

CASE REPORT

A False Positive HIV Confirmatory Test Result in Pregnancy and its Impact on Patient Management and Outcome

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SUMMARY

Background: HIV screening and diagnosis during pregnancy is routine and of paramount importance. False positive results are rare but may have catastrophic consequences on both mother's and baby's health.

Methods: The patient was tested using HIV screening assay and HIV confirmatory assay as well as HIV proviral DNA and HIV plasma viral load test.

Results: Her HIV screen tests were very low positive. Her HIV confirmatory screen tested positive, indeterminate, and negative across multiple repetitions. Her proviral DNA and plasma HIV tests were both negative.

Conclusions: This report highlights a clear example and provides guidance as to how these scenarios should be handled.

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KEYWORDS

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INTRODUCTION

Human Immunodeficiency Virus (HIV) infection has historically been tested using serology as the most reasonable and economical diagnostic modality. The Centers for Disease Control and Prevention (CDC) and the Clinical and Laboratory Standards Institute (CLSI) have published testing algorithms for HIV [1,2]. Given its significant impact on health as well as quality of life, it is paramount that performance characteristics are optimal. This is particularly the case with false positive results in a low prevalence setting. The latter is even further exacerbated in the context of pregnancy in which a false positive result can have medical and surgical consequences for both mother and the baby. Although exceedingly rare, given the importance of this matter and the paucity of the literature, we decided to report the following case which is especially important from laboratory medicine perspective.

CASE PRESENTATION

A 26-year-old married female, G3P1, who had undergone suction dilation and curettage in March 2024, was seen as part of routine prenatal assessment in June 2024. Her HIV screen test at the Cleveland Clinic main campus Immunopathology laboratory (Abbott Alinity *i* instrument), repeatedly tested reactive with signal-to-cut-off (S/Co) ratios as follows: 1.00, 1.02, 1.03 (cutoff: 1.00). Following the CDC- and CLSI-recommended algorithm, the serum specimen was tested on the confirmatory test (Geenius, BioRad) at the same laboratory with a subsequent negative result. Given her lack of HIV acquisition risk factors, a nucleic acid testing was not pursued at the time but instead HIV serology was repeated 3 weeks after the first blood draw. The 2nd HIV screen results were non-reactive that time (S/Co: 0.83) obviating the need for the confirmatory test. Given this history of false reactivity, she was rescreened in December 2024 in her late 3rd trimester (37 weeks' gestation). This time the screen was reactive with the following S/Co: 1.16, 1.20, 1.36 requiring the confirmatory test. The confirmatory test was positive this time for HIV-1 and indeterminate for HIV-2 (Figure 1A). Given the unusually low S/Co, the result was deemed suspicious, and the confirmatory test was repeated immediately. This time HIV-1 was positive with different band pattern (Figure 1B), but HIV-2 was negative. Given her pregnant status, the test resulted as positive recommending HIV nucleic acid for further confirmation. A cesarian section was planned due to her underlying pregnancy complication with placenta previa. HIV-1 RNA test subsequently returned with "target not detected" result confirming the suspicion regarding false-positive confirmatory test results. At that point, the sample was retested for the 3rd time on the confirmatory test. This time the result was indeterminate for HIV-1 and with different band pattern again (Figure 1C) and negative for HIV-2. Based on the constellation of the results and mostly based on the last confirmatory test result, the previous positive result was corrected to indeterminate. To conclude the discrepant serological results, a separate whole blood specimen was collected and sent to a reference laboratory for HIV-1 proviral DNA test which subsequently returned "target not detected". In the meantime, the patient had undergone the planned C-section with no complications, and she understood the results and had no further questions or concerns.

DISCUSSION AND CONCLUSION

To the best of our knowledge there are a few cases reported in the literature with proven false positive results with this confirmatory test [3-6]. The exact reason why this happens is not clear but heterophile antibodies and high dose anabolic steroids were found to be the only possible interfering factors. In the experience of one of the authors (KK), in many confirmatory test results re-

viewed for over a decade, a few false positive confirmatory test results were seen among patients with no risk factors with S/Co ranging from 1 - 2 to > 600 (unpublished data). Heterophile antibodies typically bind to the Fab portion of antibodies and depending on the 'design' of a given immunoassay may subvert or augment the signal. In the case of Geenius assay, which is a dual-path lateral flow immunoassay, the HIV-1/2 antigens are affixed on the solid phase. Even the presence of heterophile antibodies may not cause a false-positive result because they are not typically directed against HIV antigens so theoretically should not be able to form a bridge unless heterophile antibodies by chance recognize an epitope on a HIV antigen and antibody Fab. Additionally, the HIV antigens used in this test are from different sources: synthetic peptides, recombinant expressed in *E. coli* or Vaccinia virus; therefore, anti-vector antibodies are unlikely to cause any false positive results. This patient never received any monoclonal antibodies, did not have recent or concurrent infections, was not on any medications or had any underlying conditions other than placenta previa. To our knowledge there is no reported association with this condition and false-positive HIV results. The most likely explanation could be that given the similarities between HIV antigens and those of Human Leukocyte Antigen (HLA) and T-cell receptor (TCR) molecules [7,8] and that women with previous pregnancies, C-sections, and miscarriages [9,10] and/or placenta previa are highly likely to have anti-HLA/and-TCR antibodies (therefore cross-reacting with HIV antigens) which show false-positive or indeterminate results as described here. It is highly likely that heterophile antibodies caused the false-positive screen result, as the p24 antigen detection portion of it is in fact a sandwich assay in which heterophile antibodies notoriously can interfere. Also, different confirmatory test results may suggest low avidity and/or low titer antibodies of IgG class that bind staphylococcal protein A-coated gold particles creating the lines (bands) rather weakly and inconsistently.

Finally, it is important to reiterate that since HIV serology is done as serial testing, i.e., screen test followed by the confirmatory test, the overall specificity is calculated using the following formula (A for the screen and B for the confirmatory test):

Overall Specificity:

$$(A)_{\text{specificity}} + [1 - (A)_{\text{specificity}}] \times (B)_{\text{specificity}}$$

The screen test used here has a specificity of 99.83% (per manufacturer) while the reported specificity of the confirmatory test is 93% to 99.1% [3-6]. This means an overall specificity of 99.98% to 99.99%. If we take the mean value of 99.985%, given the prevalence of HIV in the United States (0.35%), the positive predictive value (PPV) would be calculated as 96%. This PPV is great but of course not perfect leading to a very small fraction of false-positive cases as seen in this study. This fraction is even higher among pregnant women as they are typically considered a low-risk population in developed countries. This report highlights the importance of con-



Name	Result
gp36	absent
gp140	present
p31	present
gp160	absent
p24	absent
gp41	present
Control	present

Figure 1A. Overall result: HIV-1 POSITIVE (HIV-1 Ab reactive/HIV-2 Ab indeterminate).



Name	Result
gp36	absent
gp140	absent
p31	absent
gp160	present
p24	present
gp41	present
Control	present

Figure 1B. Overall result: HIV-1 POSITIVE (HIV-1 Ab reactive/HIV-2 Ab nonreactive).



Name	Result
gp36	absent
gp140	absent
p31	present
gp160	absent
p24	absent
gp41	absent
Control	present

Figure 1C. HIV-1 INDETERMINATE (HIV-1 Ab indeterminate/HIV-2 Ab nonreactive).

A - C. Multiple repetitions using the same serum sample on the confirmatory test (Geenius) on two different days. Results generated by the Geenius software are provided.

stant vigilance regarding the veracity of HIV results by taking several parameters into account to mitigate clinical and psychological consequences.

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Declaration of Interest:

None declared.

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