

HIV Testing: 2014 U.S. Recommendations

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Incidence of false positive enzyme-linked immunosorbent assay (ELISA)/enzyme immunoassay (EIA) and indeterminate western blots are expected to rise as the United States Preventive Services Task Force (USPSTF) expands its human immunodeficiency virus HIV testing recommendations to include low-risk populations. This paper explores current HIV testing recommendations, HIV testing options, causes of false positive or indeterminate results, and how clinicians should proceed when confronted with potential false positive or indeterminate results. We report two cases in which positive ELISA/EIA and indeterminate western blots required further evaluation and prove to be false positives. The USPSTF recommends aggressive HIV testing to include screening for all persons 15 to 65 years of age, and younger or older persons who are at increased risk. They also recommend screening all pregnant women, including those who are untested, who present in labor. Indeterminate western blot tests should be followed up with nucleic acid testing (NAT) or polymerase chain reaction (PCR) tests, and repeated ELISA/EIA and western blot assays at three and six months. Fourth generation tests offer an alternative and utilize HIV-1/HIV-2 antibody differentiation immunoassays, in place of western blot assays

CASE 1

A 18-year-old male with no significant past medical history presented to a Florida academic family medicine clinic after donating blood, which required further evaluation. He was a resident of Florida and had not traveled outside the country. He denied intravenous drug use or high-risk sexual behavior, and had not received any blood products. The patient followed up at a public health clinic, which found he had a positive, reactive HIV ELISA and negative western blot. Repeat testing continued to show a reactive ELISA and negative western blot. At the time of the repeat testing, a HIV PCR ribonucleic acid (RNA) assay was performed and the patient was found to have an undetectable viral load. The patient was then referred to an infectious disease specialist, who confirmed his positive serology most likely indicated cross reactivity.

CASE 2

A 32-year-old female with no significant past medical history was seen for routine obstetric care at a Florida general hospital during her second pregnancy. During initial routine screening laboratory studies, the female was found to have a non-reactive HIV ELISA. When the patient was re-tested during her third trimester, in compliance with protocol, her ELISA was found to be repeatedly reactive. A reflex western blot was performed but was indeterminate. She stated that since the time of the original HIV ELISA she had only been sexually active with her husband, who tested negative for HIV. Additionally, both individuals denied a history of intravenous drug use or recent travel outside the country. The patient was then referred to an infectious disease specialist, where tests continued to show repeatedly reactive ELISA and indeterminate repeat western blot with 1 reactive band with a p24 antigen and then 2 indeterminate bands, p40 and p51. The viral load was undetectable.

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INTRODUCTION

As can be demonstrated by the cases described above, false positive and indeterminate human immunodeficiency virus (HIV) tests occur. False positive enzyme-linked immunosorbent assay (ELISA)/enzyme immunoassay (EIA) and indeterminate western blot occurrences are expected to rise as the United States Preventive Services Task Force (USPSTF) expands its HIV testing recommendations to include low-risk populations. This paper explores current HIV testing recommendations, HIV testing options, causes of false positive or indeterminate results, and how clinicians should proceed when confronted with potential false positive or indeterminate results.

COMMENT

In April 2013 the USPSTF made two, grade A recommendations:

- I. That “clinicians screen for HIV infection in adolescents and adults ages 15 to 65 years. Younger adolescents and older adults who are at increased risk should also be screened.”
- II. That “clinicians screen all pregnant women for HIV; including those who present in labor who are untested and whose HIV status is unknown.”¹

Currently, there are no specific screening intervals recommend by the USPSTF. The USPSTF recommends diagnosing HIV by conventional third generation serum ELISA/EIA tests demonstrating repeated reactivity, followed by confirmation by western blot or immunofluorescent assay. This conventional testing takes approximately 1 to 2 days. Rapid HIV testing, which provides results in approximately 5 to 40 minutes may also be used, but still requires conventional testing confirmation. Alternatively, fourth generation combination (p24 antigen/HIV antibody) and qualitative HIV-1 RNA tests are approved by the U.S. Food and Drug Administration for screening and diagnosing.² The USPSTF’s expansion of the recommended cohort to be tested will necessarily result in increased false positives. For this reason, it is imperative that practitioners have a working knowledge of the specific HIV testing options, causes of false positives, and how to proceed when an indeterminate test is encountered.

ELISA/EIA is the conventional screening test because it is relatively inexpensive and has a reported sensitivity of 99.3-99.7% and specificity of 99.7%.³ This low threshold produces few false negatives, however, with expanding routine testing to include a low-risk population the number of false positives will increase.⁴ In some studies, the positive predictive value (PPV) of the ELISA test may be as low as 2% in weakly reactive tests in a low-risk population to as high as 99% in strongly reactive tests in a high-risk population.⁵

Common causes of false-positives in ELISA/EIA screening for HIV are recent influenza vaccination, other viral infections, autoimmune disease, renal failure, cystic fibrosis, multiple pregnancies, blood transfusions, liver diseases, parenteral substance abuse, hemodialysis, vaccination against hepatitis B, vaccination for rabies, or experimental HIV vaccinations.^{6,7,8} (see Table 1) For this reason, a repeatedly positive ELISA/EIA test requires confirmatory testing.

Table 1: Common Causes of False-Positives in ELISA/EIA screening for HIV

| |
|---------------------------------|
| Recent influenza vaccination |
| Other viral infections |
| Autoimmune disease |
| Renal failure |
| Cystic fibrosis |
| Multiple pregnancies |
| Blood transfusions |
| Liver diseases |
| Parenteral substance abuse |
| Hemodialysis |
| Vaccination against hepatitis B |
| Vaccination to Rabies |
| Experimental HIV vaccinations |

The western blot is the most commonly used U.S. confirmatory test, and is recommended by the USPSTF to evaluate repeatedly positive ELISA/EIA.^{1,8} However, immunofluorescence or radioimmunoprecipitation are occasionally used.^{9,10} Due to cost and the unacceptable number of indeterminate results (10-49%) the western blot is only used as a confirmatory test.⁸ The Western blot detects the specific antigen which produced the antibody response detected in the ELISA/EIA.^{10,17} Several different criteria have been proposed for the interpretation of western blots by the Centers for Disease Control and Prevention (CDC), the Association of State and Territorial Public Health Laboratory Directors (ASTPHLD), the World Health Organization (WHO), and others. According to both the CDC and ASTPHLD a positive western blot requires reactivity to HIV antigens gp120/160 plus either gp41 or p24.¹¹ The western blot is considered negative when no bands are present. The results are considered indeterminate when 1 or more bands are present, but the requirements for a positive western blot are not met.^{12,13,14,15}

After the onset of the HIV infection, it takes approximately three weeks for a sufficient immune response to build up a detectable antibody titer via third generation immunoassays. This time period is referred to as the “seroconversion window.” p24 is one of the first antibodies to develop during the window period; therefore an isolated p24 band is often

seen on indeterminate western blots early in the course of HIV.¹⁶ However, in addition to early seroconversion there are numerous other causes of indeterminate HIV-1 western blots including: HIV-2, advanced AIDS, antibodies that are cross-reactive such as HTLV infection, influenza vaccination, hepatitis, rabies, history of frequent transfusions, injection drug use, liver disease, multiple pregnancies, rheumatoid factor, lymphoma, multiple sclerosis, positive rapid plasma reagent tests, chronic hemodialysis, and various autoimmune disorders.¹⁶ (see Table 2)

Table 2: Common Causes of Indeterminate HIV-1 Western Blots

| |
|------------------------------------|
| HIV-2 infection |
| Advanced AIDS |
| HTLV infection |
| Recent influenza vaccination |
| Hepatitis infection or vaccination |
| Rabies infection or vaccination |
| Blood transfusions |
| IV drug use |
| Liver diseases |
| Multiple pregnancies |
| Autoimmune disease |
| Lymphoma |
| Multiple Sclerosis |
| Positive Rapid Plasma Reagent test |
| Hemodialysis |

Indeterminate western blots should be followed up by nucleic acid testing (NAT) or polymerase chain reaction (PCR) test for viral nucleic acid sequences using the PCR-DNA method. While the PCR-DNA is the method of choice, many laboratories offer only viral load or PCR-RNA methods.^{8,17} If the follow up PCR test is positive then the patient is considered positive for HIV. If the PCR test is negative, then the patient should be retested with conventional serology testing at three monthly intervals, for a total of six months. If the western blot remains indeterminate after six months, the patient is confirmed negative.^{14,15} (see Chart 1) The CDC and the Association of Public Health Laboratories (APHL) have proposed an alternative to the third generation algorithm described above.¹⁸ In this algorithm; a HIV-1/HIV-2 EIA 4th generation screen is used initially. If it is non-reactive, then the patient is reported as negative. If it is repeatedly reactive, then a HIV-1/HIV-2 antibody differentiation immunoassay is performed. If the HIV-1 is reactive it is reported as HIV-1 positive, if the HIV-2 is reactive it is reported as HIV-2 positive, if HIV-1 and HIV-2 are both non-reactive or indeterminate, then further testing with HIV-1 RNA NAT is done. If RNA is detected then it is reported as positive; if it is not detected then it is reported as negative.¹⁸ (see Chart 2)

Chart 1: Indeterminate 3rd Generation Algorithm

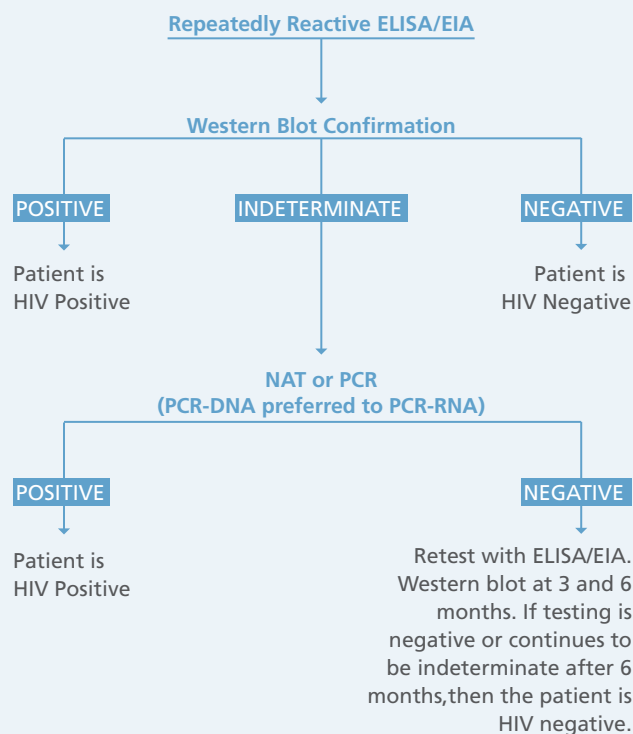
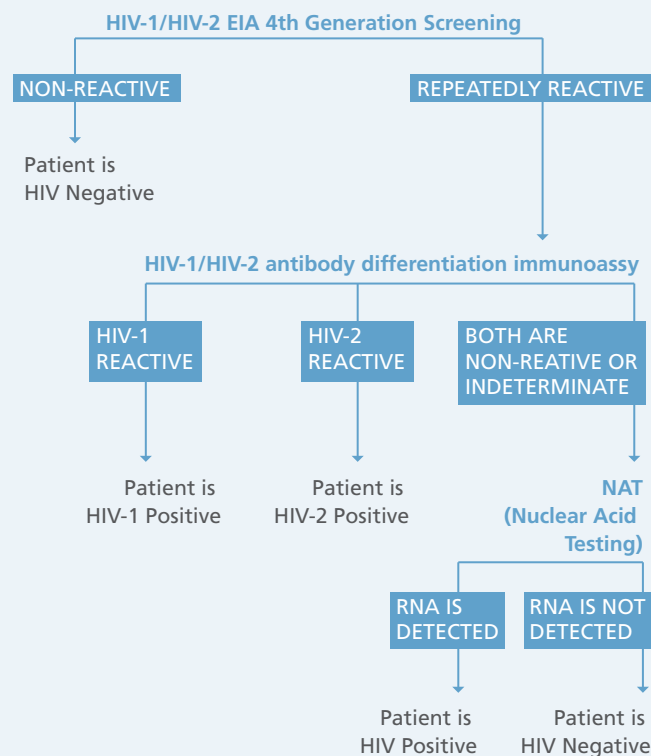


Chart 2: Indeterminate 4th Generation Algorithm



CONCLUSION

The USPSTF recommends aggressive HIV testing to include screening for all persons 15 to 65 years of age and younger or older persons who are at increased risk. They also recommend screening all pregnant women, including those who are untested, who present in labor. With this aggressive testing expanding into a low-risk population, the number of false positive ELISA/EIA screening tests increases as does the number of indeterminate western blot tests. Indeterminate western blot tests should be followed up with NAT or PCR tests and repeated ELISA/EIA and western blot assays at three and six months. Fourth generation tests offer an alternative and utilize HIV-1/HIV-2 antibody differentiation immunoassays in place of western blot assays.

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