3%) met the criteria of possible false positivity because they lacked reactivity to p31. Of these, 20 (51.3%) were proven

by PCR not to be infected with HIV-1. The false-positive prevalence was 4.8% of Western blot-positive donors and 0.0004% (1 in 251,000) of all donors (95% confidence interval, 1 in 173,000 to 1 in 379,000)...A review of the 5.02 million donations in the 1991-1995 REDS donation database revealed that 4650 were anti-HIV EIA repeat-reactive and 421 were HIV-1 Western blot positive (0.008% of all donations, 9.0% of EIA repeat-reactives) using the 1993 FDA interpretive criteria. Thirty-nine (9.3%) of the Western blots with positive results lacked the p31 band."

> Kleinman S et al. False-positive HIV-1 test results in a low-risk screening setting of voluntary blood donation. JAMA. 1998 Sep 23/30;280(12):1080-5.

"WB was considered diagnostic for HIV-1 if there was reactivity with two of three envelope bands (gp160/120 and gp 41). The rate of HIV-1 false-positive ELISAs was…63.6%…among uninfected leprosy patients…Of the cohort of 500 pregnant women, HIV-positive results were obtained by Abbott ELISA in…5.6%, Organon ELISA in…5.4%, and on both tests for…5.2%…WB were indeterminate in…83.6%…of 55 leprosy patients and...3.9%...of HIV-negative pregnant women."

> Kashala O et al. Infection with human immunodeficiency virus type 1 (HIV-1) and human T cell lymphotropic viruses among leprosy patients and contacts: correlation between HIV-1 cross-reactivity and antibodies to lipoarabinomannan. JID. 1994 Feb;169:296-304.

"This gives a false-positive rate [on ELISA] of about 4% [manufacturer quotes 0.58%]"

> Challakeree K, Rapaport MH. False positive HIV-1 ELISA results in low risk subjects. Western Journal of Medicine. 1993 Aug;159(2):214-5.

"In 1990, of 20.2 million HIV tests done in Russia only 112 were confirmed and about 20,000 were false positives, 1991 saw some 30,000 false positives out of 29.4 million tests, with only 66 confirmations."

> Voevodin A. HIV screening in Russia. Lancet. 1992;339:1548.

"HIV-1 p24 is the HIV-1 protein most prone to "false-positive" reactions…false-positive reactions have been observed with every single HIV-1 protein"

> Ng V. Serological diagnosis with recombinant peptides/proteins. Clin Chem. 1991;37(10):1667-8.

"144 dog sera were tested on Chiron Western blot strips. Of these, 72 sera (50%) reacted with one or more HIV recombinant proteins"

> Strandstrom HV et al. Studies with canine sera that contain antibodies which recognize human immunodeficiency virus structural proteins. Cancer Res. 1990 Sep 1;50(17 Suppl):5628-5630.

"we found crossreacting antibodies...to HIV-1 in patients with multiple sclerosis. Among 150 healthy Finnish persons, 1 (a woman) had antibodies to p24 and p55 of HIV-1. Some patients with multiple sclerosis, cutaneous T-cell lymphoma, or dermatologic disorders had antibodies that also reacted with the viral proteins of an HIV-2 isolate"

> Ranki A, Johansson E, Krohn K. Interpretation of antibodies reacting solely with human retroviral core proteins. NEJM. 1988;318:448-9.

"Three chimpanzees were inoculated with AIDS plasma [i.e. plasma from someone with AIDS, not purified virus] and one was inoculated only with normal human plasma. Blood was sampled biweekly from each animal and serm was tested in the standard indirect ELISA for HTLV-III [HIV] antibodies...all four of the animals were positive for passively transferred HTLV-III"

Saxinger WC, Gallo RC. Competitive ELISA for the detection of HTLV-III antibodies. US Patent Office. 1987 Apr 28;4,661,445.

"These results, supported by previous reports of false seropositivity in asymptomatic blood donors, emphasize the need to be certain of viral antigen specificity when screening for HIV antibodies. We suggest that blood banks use both HIV-infected and noninfected cell lines when confirming seropositivity by the Western Blot test and that the presence of bands on such tests not be automatically considered to indicate positive status"

Roy S et al. Need for caution in interpretation of Western blot tests for HIV. JAMA. 1987;257:1047.

"we tested 151,667 blood units by ELISA. 130 (8.6%) were confirmed positive by WB (in that they reacted with different HIV proteins, including gp41 and/or gp 110). 8 of these were false positive for gp18 and 23 were false positive for p25 [now known as p24]; in other words there was 1 false positive by WB for about 4 true positives in our population of blood donors and under our work conditions"

CouroucŽ A-M et al. False-positive Western blot tests reactions to human immunodeficiency virus in blood donors. Lancet. 1986 Oct 18;2:921-2.

"Reactivity with [HIV proteins] p24 and/or gp41 has been suggested as a minimum requirement for HIV seropositivity by WB [Western Blot]. While testing ELISA positive serum from Swedish blood donors we detected 3 sera with false-positive WB reactions to p24 and p55...The 3...had no risk factors for HIV infection"

Biberfeld G et al. Blood donor sera with false-positive western blot reactions to human immunodeficiency virus. Lancet. 1986 Aug 2;2:289-90.

"The frequences of false-positive reactions in a tricky panel of samples from patients with autoimmune and acute viral diseases...were Abbott 9.5%...Organon 1.7%...Litton 1.0%...Behring 2.7%...Wellcome 0%...and Pasteur 0%...The results of a 7th EIA (Dupont) were excluded from the study at the company's request."

Reesink HW et al. Evaluation of six enzyme immunoassays for antibody against human immunodeficiency virus. Lancet. 1986 Aug 30;2:483-6.

"Initial testing...revealed that [a 34-year old woman from rural Alabama] was positive for HTLV-III [HIV] antibody by ELISA tests on two separate occasions. Here serum was then sent for verification to the designated commercial laboratory, where three repeat ELISAs were strongly positive...as was a Western blot assay...In July 1985, the patient was informed that her serum was positive for HTLV-III antibody...Her physical examination was normal. Both she and her husband of 14 years denied any homosexual or extramarital sexual encounters, intravenous drug abuse, blood transfusions, or foreign travel. The patients T4:T8 [immune cell] ratio was 2.1:1, with a normal lymphocyte count. Her husband and their two-year-old son were both antibody negative by ELISA. More blood was drawn from the patient...Western blot, radioimmunprecipitation, and HTLV-III virus isolation studies were all negative. HTLV-III ELISAs were repeated in two laboratories, and results from both were positive...Western blot tests with positive bands at 24 and 41 kd [which this woman had, plus two others] have been used as the 'gold standard' by which other test results are judged to be falsely positive. Several articles refer to the inevitability of false positive Western blots."

Saag MS, Britz J. Asymptomatic blood donor with false-positive HTLV-III western blot. NEJM. 1986 Jan 9;314(2):118.

“we recently sent identical proficiency panels to five large commercial firms that offered HTLV-III [HIV] western blot testing...Four of the five laboratories reported at least one false-positive test result. The six false-positive results were all on different normal specimens...In the absence of a 41-kd band, a blot must show both a 24-kd and a 55-kd band to be deemed positive [by the US army]”

> Burke DS et al. False-positive Western blot tests for antibodies to HTLV-III. JAMA. 1986;256:347.

“68% to 89% of all repeatedly reactive ELISA tests are likely to represent false positive results...each year we might expected to find 175 to 209 truly antibody-positive donors [in Minnesota] and between 371 and 1701 falsely positive donors among those who have repeatedly positive screening tests”

> Osterholm MT et al. Screening donated blood and plasma for HTLV-III antibody: facing more than one crisis?. NEJM. 1985;312:1185-8.

“[during validation tests for HIV ELISA antibody tests] any positive ELISA screening result among the blood donors could be assumed to represent Òfalse-positivesÓ [but, if this same person later had the same test outside the context of a validation study, it would be assumed to be a true positive!]”

> Weiss SH et al. Screening test for HTLV-III (AIDS agent) antibodies: specificity, sensitivity, and applications. JAMA. 1985;253(2):221-5.

“Specimens were studied from a mother and her child, both with suspected transient viremia. The mother had 2 and the infant 3 positive HIV-1 cultures, but subsequently both individuals became negative for HIV-1 by nPCR, standard virus [co-]cultures, CD8+-depleted virus [co-]cultures, and enzyme-linked immunsorbent assay. HIV-1 RNA and DNA were not detected in two lymph nodes taken from the mother 3 and 4 years after the last virus-positive [co-]culture. PCR amplifaction and DNA sequence of HIV-1 env sequences from the...culture supernatants were performed in separate laboratories to eliminate the possibility of cross-contamination. Phylogenetic analysis found that none of the five isolates were genetically linked. Although it is imporbable, these 5 virus isolates appear to have arisen from 5 separate incidents of specimen contamination or mislabeling. This case remains enigmatic, however, in that both the mother and infant had strong CD8+ cytotoxic lymphocyte proliferation to multiple HIV-1 antigens”

> Frenkel LM et al. Genetic Evaluation of Suspected Cases of Transient HIV-1 Infection of Infants. Science. 15 May 1998;280:1073-1077.

Negative HIV Tests with AIDS!

There are many cases of people with AIDS defining conditions, or at least AIDS-defining low CD4 cell counts, who are continuously negative for HIV antibodies. How can this be if HIV is the undisputed cause of AIDS? It probably is not another virus, because these people are geographically scattered. Could it be that HIV is not the cause of AIDS, but most people with AIDS produce similar antibodies as a consequence of their illness?

“The syndrome of idiopathic CD4+ T lymphocytopenia is defined by the Centers for Disease Control (1992) as cases which demonstrate depressed numbers (<300/cubic millimeter) and proportions (<20% of total T cells) on at least two consecutive occasions, with no laboratory evidence of HIV-1 or HIV-2 infection, and the absence of any defined primary or secondary immunodeficiency disease or therapy associated with depressed levels of CD4+ T lymphocytes...The patients have presented with a history of severe or recurrent infections with intracellular pathogens or virus-associated malignancies which, even before the description of

AIDS, were recognised as being highly suggestive of underlying deficiency of cell-mediated immunity. Indeed, it was this constellation of clinical features...that clearly identified the new clinical entity of AIDS"

> Bird AG. Non-HIV AIDS: nature and strategies for its management. Journal of Antimicrobial Chemotherapy. 1996;37(B):171-183.

"Recently, patients have been described with profound CD4+ T-lyphocytopenia [deficiency in CD4 immune cells] but without evident HIV infection...We studied 12 patients with CD4+ T-lymphocytopenia...(10 men and 2 women) [who] ranged in age from 30 to 69 years. Eight had risk factors for HIV infection. The clinical manifestations were heterogeneous: five patients had opportunistic infections, five had syndromes of unknown cause, and two had no symptoms. Two patients died from acute complications of their immunodeficiency."

> Ho DD et al. Idiopathic CD4+ T-Lymphocytopenia - Immunodeficiency without evidence of HIV infection. NEJM. 1993 Feb 11;328(6):380-5.

"We interviewed 31 of the 47 patients identified with [HIV-negative AIDS, involving low immune cell counts (lymphocytopenia) and no record of positive HIV tests] and 23 of their contacts...Nineteen persons had AIDS-defining illnesses (18 had opportunistic infections), 25 had conditions that were not AIDS-defining [except for their low CD4 cell counts] and 3 were asymptomatic...The investigation of contacts revealed no evidence of a new transmissible agent that causes lymphocytopenia."

> Smith DK, Neal JJ, Homsberg SD. Unexplained opportunistic infections and CD4+ T-lymphocytopenia without HIV infection. NEJM. 1993;328(6):373-9.

"One infant dying of histologically confirmed HIV encepalopathy was repeatedly seronegative"

> Kind C et al. Epidemiology of vertically transmitted HIV-1 infection in Switzerland: results of a nationwide prospective study. Eur J Pediatr. 1992;151:442-8.

"A 5-month-old white girl having persistent oral candidiasis was brought to medical attention because of acute respiratory distresss, pneumonia, and hypoxia that worsened despite supportive care and antibiotics...The diagnosis of AIDS was suspected, although ELISA and Western blot tests were both negative for HIV antibody...The mother was HIV antibody positive by ELISA and Western blot but belonged to no high-risk group and was asymptomatic except for chronic diarrhea. The father was HIV antibody negative...The patient...remains HIV negative [after 6 months]"

> Goetz DW et al. Pediatric acquired immunodeficiency syndrome with negative human immunodeficiency virus antibody response by enzyme-linked immunosorbent assay and Western blot. Pediatrics. 1988;81:356-9.

"We have seen an infant, born to seropositive parents, who was persistently seronegative for HIV antibody before the onset of severe immunodeficiency..The diagnosis of AIDS in this child rests on the opportunistic infections, decreased T4/T8 [immune cell] ratios, impaired T-cell immunity, loss of functional antibody, and seropositivity in the parents"

> Marshall GS et al. AIDS in a child without antibody to HIV. Lancet. 1987;1:446-7.

"Among the 88 patients with AIDS tested, the HTLV-III [HIV] ELISA indicated a positive result in 72 cases (82%)"

Weiss SH et al. Screening test for HTLV-III (AIDS agent) antibodies: specificity, sensitivity, and applications. JAMA. 1985;253(2):221-5.

HIV Tests in Children

HIV tests in children are particularly problematic, because they get many antibodies from their mothers, especially if they nurse. This has significant consequences, because children will usually be assumed to at risk, and denied the many benefits of breastfeeding, even though most will end up HIV-negative at some point anyway.

"[the definition of HIV-uninfected in babies was] two negative enzyme-linked immunosorbent assay results at 9 months or older among those either not breastfed or who had stopped breastfeeding more than 3 months before their last sample"

Coutsoudis A et al. Method of feeding and transmission of HIV-1 from mothers to children by 15 months of age: prospective cohort study from Durban, South Africa. AIDS. 2001 Feb 16;15(3):379-87.

"Although DNA and RNA PCR and cell culture can detect very low concentrations of HIV-1, these assays yield a positive result in only 20-40% of vertically- infected infants who are tested shortly after birth"

Dunn DT et al. Interventions to prevent vertical transmission of HIV-1: effect on viral detection rate in early infant samples. AIDS. 2000 Jul 7;14(10):1421-8.

"Infants were considered to be HIV-1 infected if they had detectable HIV RNA on two separate blood draws, or a reactive EIA [ELISA antibody test] and confirmed by Western blotfor HIV-1 antibody at 18 months of age. Infants who had a single positive plasma HIV-1 RNA test were considered to have probable infection. Where possible, HIV culture...was also performed to confirm the HIV-1 infection in the infants...At birth, one of 22 infants was HIV-1 infected. One infant, who tested negative, died the day after birth leaving 21 evaluable infants for subsequent testing. At 6 months of age four of 21 infants were HIV-1 infected (one in cohort 1 and three in cohort 2). One of the four HIV-infected infants was initially positive for plasma HIV RNA at birth, two were initially HIV RNA positive at 6 weeks of age, and one was initially HIV RNA positive at 6 months of age. Two of the infants with positive plasma HIV RNA tests (one positive at birth and the other positive at 6 weeks of age) also had a confirmatory positive HIV culture; one infant had a confirmatory HIV RNA test. One of these four infants had a single positive plasma HIV RNA test, but died before a confirmatory test and was therefore considered as probably HIV-1 infected."

Musoke P et al. A phase I/II study of the safety and pharmacokinetics of nevirapine in HIV-1-infected pregnant Ugandan women and their neonates (HIVNET 006). AIDS. 1999 Mar 11;13(4):479-86.

"At birth, 5 babies had a positive PCR...and 3 of them had detectable p24 antigenaemia...Among the 45 babies who were negative by PCR, 20 were tested by HIV culture and all were negative. By 4-9 weeks of age, 16 infants had a positive PCR; 13 with the three primer sets, and 3 with two primer sets. HIV culture could be done in 14 of these cases and was positive in 11, negative in 1, and indeterminate in 2...By the age of 5-9 months...34 children previously found negative and 10 the 16 found positive [were retested]. The results remained unchanged for PCR, and culture was positive in the 2 children who had indeterminate results at 4-9 weeks. In addition, p24 antigenaemia became positive in 1 child previously found infected...The negative results obtained at birth by PCR and culture indicate that the viral load in PBMC is not sufficient to be detected."

Krivine A et al. HIV replication during the first few weeks of life. Lancet. 1992;339:1187-9.

"[HIV co-culture] was positive in 32 of 41 (78%) [HIV-positive] children"

> Kind C et al. Epidemiology of vertically transmitted HIV-1 infection in Switzerland: results of a nationwide prospective study. Eur J Pediatr. 1992;151:442-8.

"Infants were defined as infected by a positive [i.e. p24 antigen present] HIV-1 [co-]culture or by increasing anti-p24 or gp41/gp120 antibody titers after delivery...Infants were defined as not infected if HIV-1 cultures were negative, a sequential decline or loss of antibody to both p24 and gp41/gp120 proteins was measured, and immunoglobulin levels and T lymphocyte subset counts were comparable to those of control infants [no word on whether some infants were indeterminate, or whether all were arbitrarily placed in one category or the other]"

> Hutto C et al. A hospital-based prospective study of perinatal infection with human immunodeficiency virus type 1. J Pediatr. 1991;118:347-53.

"The detection of conventional IgG antibodies to HIV-1 during the first year of life may result from the passive transfer of maternal antibodies...Our inability to detect these antibodies in 10 of the 11 infants who were without evidence of AIDS in the first year of life but who had positive cord-blood cultures highlights the difficulty of diagnosing perinatal HIV-1 infection. The absence of detectable antibodies in children with obvious clinical disease has also been noted in previous studies"

> Ryder RW et al. Perinatal Transmission of the Human Immunodeficiency Virus Type 1 to Infants of Seropositive Women in Zaire. NEJM. 1989 Jun 22;320(25):1637-42.

"30 of the infants born to seropositive mothers reverted from seropositive to seronegative. The median age of these 30 infants at seroreversion...was 9 months (range 1 to 16)"

> Rogers MF et al. Use of the polymerase chain reaction for early detection of the proviral sequences of human immunodeficiency virus in infants born to seropositive mothers. NEJM. 1989 Jun 22;320(25):1649-54.

"proviral [HIV] sequences [of DNA] were detected in 9 of the 10 infants tested whose illness met the CDC case definition for HIV infection, this definition includes infants with AIDS, positive culture, positive serum antigen [p24] test, or persistent antibody beyond 15 months of age. In addition, proviral sequences were detected in one infant with nonspecicif findings who was negative on culture and serum antigen testing and less than 15 months of age"

> Rogers MF et al. Use of the polymerase chain reaction for early detection of the proviral sequences of human immunodeficiency virus in infants born to seropositive mothers. NEJM. 1989 Jun 22;320(25):1649-54.

"HIV proviral sequences were detected in 1 or more serum specimens obtained during the postnatal period from all of the 6 infants tested who later had AIDS and from 4 of the 14 infants who had non-specific findings"

> Rogers MF et al. Use of the polymerase chain reaction for early detection of the proviral sequences of human immunodeficiency virus in infants born to seropositive mothers. NEJM. 1989 Jun 22;320(25):1649-54.

"In four of five infants who had signs or symptoms of HIV infection after becoming seronegative, we detected evidence of HIV infection with use of hybridization studies carried out after the genome was amplifieid with the polymerase-chain-reaction method. One infant who became seronegative was totally free of symptoms but carried the HIV genome"

Blanche S et al. A prospective study of infants born to women seropositive for human immunodeficiency virus type 1. NEJM. 1989 Jun 22;320(25):1643-8.

"Of 1954 women screened at delivery, 12% were seropositive for HIV-1...At labour none of the 109 seropositive mothers for whom follow up was possible had AIDS...during two years of follow up, 4% developed AIDS, 28% had AIDS related complex, 46% had generalized lymphadenopathy, and the remaining 25 had no symptoms...Of 61 children who became seronegative at 8 months, antibodies to HIV-1 reappeared in nine at 12 months. By comparison none of the nine children who became seronegative at 12 months showed a reappearance of antibodies. On the other hand, 18% of children who were seropositive up to 12 months became negative at 18 months and were free of symptoms after 24 months...of 13 neonates who were seronegative at birth, four converted to become seropositive at different ages...HIV-1 serology is unreliable among children under 18 months of life"

Hira SK et al. Perinatal transmission of HIV-1 in Zambia. BMJ. 1989;299:1250-.

Validation: Search for the Gold Standard

HIV tests should be validated against direct isolation of the virus from people that test positive, and lack of isolation in people who test negative. Instead of just comparing people with AIDS against healthy people, tests should also be validated on people with non-AIDS diseases that may cross-react (such as leprosy or malaria) and with other conditions that may generate a higher load than normal of antibodies (e.g. recent vaccination, pregnancy, auto-immune disease). Isolation should be validated by purification of HIV particles, followed by microscope verification of purity, and then by analysis of the constituent proteins and genetic material.

"Primary infection was defined as a confirmed positive virologic test result with either a negtaive HIV antibody assay result or an indeterminate Western blot. Because there is no virologic gold standard, we assumed that levels of plasma HIV RNA had a sensitivity of 100% for diagnosing primary infection [bonus marks for detecting the flaw in this logic].False-positive HIV RNA measurements were defined as those that were negative on repeated testing and those obtained in patients who did not undergo seroconversion [note the contradiction with the previous sentence]...Eight of 303 uninfected patients (2.6%) had false-positive results on HIV RNA testing"

Daar ES et al. Diagnosis of primary HIV-1 infection. Ann Int Med. 2001 Jan 2;134(1).

"At present there is no recognized standard for establishing the presence and absence of HIV-1 antibody in human blood."

Human Immunodeficiency Virus Type 1 HIVAB HIV-1 EIA. Abbott Laboratories. 1997 Jan.

"This study assumed that the investigated samples were from non-HIV infected individuals. While we were unable to unequivocally prove this, samples were obtained from normal blood donors who signed a declaration form stating that they had not engaged in any HIV-related risk behaviour. Because these individuals were healthy enough to present for blood donation, it is unlikely that their indeterminant WB reactivities could have resulted from the loss of anti-core antibodies, which has been associated with late-stage AIDS...the use of WB interpretive criteria, such as those proposed by the World Health Organization [would] allow individuals reactive to two glycoprotein bands to be considered anti-HIV-1-seropositive, and would inappropriately classify 11 out of the 13 samples described in this study as anti-HIV-1-seropositive by the DB [Diagnostic Biotechnology] WB"

Healey DS, Bolton WV. Apparent HIV-1 glyco-protein reactivity on Western Blot in uninfected blood donors. AIDS. 1993;7:655-8.

"Alloimmune mice...were shown to make antibodies against gp120 and p24 of human immunodeficiency virus (HIV), and mice of [two] autoimmune strains...made antibodies against gp120. This is surprising because the mice were not exposed to HIV. [i.e. HIV proteins are found in uninfected mice!!]"

> Kion TA, Hoffmann GW. Anti-HIV and anti-anti-MHC antibodies in alloimmune and autoimmune mice. Science. 1991 Sep 6;253:1138-40.

"Serologic assays identify persons with prior exposure to human immunodeficiency virus (HIV-1), they do not specifically determine current infection...The number of peripheral blood lymphocytes expressing viral RNA, as detected by in situ hybridization in an infected person is less than 1 in 10,000 cells...Defective provirus would be detected by the PCR technique provided the region targeted for amplification was preserved [i.e. antibody tests do not prove current infection, and viral load/PCR tests cannot distinguish between defective and infectious HIV!]"

> Ou CY et al. DNA amplification for direct detection of HIV-1 in DNA of peripheral blood mononuclear cells. Science. 1988 Jan 15;239(4837):295-7.

"for HIV infection, there is no independent, unequivocal way of identifying a group of individuals who are all assuredly infected or uninfected"

> Cleary PD et al. Compulsory premarital screening for the human immunodeficiency virus: Technical and public health considerations. JAMA. 1987;258:1757-62.

"The meaning of positive tests will depend on the joint [ELISA/WB] false positive rate. Because we lack a gold standard, we do not know what that rate is now. We cannot know what it will be in a large-scale screening program"

> Meyer KB, Pauker SG. Screening for HIV: can we afford the false positive rate?. NEJM. 1987;317(4):238-41.

"We evaluated the validity of the test by determining whether the test could distinguish known patients with AIDS from the normal population and from groups that might pose cross-reactivity problems [but not against actual detection of a virus]...Since the ELISA [antibody test] ratio [indicating the intensity of the antibody reaction] was less than 5.0 in approximately 99% of these controls, serum samples with ratios of 5.0 or greater were defined as positive for HTLV-III [HIV] antibodies"

> Weiss SH et al. Screening test for HTLV-III (AIDS agent) antibodies: specificity, sensitivity, and applications. JAMA. 1985;253(2):221-5.

Surrogate Markers

Surrogate Markers are lab measurements that substitute for real measures of health. The commonest surrogate markers used in HIV/AIDS research are CD4/CD8 cell counts/ratios and Viral Load. Decisions made on the basis of these lab counts should be interpreted with caution, particularly decisions that could prove damaging, such as starting antiretroviral medications in a healthy person.

"until controlled trials are able to prove the utility of an undetectable viral load as a surrogate marker for clinically relevant outcomes in heavily pre-treated patients, we bleieve that clinicians should show caution before striving for complete viral suppression at any cost"

> Deeks SG, Martin JN. Editorial Comment: Reassessing the goal of antiretroviral therapy in the heavily pre-treated HIV-infected patient. AIDS. 2001;15(1):117-9.

"Significant improvements in CD4 cell count and plasma HIV RNA in recipients of IL-2 relative to control patients were associated with a nonsignificant trend toward improved clinical outcome [normally statistically insignificant trends are ignored, but if you really, really want to believe that your therapy is working...]"

> Emery S et al. Pooled Analysis of 3 Randomized, Controlled Trials of Interleukin-2 Therapy in Adult Human Immunodeficiency Virus Type 1 Disease. JID. 2000 Aug;182(2):428-434.

"CD4 and CD8 T cell counts, and HIV-1 plasma v iremia were quaantitated before, during, and after episodes of STI [Sexually Transmitted Infections]. Increases in...viremia [viral load] and a decline in CD4+ T cell counts occurred during gonococcal cervicitis and returned to baseline after treatment...Similar changes were seen in women with pelvic inflammatory disease. Acute bacterial STI resulted in increased HIV-1 viremia"

> Anzala AO et al. Acute Sexually Transmitted Infections Increase Human Immunodeficiency Virus Type 1 Plasma Viremia, Increase Plasma Type 2 Cytokines, and Decrease CD4 Cell Counts. JID. 2000 Aug;182(2):459-466.

"The clinical state (if the person is without symptoms) is not a major detriment [to administering anti-HIV drugs]: it is the [viral load surrogate marker] numbers that appear to decide the therapeutic course. I take issue with that approach...These drugs can be toxic and can be directly detrimental to a natural immune response to HIV…. This effective antiviral immune response is characteristic of long-term survivors who…have not been on any therapy. …[T]he current antiviral therapies…do not bring about the results achieved by a natural host anti-HIV response. This immune response, observed in long-term survivors, maintains control of HIV replication without the need for antiviral treatment."

> Levy JA. Caution: should we be treating HIV infection early?. Lancet. 1998 Sep 19;352:982-3.

"surrogate end points have been misleading about the actual effects that treatments have on the health of patients...Surrogate end points are rarely, if ever, adequate substitutes for the definitive clinical outcome in phase 3 trials"

> Fleming TR, DeMets DL. Surrogate End Points in Clinical Trials: Are We Being Misled?. Ann Int Med. 1996 Oct 1;125(7):605-13.

"At present there is no convincing evidence that the current surrogate markers [including CD4, CD8, viral load measurements] can be reliably used to predict the clinical efficacy of new treatments."

> Peto T. Surrogate markers in HIV disease. Journal of Antimicrobial Chemotherapy. 1996 May;37 (Suppl. B):161-170.

CD4/CD8 Cell Counts

Low CD4 cell counts (or abnormal CD4/CD8 ratios) are considered to be an unambiguous sign of the progression of AIDS, yet science does not support this. There are people with AIDS with normal CD4 cell counts and healthy people with low CD4 cell counts. Over a large group of people, it may well be true that on average, low CD4 cell counts identify a group of people who are more likely to be in ill health, but this logic does not apply to all individuals. Furthermore, even if low CD4 cell counts were always associated with ill health, it would not necessarily follow that artificially raising these counts with toxic drugs would be beneficial.

CD4 cell counts are a type of Surrogate Marker, a lab measurement that substitutes for a real measure of health. Consequently, decisions made on the basis of CD4 cell counts should be interpreted with caution, particularly decisions that could prove damaging, such as starting antiretroviral medications.

"Two years before the virus that causes AIDS was discovered, physicians in the United States and Europe realized that the defining symptom of the mysterious new disease was the loss of a specific immune system cell population, called CD4 cells. Twenty years later scientists still don't know what kills the cells [but, gee, we all thought it was HIV!]. And its eventual discovery will be key to understanding AIDS - defined as a rise in the numbers of viruses in the bloodstream coupled with a fall in the CD4 cell count. [However]...it is not in HIV's interests to kill off its "home" [CD4] cell, most scientists believe a complex interaction is at play, causing the cells' deaths [translation: HIV doesn't kill CD4 cells and therefore cannot be the cause of AIDS]"

Garrett L. Immune cell deaths still a stubborn puzzle. Newsday. 2001 Jun 5;C08.

"Declining host selenium levels...inhibit CD4 T cell production, which permits opportunistic infectious pathogens to proliferate. These pathogens in turn lead to further depression of serum selenium levels and associated decline in CD4 T cell count"

Foster HD. AIDS and the Selenium-CD4 T Cell Tailspin, the geography of a pandemic. Townsend Letter. 2000 Dec;94-9.

"HIV-1 infects CD4+ T cells but direct infection and killing of these cells can only partly account for HIV-1-associated lymphocyte depletion. The actual number of productively infected cells is estimated to be relatively low, in the order of 5 X 10^7 to 5 X 10^8 CD4+ T cells whereas the human body contains an average of 2.5 X 10^11 CD4+ T cells. Direct infection of CD4+ T cells does not explain the loss of naive CD8+ T cells that parallels the decline in naive CD4+ T cells during asymptomatic HIV-1 infection.

More important in this respect may be the response of the immune system. HIV-1 activates the immune system persistently because of high and continuous virion production and possibly by viral gene products such as Nef."

Mette D et al. T cell depletion in HIV-1 infection: how CD4+ cells go out of stock. Nature Immunology. 2000 Oct;1:285-9.

"Previously published data on CD4 cell count changes during therapy interruptions have mainly consisted of reports on very small numbers, but there has been a tendency to observe a distinct fall in numbers. The relatively rapid early fall in CD4 cell count after interrupting therapy may correspond to the relatively rapid increase in CD4 cell count after initiating therapy, which has been ascribed to the redistribution of cells from the lymphoid tissue [i.e. CD4 cells may not be increased with antiretroviral therapy, merely shuffled around the body to places where they are easier to measure]"

Youle M et al. (title missing). AIDS. 2000 Aug 18;14:1717-1720.

"The data should also serve as a strong reminder that using the CD4+ cell count as a surrogate for HIV testing has a risk of markedly overestimating the diagnosis of HIV. Therefore, CD4+ cell counts should not be relied on for a presumptive diagnosis of HIV in lieu of consent for serologic testing. Finally, a larger study of CD4+ cell counts in critically ill individuals might better define the prognostic value of a low result."

Aldrich J et al. The Effect of Acute Severe Illness on CD4+ Lymphocyte Counts in Nonimmunocompromised Patients Ê. Arch Intern Med. 2000 Mar 13;160(5):715-6,

archinte.ama-assn.org/issues/v160n5/full/ilt0313-6.html#rc1r2.

"Several of the features of Leishmania infection are reminiscent of HIV infection. In both, there is a decrease of CD4 lymphocytes, the immune activation profile is similar, and a dominant TH2 profile is present...All helminthic infections are associated with strong chronic immune responses... [including] dcreased CD4 and CD4:CD8 ratios"

Bentwich et al. EDITORIAL REVIEW: Concurrent infections and HIV pathogenesis. AIDS. 2000;14:2071-81.

"Controversy continues regarding the effects of HAART on CD4 T-cell production...our understanding the mechanisms that lead to depletion of CD4 T cells remains incomplete. The observation that T-cell turnover in SIV-infected mangabeys appears to be normal, despite high viral loads, has reinforced the hypothesis that indirect mechanisms may be largely responsible for increases in T-cell turnover, yet there is little solid evidence on how HIV and SIV mediate this effect [translation: we don't have a clue how HIV kills CD4 cells]"

Johnson RP. The dynamics of T-Lymhyocte turnover in AIDS. AIDS. 2000;14(suppl 3):S3-9.

"Tenfold (1 log10) incremental increases in maternal HIV RNA were associated with a 1.9-fold increase (95% confidence interval [CI], 1.2-2.9) in transmission and a 2.1-fold increase (95% CI, 1.3-3.5) in infant mortality (P<.01). Maternal CD4 cell counts and demographic and medical characteristics were not significant predictors of transmission. However, maternal CD4 cell counts below the median (400/mm3) were significantly associated with infant mortality (P=.035, Fisher's exact test) [i.e. CD4 cell counts might predict ill-health, but don't seem to be particularly associated with HIV/AIDS]"

Katzenstein DA et al. Serum Level of Maternal Human Immunodeficiency Virus (HIV) RNA, Infant Mortality, and Vertical Transmission of HIV in Zimbabwe. JID. 1999 Jun;179(6):1382-7.

"breast-fed [healthy, HIV-negative] infants had fewer CD4 T cells and a great number of NK [natural killer] cells than the age-matched formula-fed infants...suggesting greater maturity in the development of the immune system of breast-fed infants"

Hawkes JS et al. The effect of breast feeding on lymphocyte subpopulations in healthy term infants at 6 months of age. Pediatr Res. 1999 May;45(5):648-51.

"This analysis confirms that the rate of removal of CD4+ T cells is indeed elevated and the half-life is indeed shortened in the HAART group [i.e. therapy might raise the level of CD4+ cell counts, but they are not the same as normal immune cells, and might not be doing any good]"

Hellerstein M et al. Directly measured kinetics of circulating T lymphocytes in normal and HIV-1 infected humans. Nat Med. 1999 Jan;5(1):83-9.

"During HIV infection, CD4+-cell numbers and CD4/CD8 ratios decline in the blood. This is usually attributed to direct viral killing or to other indirect mechanisms. However, current models generally assume that such changes in the blood are representative of changes in total CD4+-cell numbers throughout the body. This article discusses the importance of alterations in CD4+- and CD8+-cell migration in regulating blood lymphocyte levels and questions the extent of virus-mediated CD4+-cell destruction"

Rosenberg YJ, Anderson AO, Pabst R. HIV-induced decline in blood CD4/CD8 ratios: viral killing or altered lymphocyte trafficking?. Immunology Today. 1998;19:10-17.

"Although progression to AIDS has generally been believed to be related to exhaustion of the capacity for CD4+ T-cell renewal due to persistently enhanced CD4+ T-cell turnover, this view is now increasingly being challenged. This paper discusses these new experimental findings and proposes alternative explanations for CD4+ T-cell depletion in human HIV-1 infection."

> Wolthers KC et al. Rapid CD4+ T-cell turnover in HIV-1 infection: a paradigm revisited. Immunology Today. 1998;19:44-48.

"Margolick...and, independently, Adleman and Wofsy proposed the BH [Blind Homeostasis] hypothesis. They postulated that, normally, a constant number of T lymphocytes is maintained regardless of the CD4-toCD8 ratio...[and] would necessarily lead to a progressive depletion of the CD4 compartment in HIV infection if CD4 cells are preferentially destroyed by the virus...[based on the detailed analysis in this paper] we consider the original BH hypothesis and also its more elaborate version to be biologically rather implausible...If correct, the BH hypotheses, but not the alternative hypothesis, could account for CD4 cell depletion by HIV. However, as we have discussed, there is no compelling evidence in favor of BH, either inherent or HIV imposed...these considerations also suggest a need to reevaluate current concepts about HIV pathogenesis, including the concept that a systemic depletion of CD4 T cells is the hallmark of the disease."

> Grossman Z et al. Conservation of total T-cell counts during HIV infection: alternative hypotheses and implications. JAIDS. 1998;17(5):450-7.

"The summary of results from a 1993 state-of-the-art conference shows that the effect of treatment on the most popular surrogate, the CD4 cell count, did not accurately predict the effect of treatment on the clinical outcomes, that is, progression to AIDS or time to death"

> Fleming TR, DeMets DL. Surrogate End Points in Clinical Trials: Are We Being Misled?. Ann Int Med. 1996 Oct 1;125(7):605-13.

"The CDC definition of idiopathic CD4 lymphocytopenia [HIV-free AIDS] does not include any clinical parameters. [One group] comprises a series of individuals...who have CD4 counts which are low but in whom there is no clinical evidence suggestive of immunodeficiency...[Some of these are] individuals whose CD4 counts are below the lower end of the normal range and who have constitutionally low CD4 blood levels consistently over a period of time without ill effect...their low CD4 counts may have no prognostic significance"

> Bird AG. Non-HIV AIDS: nature and strategies for its management. Journal of Antimicrobial Chemotherapy. 1996;37(B):171-183.

"CONCLUSIONS: Acute illness alone, in the absence of HIV infection, can be associated with profound decreases of T-lymphocyte populations [e.g. CD4 cells]. This problem is unpredictable and does not correlate with severity of illness, predicted mortality rate, or actual mortality rate. No conclusions regarding HIV serostatus or survival can be made based on single measurements of T-cell concentrations in acutely ill hospitalized patients."

> Feeney C et al. T-lymphocyte subsets in acute illness. Crit Care Med. 1995 Oct;23(10):1680-5.

"The understanding of the pathogenesis of AIDS has probably suffered seriously from two major shortcomings. First, HIV has been wrongly...assumed to be cytopathic in vivo and second, for obvious practical reasons, only peripheral blood has been analyzed and therefore CD4+ T-cell counts in blood have captured too much attention. As stated above, there is good evidence that many host cells other than CD4+ T cells are infected by HIV, and there is no convincing evidence that HIV is cytopathic in vivo"

Zinkernagel R. Are HIV-specific CTL responses salutary or pathogenic?. Curr Opin Immunol. 1995;7:462-70.

"In a small percentage of persons infected with human immunodeficiency virus type 1 (HIV-1), there is no progression of disease and CD4+ T-cell counts remain stable for many years...We studied 15 subjects with long-term nonprogressive HIV infection and 18 subjects with progressive HIV disease. Nonprogressive infection was defined as seven or more years of documented HIV infection, with more than 600 CD4+ T cells per cubic millimeter, no antiretroviral therapy, and no HIV-related disease."

Pantaleo G et al. Studies in subjects with long-term nonprogressive Human Immunodeficiency Virus Infection. NEJM. 1995;332(4):209-16.

"The results of Concorde [a clinical trial of AZT] do not encourage the early use of zidovudine in symptom-free HIV-infected adults. They also call into question the uncritical use of CD4 cell counts as a surrogate endpoint for assessment of benefit from long-term antiretroviral therapy"

Concorde Coordinating Committee. Concorde: MRC/ANRS randomised double-blind controlled trial of immediate and deferred zidovudine in symptom-free HIV infection. Lancet. April 9, 1994;343:871-881.

"We found that among those with CD4 lymphocyte counts less than [200 per cubic millimeter], survival was highly dependent on clinical status [i.e. current health]. Those who were asymptomatic or were symptomatic but with no AIDS-defining clinical conditions had considerably better survival outcomes than those who had clinical AIDS, suggesting that while CD4 lymphocyte count is a reasonable predictor of duration of survival among homogenous clinical groups, the presence of a clinical AIDS-defining condition plays a major role [i.e. CD4 cell counts, by themselves, are not good predictors of survival in HIV-positive people]"

Vella S et al. Differential Survival of Patients With AIDS According to the 1987 and 1993 CDC Case Definitions. JAMA. 1994 Apr 20;271(15):1197-9.

"The median CD4 lymphocyte count did not differ in the 3 groups: 54 for the group receiving neither antiretroviral nor P. carinii pneumonia prophylaxis, 53 for the group receiving only antiretroviral therapy, and 52 for the combined treatment group. There were also no major differences in the median CD8 lymphocyte count of the 3 groups [indicates that CD4 cell counts are not always improved by treatment]"

Bacellar H et al. Incidence of clinical AIDS conditions in a cohort of homosexual men with CD4+ cell counts < 100/cubic mm. JID. 1994;170:1284-7.

"In this report, we present data on the prevalence of CD4+ T-lymphocytopenia [low CD4 cell counts in HIV-negative people] among healthy, volunteer, anti-HIV-1-negative blood donors...Five (0.25%) of 2030 index donations had a CD4+ count <300 cells per microliter or a CD4+ percentage <20%"

Busch MP et al. Screening of blood donors for idiopathic CD4+ T-lymphocytopenia. Transfusion. 1994;34(3):192.

"More than 15% of the HIV-1-seropositive individuals had plasma vitamin A levels less than 1.05 micromol/L, a level consistent with vitamin A deficiency. The HIV-1-seropositive individuals had lower mean plasma vitamin A levels than HIV-1-seronegative individuals ($p<.001$). Vitamin A deficiency was associated with lower CD4 levels among both seronegative individuals ($p<.05$) and seropositive individuals ($p<.05$). In the HIV-seropositive participants, vitamin A deficiency was

associated with increase [indicates that CD4 cell counts can be modified by things other than HIV]"

> Semba RD et al. Increased Mortality Associated with Vitamin A Deficiency During HIV 1 Infection. Arch Intern Med. 1993 Sep 27;153:2149-54.

"Some HIV-infected individuals have remained healthy for more than 15 years following seroconversion. Lower numbers of CD4+ peripheral blood lymphoctyes have generally been found to indicate the advancement of HIV disease... [but] The CD4+ cell counts vary from day to day and laboratory to laboratory, and similar levels do not necessarily reflect the same disease status in all patients. For example, very low CD4+ cell counts (less than 0.05x10**9/L (50/microL)) usually indicate advanced disease; however, some patients with these levels remain asymptomatic for extended periods of time while others succumb rapidly"

> National Institute of Allergy and Infectious Diseases State-of-the-Art Panel on Anti-Retroviral Therapy for Adult HIV-Infected Patients. Anti-retroviral therapy for adult HIV-infected patients. Recommendations from a state-of-the-art conference. JAMA. 1993;270:2583-9.

"CD4 cell counts and percentages were obtained from 1,246 HIV-seronegative subjects...Nine had at least one CD4 cell count of <300 cells/microliter or a CD4[:CD8 ratio] <20%...Four subjects had CD4 counts <300 cells/microliter or CD4 < 20% for two points in time, meeting the current surveillance definition for ICL [HIV-free AIDS]...We also examined the data for the 21 subjects who had one CD4 count between 300 and 500 cells/microliter and for whom there was at least one follow-up data collection. None of these subjects progressed to a CD4 cell count of <300 cells/microliter or a CD4<20% at any follow-up visit. Five of these 21 subjects later seroconverted for HIV [indicating that low CD4 cell counts preceded infection, and wasn't caused by HIV]"

> Des Jarlais DC et al. CD4 lymphocytopenia among injecting drug users in New York City. JAIDS. 1993;6:820-2.

"47 cases of AIDS were identified among the seropositive [IV drug using] subjects during the study period. The likelihood of developing AIDS was inversely related to the CD4 cell count at baseline...[but]...The principal finding was the slow rate of decline in CD4 lymphocyte counts in HIV-1 seropositive IVDUs over a 2.5 year period [and some cases of AIDS occurred at quite high CD4 cell counts]"

> Margolick JB et al. Changes in T-Lymphocyte subsets in intravenous drug users with HIV-1 infection. JAMA. 1992;267:1631.

"In the group [of 10 asymptomatic HIV-positive hemophiliacs] switched to the very high purity [Factor VIII] concentrate there was no significant change of the CD4 cell counts over the 96-week follow-up period, whereas in the group continued on the intermediate purity concentrate [also 10 asymptomatic HIV-positive hemophiliacs], a highly significant decline was detected ($p < .013$) [indicates that CD4 cell counts are affected by the purity of blood products]"

> Biasi R et al. The impact of a very high purity of factor VIII concentrate on the immune system of Human Immunodeficiency Virus-infected hemophiliacs: a randomized, prospective, two-year comparison with an intermedate purity concentrate. Blood. 1991;78(8):1919-22.

"some healthy seropositive individuals lose a large proportion of their CD4+ lymphocytes, yet do not develop symptoms...Two patients followed at our medical center have had only 5% of their normal CD4+ cell number for over a year without any new symptoms. Thus, predictions on the

development of opportunistic infections or cancers based solely on a decrease in CD4+ cells could be misleading."

> Levy JA. Mysteries of HIV: challenges for therapy and prevention. Nature. 1988 Jun 9;333:519-22.

"[A] real T-helper [CD4] lyphopenia is only consistent with and not diagnostic of AIDS [even though it is now used to diagnose AIDS]; other diseases and some treatment regimens also can express a T-helper lymphopenia [deficiency], such as hospitalized non-AIDS IV drug abusers"

> Layon J, Warzynski M, Idris A. Acquired immunodeficiency syndrome in the United States: a selective review. Critical Care Medicine. 1986;14(9):819-27.

"The 53 patients studied includ 15 homo- and 11 heterosexual men with chronic HBV [Hepatitis-B Virus] infection, as well as 11 homo- and 16 heterosexual men who were in apparent good health...Only 4 patients in this study had detectable anti-HIV [HIV antibodies] by ELISA...The mean [CD4/CD8] ratio was significantly lower in homosexual men, independent of HBV infection...Although 4 patients in this study had [HIV antibodies], the association of the abnormalities of T lymphocyte subsets [CD4/CD8] and NK [Natural Killer] activity with homosexuality remained significant after exclusion of these 4 patients from the data analysis [this paper does not consider the possibility that nitrite inhalants, almost exclusively marketed and used by homosexual men, could have affected the CD4/CD8 cell counts]"

> Novick DM et al. Influence of Sexual Preference and Chronic Hepatitis B Virus Infection on T Lymphocyte Subsets, Natural Killer Activity, and Suppressor Cell activity. J Heptaol. 1986;3:363-370.

"Between April and October, 1984, anti-HTLV-III [HIV antibodies] developed in 16 patients with hemophilia A...[of whom] all but one had received a common batch [of clotting] factor VIII...a further eighteen patients received the implicated batch A...[but] have been negative for [HIV antibodies]...Lymphocyte subsets [CD4/CD8 cells] were investigated in 24 of the patients...the patients in whom [HIV antibodies] later developed had lower [CD4/CD8] ratios than those who did not seroconvert and the controls. The difference...just failed to reach statistical significance. In 1983 the absolute [CD4] cell numbers in those who subsequently seroconverted were significantly lower than those in the controls...The 15 patients who seroconverted used significantly more vials of batch A and also had a higher annual factor VIII consumption than the eighteen patients who did not seroconvert...Our finding in this study that T-helper-cell numbers and the helper/suppressor ratio did not change after infection supports our previous conclusion that the abnormal T-lymphocyte subsets [CD4/CD8 cells] are a result of the intravenous infusion of factor VII concentrates per se, not [HIV] infection"

> Ludlam CA et al. Human T-Lymphotropic Virus Type-III (HTLV-III) Infection in Seronegative Hemophiliacs after Transfusion of Factor VIII. Lancet. 1985 Aug 3;2(8449):233-236.

"we tested six asymptomatic asthmatics who were sensitive to mixed grass (positive skin test) with mixed grass extract, methacholine, and an antigen to which they were not sensitized (negative skin test). Levels of OKT4 [CD4] (helper T lymphocytes) were reduced in the peripheral blood immediately after the challenge with mixed grass extract, and remained low for at least 72 hours [note that low CD4 cell counts are supposed to be unique to AIDS by many accounts]"

> Adi A et al. Change in T-cell Lymphocyte Subpopulations after Antigenic Bronchial Provocation in Asthmatics. NEJM. 1984 May;310(21):1349-52.

"Ten patients (6 males and 4 females)...were studied during the acute...or convalescent phases of CMV[Cytomegalovirus]-mononucleosis..Analysis of the T lymphocyte subsets..indicated both relative and absolute reductions in the T helper [CD4] subset and simultaneous increases in the T suppressor [CD8] population...Recent studies suggest that a delicate balance exists between helper [CD4] and suppressor [CD8] T lymphocytes in the maintenance of immune homeostatsis. Several disease states have been associated with alterations in this balance [not just HIV, even though an imbalance in CD4/CD8 cell counts is now considered, along with an HIV test, diagnostic for AIDS, at least in the USA]"

Carney WP et al. Analysis of T lymphocyte subsets in Cytomegalovirus mononucleosis. The Journal of Immunology. 1981;126(6):2114-6.

VIRUSMYTH HOMEPAGE