

I. INDETERMINATE HIV-1 WESTERN BLOTS WITH ONLY NON-VIRAL BANDS

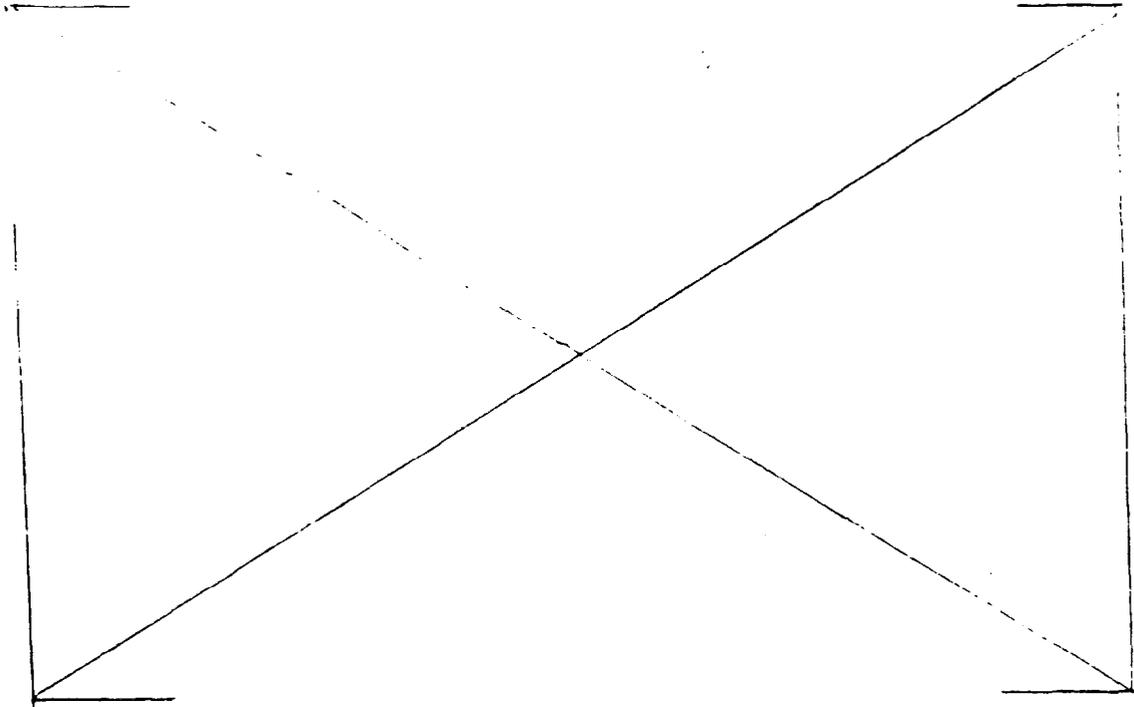
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Holiday Inn, Silver Spring
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Indeterminate HIV-1 Western Blots with Only Non-Viral Bands

Blood Products Advisory Committee

March 16, 2000



Background

In an MMWR of July 21, 1989 entitled, "Interpretation and Use of the Western Blot Assay for Serodiagnosis of Human Immunodeficiency Type 1 Infections," the Public Health Service recommended the use of the CDC/ASTPHLD criteria for blot interpretation which state that the presence of any band or bands that do not meet the criteria for a positive blot results in an indeterminate interpretation, and that for a sample to be reported as negative there must be no bands at all visible on the blot. This includes the absence of any non-viral bands that often appear on Western blots, usually as very narrow bands at defined molecular weights. In most cases, non-viral bands result from the binding of certain antibodies in the serum to contaminating cellular proteins that are by-products of the production of the whole viral lysate that is used to manufacture the Western blot strips. Currently, the package inserts for all four licensed Western blots state that the criterion for a negative blot is "no bands present" or "the absence of any band reactivity."

Non-viral bands on a Western blot are to some degree kit specific. For the Calypte HIV-1 Western Blot Kit, the non-viral bands most commonly seen are bands above gp120/160, p70, p7 and p5. For the Bio-Rad Novapath HIV-1 Immunoblot, the bands are a thin p110, a p90, and occasionally p70 or p40; for the Genetic Systems HIV-1 Western Blot, p42 is the most frequent non-viral band; for the _____

_____ In comparison, the virus specific bands on the HIV-1

Western blot are p17 and p24 (gag or core proteins), p31 (the endonuclease component of the polymerase translate), gp41 (transmembrane envelope glycoproteins), p51 and p65 (reverse transcriptase components of the polymerase gene translate), p55 (a precursor of gag or core proteins), gp120 (the outer envelope glycoprotein) and gp160 (a precursor of the envelope glycoprotein).

<u>Virus specific bands</u>	<u>Non-viral bands</u>
	>gp160
gp160	
gp120	
	p110
	p90
	p70
p65	
p55	
p51	
	gp45
	p42
gp41	p40
p31	
p24	
p17	
	p14
	p7
	p5

Impact of a Change in Policy

Currently, if a repeatedly reactive donation is Western blot indeterminate, due to the presence of non-viral bands or viral bands that do not meet the criteria for a positive blot interpretation, the donor is deferred indefinitely and is not eligible for reentry. The donor is counseled that there is a chance that they are infected with HIV, and that they should receive follow-up testing. If the Western blot pattern is stable for 6 months, they are reassured that they are almost certainly not infected with HIV-1, but will remain deferred indefinitely because of their test results. Since almost all HIV-infected persons with initial indeterminate Western blot results will develop detectable HIV antibody within 1 month, this 6-month time period may soon be shortened. A draft PHS guideline on HIV counseling, testing, and referral proposes to recommend that persons with an initial indeterminate Western blot result be retested for HIV infection at least 1 month after the first indeterminate result and that persons with continued indeterminate Western blot results after 1 month are highly unlikely to be infected and may be counseled as such.

It has been reported that approximately 15% of all indeterminate Western blots have non-viral bands only. Since indeterminates represent about 44.5% of all repeatedly reactive samples, approximately 6.7% of all repeatedly reactive samples are non-viral band only indeterminates. Out of 12 million donations nationwide per year, with a repeatedly reactive rate of approximately 0.09%, about 700 non-viral band only indeterminate donors per year are currently deferred indefinitely because of their indeterminate blot results. As for all other indeterminate blots, these donors are given a counseling message that there is a chance that they are infected with HIV but that they should get retested in about a month.

If Western blots that exhibit non-viral bands only were to be interpreted and reported as negative, the donors could be reentered using the current reentry algorithm if a subsequent sample is negative on the EIA and on a Western blot. It has been reported that due to persistent repeatedly reactive results on the EIA, less than 10 % of all donors for which reentry is attempted are actually reentered and eligible for future donation. Thus, the major benefit to interpreting the Western blots for these 700 or so donors per year as negative is that they would receive a counseling message that says they are not infected with HIV rather than donor reentry in a small number of cases.

In June 1996, FDA presented to the Blood Products Advisory Committee a modified algorithm to reenter donors who have an indeterminate HIV-1 Western blot, whether due to viral or non-viral bands. This algorithm was based on data that showed that the vast majority of indeterminate patterns do persist, and represent uninfected individuals. By the new HIV reentry algorithm, which was endorsed unanimously by the Committee but has yet to be recommended by FDA due to continued absence of an EIA approved for sensitive detection of HIV-1 group O, donors with indeterminate blots, whether due to viral or non-viral bands, eventually could be reentered if their subsequent sample and then donation are EIA negative, without re-running a Western blot.

Safety Considerations

It should be pointed out that in the event of a repeatedly reactive EIA screening test, regardless of the Western blot result, the current donation is discarded so there would be no danger to a recipient that would result from any change in this blot interpretation policy

At their meeting last March, APHL (the Association of Public Health Laboratories) recommended that "An interpretation of negative should mean no viral bands. Non-viral bands (e.g., p70) should not be required to be reported [because] since 1991 no individual exhibiting non-viral banding has been associated with either early seroconversion, detection of different HIV-1 subtypes, or other disease agents."

In this session we will hear statements that reflect the widely held belief that such a change to the policy of interpreting non-viral band only Western blots does not represent a threat to the safety of the blood supply. In this session, we will see that early seroconverters exhibit specific viral band patterns such as a weak p24 band or a weak

gp120/gp160 band, or both, and that these patterns are readily identifiable without confusion due to non-viral bands.

We will also hear concerns that there is a danger of non-viral band only blots being misinterpreted by small volume testing laboratories whose personnel may not be proficient in interpreting Western blot patterns. Specifically, concern exists that a viral band such as a p65 may be misread as a non-viral p70, or an uncharacteristically thin gp41 viral band may be misread as a non-viral p40 band, and as a result the blot misinterpreted as negative. It should be pointed out that the occurrence of blots that exhibit these bands only, without any other bands present, is reportedly extremely rare. This concern regarding possible misinterpretation of blots could be alleviated by focusing on effective training and proficiency testing of new or inexperienced Western blot users. In the interim, the recent introduction of Nucleic Acid Testing (NAT) provides an added layer of safety in the event the donor is a seroconverter with an indeterminate Western blot. Currently, the industry estimates that 99% of all blood donations in the U.S. are being screened by NAT for HIV-1 RNA using minipool testing of serum from those donations. Small pool sizes such as 16 and 24 unit pools are being used, so NAT testing has the high sensitivity to provide added assurance that a donation from a seroconverter will be interdicted.

An additional concern has been raised that an HIV-1 Western blot from an individual infected with HIV-2 that shows viral HIV-2 bands could be misinterpreted as negative. However, blots from an individual infected with HIV-2 usually show both gag and pol bands, and would at least be interpreted as indeterminate on an HIV-1 blot.

And so, in considering the question of whether to permit HIV-1 Western blots with only non-viral bands to be interpreted as negative, we are faced with a scientific argument that must be weighed against a potential public health concern. The scientific argument is that individuals with non-viral band only Western blots are not infected with HIV. The public health concern is that indeterminate blots with viral bands may be misinterpreted as negative by inexperienced Western blot users.

A trained individual can readily distinguish a non-viral banding pattern and interpret the blot as negative. However, with the possibility of less experienced individuals misinterpreting the blot, the question is, is it better public health practice to take the conservative approach and perform the follow-up testing or to notify the donor that the test was negative ?

A middle ground approach also is possible whereby the counseling message could be stratified based on the band pattern. That is, different counseling messages that reflect the likelihood of infection could be provided to donors with indeterminate blots with viral bands present and to donors with indeterminate blots with viral bands absent.

QUESTIONS FOR THE COMMITTEE:

1. Should FDA permit indeterminate blots with only non-viral bands to be interpreted as negative?
2. If not, should blot interpretations such as “Indeterminate (Viral Bands Present)” and “Indeterminate (Viral Bands Absent)” be reported with distinct counseling messages?
3. Does the Committee see the need for additional studies?

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*Recommendations
and
Reports*

MORBIDITY AND MORTALITY WEEKLY REPORT

**Interpretation and Use of the
Western Blot Assay
for Serodiagnosis of
Human Immunodeficiency
Virus Type 1 Infections**

**U.S. Department of Health and Human Services
Public Health Service
Centers for Disease Control
Center for Infectious Diseases
AIDS Program
Atlanta, Georgia 30333**

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Interpretation and Use of the Western Blot Assay for Serodiagnosis of Human Immunodeficiency Virus Type 1 Infections

Reported by

Association of State and Territorial Public Health
Laboratory Directors and AIDS Program,
Center for Infectious Diseases,
Public Health Practice Program Office,
Centers for Disease Control*

The Association of State and Territorial Public Health Laboratory Directors (ASTPHLD) and CDC have collaborated in preparing this report. It includes a description of various interpretive criteria associated with the Western blot test for HIV-1, evaluates the sensitivity and specificity of these criteria as tools for public health practice, and provides recommendations for use of the Western blot and the manner in which to report results in order to provide clinicians and public health policy officials with useful information in their efforts to reach an accurate diagnosis for persons tested for HIV-1 infection.

INTRODUCTION

The development of sensitive and specific tests for antibody to human immunodeficiency virus type 1 (HIV-1) progressed rapidly after this retrovirus was identified as the cause of acquired immunodeficiency syndrome (AIDS). These tests have been used for various purposes, including clinical diagnosis of HIV-1 infection—for symptomatic and asymptomatic patients in counseling and testing programs—for seroprevalence surveys, and for blood-donor screening.

Enzyme immunoassay (EIA) is the most widely used serologic test for detecting antibody to HIV-1. Serum samples that are repeatedly reactive in the EIA for HIV-1 antibody are then retested with a supplemental and more specific test, the most common of which is the Western blot (1-3). To date, only one commercial Western blot test (Du Pont®) has been licensed by the Food and Drug Administration (FDA). The purpose of this report is to provide guidance for interpreting Western blot test results and their use in diagnosing HIV-1 infection.

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THE WESTERN BLOT ASSAY

The Western blot assay is a method in which individual proteins of an HIV-1 lysate are separated according to size by polyacrylamide gel electrophoresis. The viral proteins are then transferred onto nitrocellulose paper and reacted with the patient's serum. Any HIV antibody from the patient's serum is detected by an antihuman immunoglobulin G (IgG) antibody conjugated with an enzyme that in the presence of substrate will produce a colored band. Positive and negative control serum specimens are run simultaneously to allow identification of viral proteins.

Table 1 lists the major structural proteins coded for by the HIV genome. Antibodies to the HIV-1 major group-specific antigen (GAG) protein p24, and its precursor p55, are the earliest detected after infection by Western blot and tend to decrease or become undetectable with onset or progression of clinical symptoms (4-9). In contrast, antibodies to the envelope (ENV) precursor protein gp160 and the final ENV proteins (gp120 and gp41) can be detected in specimens from virtually all HIV-infected persons regardless of clinical stage (4-9). Antibodies to the polymerase (POL) gene products (p31, p51, and p66) are also commonly detected if these antigens are present on the Western blot strips. However, in a recent study, the protein with a mobility of 160 kilodaltons (kd) present in commercially available Western blots and in viral lysate antigen preparations was identified as a multimer of the gp41 protein (10,11). Furthermore, this study presented evidence that the reaction observed against the gp120 on certain Western blots may have resulted in part from a reaction with a multimeric form of the gp41. In fact, the true gp120 was shown to be absent from some commercial Western blot antigens. When these reagents were used, serum specimens with only gp41 antibodies produced bands at the 41-, 120-, and 160-kd positions.

Interpretive Criteria

Although the overall sensitivity and specificity of the Western blot for detection of antibodies to the various viral proteins are high, there has been substantial debate regarding the interpretive criteria. The currently licensed Du Pont Western blot test specifies that the test result should be interpreted as positive only when the detected bands include p24 and p31, and gp41 or gp120/160 (12) (see Table 2). Conversely, a negative Du Pont Western blot test result requires the absence of any and all bands—not just viral-bands. All other patterns are regarded as indeterminate. This interpretation scheme maximizes the specificity of the assay and is mainly intended for use with samples from persons, such as blood donors, for whom there is usually little clinical or virologic information available. (Donated units of blood that are

TABLE 1. Major genes and gene products of HIV-1

Gene	Gene products*
Group-specific antigen/core (GAG)	p18, p24, p55
Polymerase (POL)	p31, p51, p66
Envelope (ENV)	gp41, gp120, gp160

*p = protein; gp = glycoprotein. Numbers indicate the approximate molecular weights of the antigens in kilodaltons.

repeatedly reactive by EIA are discarded; Western blot results are used to guide donor notification and deferral.) These criteria are not ideal for all situations, especially the testing of persons at increased risk for HIV infection, or with symptoms suggestive of this infection.

Alternative criteria have been proposed by various groups. ASTPHLD has proposed that a positive test result be defined by the presence of any two of the following bands: p24, gp41, and gp120/160 (13). The Consortium for Retrovirus Serology Standardization (CRSS) has defined a positive test result as the presence of either p24 or p31, plus a diffuse envelope band (i.e., gp41 or gp120/160) (14). The American Red Cross has defined a positive test result as ≥ 1 band from each of the GAG, POL, and ENV gene-product groups (15). These three groups and DuPont all agree that an indeterminate result is the presence of any other band or bands that fail to meet the positive criteria, and that a negative result is the absence of all bands.

The criteria for a negative Western blot interpretation specify "no bands." This interpretation is essential because some observed bands may reflect the presence of antibodies to HIV regulatory proteins or may indicate partially processed or degraded viral structural proteins. Furthermore, different Western blots (commercial, as well as "in-house" preparations) and different virus-antigen preparations used to prepare Western blots may contain different numbers and concentrations of both viral-specific and contaminating cellular proteins that may have unpredictable molecular weights.

Evaluation of Criteria

To compare the four sets of criteria for Western blot interpretation, CDC selected 424 serum samples on the basis of the patients' clinical status and EIA results only, and analyzed them using the licensed Du Pont Western blot test (CDC unpublished data). The samples were scored according to each of the criteria (Table 3). For all three categories with repeatedly reactive EIA test results, the Western blot results demonstrate that the ASTPHLD definition gives the highest percentage of positive and the lowest percentage of indeterminate results. The interpretive standards that require

TABLE 2. Criteria for positive interpretation of Western blot tests

Organization	Criteria
Association of State and Territorial Public Health Laboratory Directors/CDC	Any two of: <ul style="list-style-type: none"> • p24 • gp41 • gp120/gp160*
FDA-licensed Du Pont test	p24 and p31 and gp41 or gp120/gp160
American Red Cross	≥ 3 bands—1 from each gene-product group: <ul style="list-style-type: none"> • GAG and • POL and • ENV
Consortium for Retrovirus Serology Standardization	≥ 2 bands: p24 or p31, plus <ul style="list-style-type: none"> • gp41 or • gp120/gp160

*Distinguishing the gp120 band from the gp160 band is often very difficult. These two glycoproteins can be considered as one reactant for purposes of interpreting Western blot test results.

the identification of bands from each of the three groups of gene products tend to have indeterminate results for some AIDS and other symptomatic patients due to absence of antibodies to p24 (n=5) or to p31 (n=14) or absence of both types of antibodies (n=2). Since these patients clearly are infected with HIV, the three-gene-product approach to Western blot interpretation is not sensitive enough for public health or clinical practice.

The ASTPHLD/CDC criteria for a positive Western blot differ from the CRSS criteria in two ways: first, ASTPHLD/CDC deletes p31, a change that does not affect the sensitivity or specificity of the criteria (Table 3), and second, ASTPHLD/CDC adds "gp41 and gp120/160," a combination not interpreted as positive with the CRSS criteria. This latter combination of bands represents antibody to envelope glycoproteins only. In practice, this is a rare finding for asymptotically infected persons, but it has been reported to be specific for HIV-infected persons and should be included in the positive criteria (9). However, when a Western blot test has only the multimeric form of gp41 and no true gp120 present, a serum sample would be scored as positive on the basis of the presence of antibody to a single envelope glycoprotein, gp41. HIV-1-infected persons with this profile have lost their antibodies to the GAG proteins and are usually symptomatic and do not present a diagnostic problem.

The ASTPHLD/CDC interpretive criteria for a negative result are identical to the FDA recommendation for blood-donor reentry or the Western blot interpretive criteria that are specified in the licensed Western blot kit package insert.

RECOMMENDATIONS

On the basis of the results described above, CDC concurred with the ASTPHLD criteria and recommends their use in public health and clinical practice.

Laboratories should report test results as positive, indeterminate, or negative. The Public Health Service recommends that no positive test results be given to clients/patients until a screening test has been repeatedly reactive (i.e., \geq two tests) on the

TABLE 3. Western blot results of 424 serum samples by four interpretive standards

Clinical status	EIA results	ASTPHLD/CDC (%)			CRSS (%)			Du Pont (%)			Red Cross (%)		
		P*	I*	N*	P	I	N	P	I	N	P	I	N
Group A (n=86)	Repeatedly reactive	83(97)	3(3)	—	76(88)	10(12)	—	62(72)	24(28)	—	74(86)	12(14)	—
Group B (n=40)	Repeatedly reactive	39(98)	1(2)	—	38(95)	2(5)	—	34(85)	6(15)	—	36(90)	4(10)	—
Group C (n=58)	Repeatedly reactive	45(78)	13(22)	—	43(74)	15(26)	—	39(67)	19(33)	—	41(71)	17(29)	—
Group D (n=240)	Nonreactive	0	7(3)	233(97)	0	7(3)	233(97)	0	7(3)	233(97)	0	7(3)	233(97)

*P = positive, I = indeterminate, N = negative.

Group A = AIDS patients.

Group B = Other symptomatic patients.

Group C = Asymptomatic homosexual men.

Group D = Volunteer blood donors.

same specimen and a supplemental, more specific test such as the Western blot has been used to validate those results (3). Upon request, laboratory reports may also contain a list of the bands detected and reference to the interpretive criteria the laboratory uses. Because of the variability of unlicensed reagents, laboratories using non-FDA-licensed Western blots should compare, on a routine basis, their tests with the FDA-licensed Western blot kit using well-characterized serum specimens.

Clinical diagnosis and follow-up of patients is the responsibility of the clinical practitioner. Serologic test results are but one contribution to a patient's data base, which contains medical history (including high-risk behavior or exposure to HIV), results of physical examination, and other clinical findings. Clinicians must consider the total profile for a client when attempting to make a diagnosis after indeterminate Western blot results have been obtained. Accurate diagnosis for such persons can be challenging—and the challenge can be complicated by the tendency of some clients to become distressed by the apparent "uncertainty" of their test results.

Clinical follow-up of patients with indeterminate Western blot results may require many months of observation, interviewing, and testing. Most indeterminate patterns involve p18 (also referred to as p17), p24, or p55, or any combination of these three proteins (16-18). In one study of 390 "atypical" or indeterminate samples, 53% reacted against p24, with or without p18 or p55; 47% reacted against p18 (but not p24), with or without p55 (18).

Some indeterminate results may be obtained with serum samples from persons who are in the process of seroconverting. A compilation of 209 volunteer blood donors with GAG-only indeterminate Western blot results were followed for as long as 2 years (17-21). During that time, only five of 134 persons who had initially reacted to p24 developed additional bands on the Western blot test. None of the 75 persons who initially reacted against p18 (but not p24) developed additional bands. The five persons who did seroconvert had positive results when their first follow-up samples were tested. The intervals between initial and follow-up tests were 8 weeks (two persons), 20 weeks (two persons), and 32 weeks (one person). The three longest intervals reflected delays in follow-up testing and not the actual time to seroconversion. These results do not refute earlier findings that seroconversion typically occurs within 3 months of infection (5,22). The importance of careful risk assessment for persons with indeterminate Western blot patterns was reemphasized when in one study (18) two of three people who initially had indeterminate results (but later seroconverted) disclosed histories of risk behavior when they were reinterviewed during follow-up.

A person whose Western blot test results continue to be consistently indeterminate for at least 6 months—in the absence of any known risk factors, clinical symptoms, or other findings—may be considered to be negative for antibodies to HIV-1. Such persons should be reassured that they are almost certainly not infected with HIV-1. However, no large-scale studies have been done to provide virologic data to confirm independently the serologic findings from the studies of clients whose Western blot test results are consistently indeterminate. In contrast, an asymptomatic person who has an indeterminate Western blot test result and a history of possible exposure to or symptoms compatible with HIV infection requires additional diagnostic follow-up. This should include conducting serial Western blot testing, assessing the function of the individual's immune system, and eliciting the cooperation of the person's sexual and needle-sharing partners to determine whether they are infected.

Individuals with a pattern of indeterminate Western blot test results should not donate blood or plasma for either transfusion or use in manufactured blood products.

As the HIV/AIDS epidemic continues, additional tests of higher specificity will be needed to decrease the number of false-positive reactions and to permit correct diagnosis of HIV infection in a larger spectrum of clinical situations in which an indeterminate antibody profile exists. The use of new antibody tests based on antigens derived by recombinant deoxyribonucleic acid (DNA) technology or the application of DNA probe technology—particularly DNA amplification by the polymerase chain reaction (PCR)—already shows promise in this area (23).

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Frequency of human immunodeficiency virus (HIV) infection
among contemporary anti-HIV-1 and anti-HIV-1/2
supplemental test-indeterminate blood donors

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C.-P. PAU, G.B. SCHREIBER, AND THE RETROVIRUS EPIDEMIOLOGY DONOR STUDY

HIV-1: Absence of infection in subjects with indeterminate Western Blot

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Long-term follow-up of blood donors with indeterminate human immunodeficiency virus type 1 results on Western blot

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