

False-Positive and Indeterminate Human Immunodeficiency Virus Test Results in Pregnant Women

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Guidelines from the Centers for Disease Control and Prevention, Atlanta, Ga, recommend that all pregnant women be offered human immunodeficiency virus (HIV) testing to ensure that they have the opportunity to use currently available therapeutic interventions to reduce the risk infecting their offspring with HIV. These recommendations have resulted in an increased number of low-risk women being tested and a significant rise in the percentage of false-positive results from HIV antibody screening tests and ambiguous (indeterminate) findings from confirmatory tests. Women receiving such results are generally in emotional turmoil yet must make treatment choices if they prove to be infected. This article provides guidelines to help general medical practitioners to understand the nature of HIV testing, to assess a woman's infection status when initial tests are ambiguous, and to determine when treatment is appropriate.

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The Centers for Disease Control and Prevention (CDC), Atlanta, Ga, have recommended that all pregnant women be screened for human immunodeficiency virus (HIV).^{1,2} In response, physicians have increased their efforts to screen patients during the prenatal period. As the number of women being screened has increased, the proportion of false-positive and ambiguous (indeterminate) test results has increased and the positive predictive value (PPV) of the standard HIV test has decreased. Physicians need to be alert to such problems when testing pregnant women for HIV and know how to verify unclear test results to avoid misinforming patients about their HIV status. This article provides a protocol for verifying test results that are positive for HIV and recommends counseling strategies.

Overall, 91% of acquired immunodeficiency syndrome (AIDS) cases in US children have resulted from vertical trans-

mission.³ Since the mid 1990s, there has been a notable decrease in both the rate of mother-to-child HIV transmission and in the number of children with AIDS. The AIDS Clinical Trial Group 076 study demonstrated that use of the antiretroviral medication zidovudine (ZDV, sometimes referred to as AZT) can reduce the risk of vertical HIV transmission by nearly two thirds, from 25% to 8%.⁴⁻⁷ Recently, other interventions have been shown to decrease the risk of vertical transmission, including abbreviated ZDV regimens, the use of the antiretroviral medication nevirapine in the perinatal period, and elective cesarean delivery.⁷⁻¹² Interventions that prevent transmission of HIV infection to children have prompted recommendations to test women early in pregnancy and offer such interventions to those who are infected. The US Public Health Service, Washington, DC, recommends that health care providers counsel and encourage all pregnant women to undergo testing for HIV.¹ Furthermore, such testing is to be voluntary for women and should ideally

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be done at the first prenatal care visit. Physicians need to emphasize the importance of HIV counseling for pregnant women, understand the methods used for testing pregnant women for HIV, be familiar with treatment strategies to prevent the vertical transmission of HIV, and be cognizant of the pitfalls of HIV testing.

As a consequence of the increased effort for prenatal screening for HIV, more women at low risk for infection are being tested. Since pregnancy itself can on occasion cause a false-positive HIV test result, the PPV of HIV testing will be reduced as more low-risk women are screened. In most cases, this type of false-positive result will yield a positive enzyme-linked immunosorbent assay (ELISA) and an indeterminate Western blot (WB) confirmatory test. Sometimes these results are misinterpreted as evidence of infection, when often the person is not infected. This article addresses issues related to such ambiguous or indeterminate HIV test results occurring in pregnant women and provides a protocol for discriminating between false- and true-positive tests. This will assist clinicians in counseling patients about HIV test results.

HIV SEROLOGIC TESTING

The standard procedure for HIV testing includes a screening test, the ELISA, or enzyme immunoassay. The ELISA tests for antibodies produced against one or more HIV protein. Most laboratories use commercially available ELISA kits. The result from the ELISA is a color reaction, the density of which is based on the degree of antibody-antigen reaction. A colorimeter measures the strength of the color. The test results are reported as positive (reactive) or negative (nonreactive) depending on whether the result is above or below a standardized color intensity used as a cutoff value. The

Table 1. Minimal Criteria for a Positive Human Immunodeficiency Virus Western Blot Test Result by Certifying Organizations

Organization	Western Blot Test Result Criteria	Minimum No. of Bands
American Red Cross, Washington, DC	At least 1 band from each gene product group: gag, pol, and env*	3
Association of State and Territorial Public Health Laboratory Directors/Centers for Disease Control and Prevention, Atlanta, Ga	Any 2 of the following: p24, † gp41, † or gp120/160	2
Consortium for Retrovirus Serology Standardization, Davis, Calif	p24 or p31 and either gp41 or gp120/160	2
World Health Organization, Geneva, Switzerland	2 env bands ± gag or pol	2

*gag (viral core antigens p55, p24, and p17), pol (viral reverse transcriptase 66/51 and p31), and env (viral envelope antigens gp160, gp120, and gp41) are virally encoded classes of proteins that may form bands on Western blot tests in the presence of a specific antibody.

†The designations p (protein) and gp (glycoprotein) are combined with the molecular size in kilodaltons.

ELISA tests for HIV have improved in sensitivity and specificity after several generations in the development of materials and techniques. A 1995 analysis of 6 available ELISA test kits showed that all were 100% sensitive with specificities of 94.6% to 100%.¹³ Numerous articles cite sensitivities exceeding 98%.¹³⁻¹⁹

A strongly reactive ELISA result is more likely to indicate that the person is infected (true-positive result) than a weakly reactive result.^{16,17} The ELISA screening test is inexpensive and is designed with a low threshold so that few infected individuals will be missed. However, like other screening tests, it will also capture some uninfected individuals. This is especially a problem when testing is expanded from a high-risk population to a large low-risk population.¹⁸ The PPV of the ELISA test has been reported to be as low as 2% for a weakly reactive ELISA result in a low-risk population and as high as 99% for a strongly positive ELISA result in a high-risk population.¹⁴ Expanding the test population to include more low-risk people can be expected to result in a proportionate increase in false-positive test results and to decrease the PPV. False-positive ELISA test results can be caused by alloantibodies resulting from transfusions, transplantation, or

pregnancy,^{13,19,20} autoimmune disorders,^{13,15,20} malignancies,^{15,19} alcoholic liver disease,^{15,19,21} or for reasons that are unclear.

A positive ELISA screening test result must be followed by a more specific confirmatory antibody test. Ideally, a confirmatory test will separate the infected from the uninfected individuals. The most common confirmatory method for HIV is a WB test. Other confirmatory methods such as immunofluorescence assay²² or radioimmunoprecipitation assay²³ are occasionally used. Several WB assay kits are commercially available. Viral proteins (antigens) and patient's antibodies combine to form visible "bands" in the WB test. The discrete bands are interpreted using one or more sets of published guidelines given in **Table 1**.^{24,25} Using these guidelines, laboratories will typically report that the WB results are positive, negative, or indeterminate. Typically, a serum sample that is positive by one set of guidelines will be positive by the other guidelines as well; however, there may be exceptions.^{24,25}

The WB is not used as a screening tool because it is costly when used in this manner and because when used for screening, it yields an unacceptably high percentage of inde-

Table 2. Human Immunodeficiency Virus (HIV) Proteins That Are Available to Form Antigen-Antibody Bands on HIV Western Blot Tests

Band*	Gene	Characteristics
gp160†	env	An outer membrane glycoprotein (envelope). Binds with CD4 receptor. A complex of gp120 and gp41.
gp120	env	The outermost portion of the gp160 outer membrane glycoprotein.
p66	pol	A form of the reverse transcriptase enzyme.
p55	gag	A structural protein comprised of the final p17 and p24 structural protein products.
p51	pol	Another form of the reverse transcriptase enzyme.
gp41	env	The portion of the outer membrane gp160 glycoprotein that spans the viral membrane and anchors gp120 to the membrane.
p31	pol	The integrase enzyme.
p24	gag	Structural core protein.
p17	gag	Structural core protein.

*There are numerous other viral proteins that are nonantigenic or too small to appear on the blot.

†The number represents the molecular weight of the protein in kilodaltons. p indicates protein; gp, glycoprotein.

terminate results (10%-49%).^{15,18,25,26} When used as a confirmatory test following a positive result from ELISA, the WB is quite effective at identifying infected individuals from high-risk populations. A positive WB test result must meet at least the minimum criteria, typically 3 or more bands.^{24,25,27} However, it is possible for 2 bands to be interpreted as positive by some criteria. A negative WB test result is one in which no bands are present. An indeterminate test result will have 1 or more bands present, but fewer than needed to meet the assay criteria for a positive test result, or it will have bands that do not align with those in the positive control.^{24,25,27,28}

In the early weeks or months following the onset of HIV infection, the WB test may be indeterminate owing to the initial appearance of antibodies since different viral proteins appear at different times. Some women with indeterminate test results have been infected and have transmitted HIV to their infants.²⁹ Also, in late-stage AIDS, a patient may have a failing immune system with impaired antibody production. The WB may revert to indeterminate or even negative results.^{14,30,31} Such late-stage cases are not likely to present a serious problem for the physician attempting to sort out an ambigu-

ous pregnancy-related test, especially since late-stage patients are typically symptomatic.

Ideally, the laboratory will list the number and identities of bands present (**Table 2**). It is possible for serum to be positive by one set of criteria and indeterminate by another.^{24,25,27} Additionally, a person who is not infected may on occasion have an HIV test result that meets positive criteria for both ELISA and WB tests. In these uncommon instances, the WB result will usually show 2 bands, the weakest criteria for a positive test result. In rare cases, an uninfected person may even have more than 2 bands present.²⁸ The WB criteria (Table 1) used to define a positive test result were originally designed for use with high-risk individuals to capture early-stage infections in individuals with few antibodies present.^{19,27} For lower-risk populations, these tests can be problematic. A woman who receives indeterminate WB test results needs guidance on how test ambiguity will be resolved and recommendations regarding treatment in the interim.

HOW TO PROCEED WITH AN AMBIGUOUS TEST RESULT

The physician should wait for both the ELISA and WB test results be-

fore the diagnosis of infection is made. An algorithm is provided to help physicians evaluate positive, negative, or indeterminate WB test results in pregnant women (**Figure**

). When confronted with an indeterminate WB test result, the gestational stage is an important starting point. In the first and second trimesters, there is less urgency to begin treatment than in the third trimester. At this stage of pregnancy, a polymerase chain reaction (PCR) test for viral nucleic acid sequences should be performed using the PCR-DNA method. Some laboratories may offer only viral load tests such as the PCR-RNA method, which is typically used to follow the course of HIV disease by measuring the amount of virus produced in an infected person. It is reasonable to use this method as a diagnostic tool as well, but at present, the PCR-DNA is considered the method of choice.

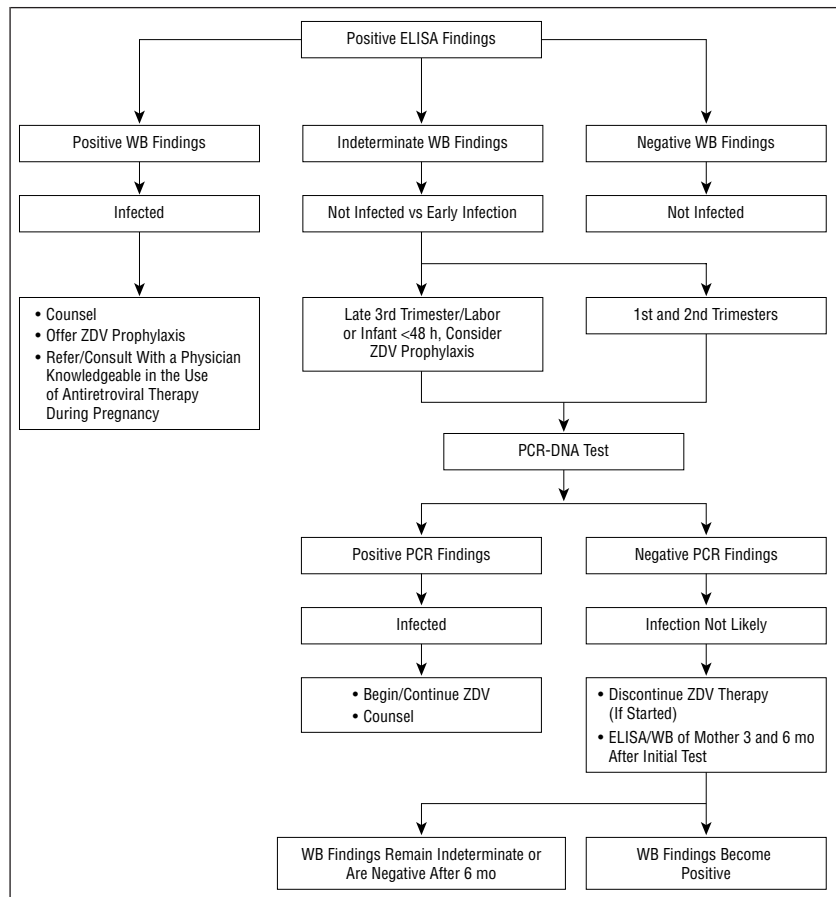
A positive PCR test result confirms infection, and appropriate counseling and therapy, including the ZDV protocol, should be offered. A negative PCR test result indicates that infection is highly unlikely, and ZDV treatment does not need to be initiated. After an initial negative PCR result, performing the ELISA and WB tests at 3-month intervals will resolve any lingering doubt about the patient's HIV status. Most patients who are infected will have clearly positive WB results by 3 months after the initial indeterminate test result.²⁷ If the WB remains indeterminate after 6 months, the patient is not infected and no further testing is necessary.^{27,28} Often such test results revert to negative within a few weeks or months after delivery.

In the third trimester, rapid decisions regarding treatment are necessary. The physician should review risk factors for HIV, gauge the estimated time until delivery, and determine the laboratory turnaround time for a PCR test. The am-

biguity of the ELISA and WB test results needs to be explained to the patient along with the steps describing how it will be resolved. It may not be possible to resolve the issue before delivery, and the patient's preferences for ZDV treatment must be explored. Such a patient, especially one with known risks for HIV, including a history of injection drug use or a sexual partner with HIV, should have ZDV recommended, pending clarification of her infection status. If she chooses to receive ZDV and give it to her infant, it should be discontinued if the PCR test results are negative. A woman with no evident risks may choose not to receive ZDV. Coercion should be avoided and her choice regarding therapy respected.

Occasionally, a woman with no prenatal care or who had an initial negative test result during prenatal care will have a positive ELISA result at the time of labor or shortly after delivery. The physician is then faced with the difficulty of making treatment decisions while waiting for the WB test result, which may take days. Once ZDV therapy is initiated, the need for continued therapy is guided by the WB result. By 48 hours after delivery, any potential benefit of ZDV for the infant is lost, and ZDV is not recommended. A positive WB test result suggests infection, and the physician should proceed with medical care similar to what would be done for other infected women. A subsequent PCR-DNA test can clarify the infection status of the mother.

Rapid testing for HIV is possible, but the available tests do not resolve the issue of indeterminate results for pregnant women. There are 3 Federal Drug Administration-licensed HIV test kits currently available.³² The accuracy is approximately equivalent to the traditional ELISA test (99.9% sensitivity and 99.6% specificity). Results are typically available in about 10 minutes. A negative test result effectively rules



Algorithm for clinical decisions related to positive, negative, and indeterminate findings from human immunodeficiency virus (HIV) Western blot (WB) tests following positive findings from HIV enzyme-linked immunosorbent assay (ELISA). Two to 3 bands constitute a minimally positive WB test result; findings are interpreted with caution since they can be false-positive results. Individuals infected with HIV are unlikely to have positive findings from ELISA and negative findings from the WB test. A polymerase chain reaction (PCR) test is generally unnecessary in such cases but can be considered if there is considerable patient anxiety. The break point between the second and third trimester is artificial and is used for simplicity, meant to distinguish less urgent (early pregnancy) from more urgent (late pregnancy, labor, and delivery) situations. ZDV indicates zidovudine.

out HIV except in the case of recent infection in which antibodies have not yet developed. A positive HIV rapid test result still requires a confirmatory test such as the WB, which typically will take several days. For rapid testing to be useful, there is also a requirement for in-house laboratory staff available 24 hours per day with the skills to perform such a test. Hospitals in small and mid-sized towns, rural areas, and smaller hospitals in urban areas may have few laboratory personnel, and HIV tests are often sent to outside laboratories. Rapid screening tests might be useful for select situations in making a preliminary diagnosis of HIV positive or negative re-

sults. Until rapid confirmatory tests are also developed and become widely available, currently available rapid tests will not alleviate the problem of false-positive or indeterminate test results.

RECOMMENDATIONS AND COUNSELING

Findings from HIV tests should always be given in person. Under no circumstances should a patient be informed that she is infected unless both the ELISA and WB test results are positive. When the WB is indeterminate, the ambiguity of the test result should be discussed along with methods to clarify the result and pos-

sible treatment options. The patient should understand from pretest counseling that occasional false-positive and indeterminate test results can occur in pregnancy. It is helpful to review potential HIV risks, but many people are unaware of exposures such as previous sexual partners at high risk, or they deny or underestimate risks. These risks include injection drug use, having a sexual partner who injects drugs or is HIV positive, or a history of multiple sexual partners or sexually transmitted disease. It is common for HIV-infected women to have no risk other than an infected partner whose risk was unknown to her. The presence or absence of risk factors in the medical history may help sway a decision about treatment but does not obviate the need for further testing.

As noted previously, when an ELISA is performed near the time of delivery and the results are positive, it may not be possible to get the WB result back in a timely manner. Treatment decisions may need to be made on the basis of incomplete information. During counseling it should be explained that the test is incomplete. Risk factors should be reviewed, and the woman and her infant should be offered the option of ZDV treatment while awaiting the WB result, recognizing the need to start no later than 48 hours after delivery to receive any benefit.⁸ If the WB result proves to be positive, ZDV therapy is continued. If the result is indeterminate, the woman may opt to continue treatment for herself or her infant depending on personal preference and risk factors. The PCR-DNA testing is useful to confirm infection in these situations. We do not recommend testing the infant until the mother's infection status is known. It will not change initial treatment, and testing the infant adds both expense and possibly unnecessary venipuncture.

A woman's perception of her own risk, her perception of harm vs

benefit of ZDV for the infant, and personal fears all enter into the decision. She should be strongly encouraged but not coerced into the use of ZDV and should be reassured that if she elects to not take medication that her infant will continue to receive care. The decision to use medications in pregnancy is a personal one in which the patient should be made aware of potential risks, including anemia with ZDV, as well as benefits. She should know that birth defects have not been documented when ZDV is used in pregnancy.

Since cesarean delivery has also been shown to decrease risk,¹¹ particularly when coupled with the use of ZDV, this option should be discussed as well. It is important for the physician and patient to recognize that cesarean delivery only is of benefit as a planned procedure performed prior to labor. An emergency procedure offers no benefit. While it is somewhat difficult to encourage a surgical procedure when the HIV test results remain unresolved, each patient should be apprised of risks and benefits in light of an uncertain test result.

Breastfeeding increases the risk for HIV transmission to an infant by 14% when the mother is infected prior to delivery and by 29% if she becomes infected during the postpartum period.³³ With the option of formula feeding available to women in developed countries, it is reasonable to discourage breastfeeding, though again this is a highly personal decision. One possible option is to pump the breasts and store or discard the milk pending a clarification of HIV status.

Testing for HIV is an emotional experience. An HIV diagnosis may lead to depression, fear, anger, and suicidal ideation.³⁴ Family, friends, and community may ostracize infected people, and relationships with spouses or partners may be jeopardized. An indeterminate result can cause the same problems if

the physician misinterprets the result as being indicative of infection. Posttest counseling should always be performed, regardless of the result, but is especially important in this event. Even correctly interpreted and explained, an indeterminate test result is emotionally laden and frightening because of the uncertainty it creates for the woman and her family. A physician should be sensitive to these issues and take time to explain the steps to be taken in clarifying the diagnosis, perhaps also explaining how best to communicate the situation to family and partners.

Testing for HIV for most adults consists of pretest counseling, a screening ELISA test, a confirmatory WB test if the screening test result is positive, and posttest counseling. Issues become somewhat more urgent when dealing with a pregnant woman since it is an emotional time, and interventions to reduce risk to the infant need to be addressed. With the increased effort to screen pregnant women for HIV, more low-risk women will be tested. Indeterminate WB results will inevitably increase. We have presented an algorithm for evaluating indeterminate results.

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