Interpretation of HIV Serologies in the Era of PrEP: Two Cases of False Positives

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ABSTRACT

Introduction: More availability of HIV pre-exposure prophylaxis (PrEP) is needed to end the HIV epidemic, but this means more clinicians will encounter false positive HIV test results. These cases highlight such occurrences and the steps a clinician may take to determine the significance of such results.

Case Presentations: We present the case of a 68-year-old male who presented for routine follow-up for HIV PrEP and a 41-year-old transgender male who presented to establish care with a primary care clinician. On labs, both had repeatedly positive HIV antigen/antibody tests with undetectable viral loads.

Discussion: With increasing prescription of HIV PrEP comes a need for accurate interpretation of HIV serologies. HIV PrEP users may have altered stages of seroconversion. Additionally, heterophile antibody interference can lead to false positive or negative results.

Conclusions: The reader should gain an understanding of HIV testing, potential pitfalls, and next steps amidst unclear results.

INTRODUCTION

HIV pre-exposure prophylaxis (PrEP) refers to the use of medication taken with the purpose of preventing the acquisition of HIV.[1] It is highly effective (approximately 99%) at preventing sexual transmission of HIV.[1] The rate of prescription of HIV PrEP in the United States is increasing – the percentage of 16 to 20 year olds who are accessing PrEP increased from 8% in 2017 to 20% in 2021.[2] Though these statistics are encouraging, even more PrEP prescription and better targeting of PrEP is needed–especially given the significant racial disparities associated with PrEP prescription, as Black and Hispanic/Latino people are much less likely to be prescribed PrEP than their White counterparts.[2] Nonetheless, with increasing PrEP prescription and awareness comes the need for increasing familiarity with various HIV testing modalities among primary care clinicians.

The Centers for Disease Control and Prevention (CDC) has published clinical guidelines (as of 2021) for the prescription of oral and injectable PrEP, with the recommendation to check an HIV antigen/antibody (Ag/Ab) test every 3 months in patients taking oral PrEP to ensure seroconversion has not occurred.[3] In the case of long-acting injectable cabotegravir (Apretude) for PrEP, these guidelines call for HIV RNA testing prior to bimonthly administration (and prior to subsequent doses given if scheduled doses have been missed) given the pharmacokinetics of this drug and the risk of development of HIV resistance.[3] On the whole, lab monitoring may be challenged by false positive or negative results or the rare case of true seroconversion in a person appropriately taking PrEP. This is further complicated by the fact that those taking PrEP may have altered stages of seroconversion, lower viral loads, and less symptoms compared to those not taking PrEP.[4,5]

This report details one such occurrence of a false positive result in the context of PrEP utilization, one in a lower risk patient not taking PrEP, and the steps clinicians may consider in handling such clinical scenarios.

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CASE PRESENTATIONS

Case 1

The first patient is a 68-year-old man who presented to clinic in January 2022 for lab work for routine PrEP follow-up. His labs

were notable for an HIV Ag/Ab test, which returned repeatedly reactive, and reflex HIV-1 and HIV-2 antibody differentiation resulted indeterminate. The patient was contacted via phone. At that time, he reported taking his PrEP, emtricitabine/tenofovir-alafenamide (Descovy), every day without missed doses. He reported the last time he engaged in condomless receptive anal sex was several months prior (April 2021) with a male partner of unknown HIV status and that he had engaged only in condomless oral sex with that same partner since then. An HIV RNA polymerase chain reaction (PCR) test (viral load) was checked, which was not detectable; he was deemed to be HIV negative and was continued on PrEP.

Three months later (April 2022), he returned for an in-person visit at which time he reiterated his adherence to PrEP and disclosed no new sexual partners. Labs were checked and his HIV Ag/Ab test again returned reactive with indeterminate antibody differentiation. An HIV viral load was again checked and was undetectable. He was instructed to stop taking PrEP for 1 month and to return for repeat testing at that time.

He ultimately followed up 3 months later (July 2022) for labs. Again, an HIV Ag/Ab test returned repeatedly positive, this time with negative HIV-1 and HIV-2 antibody differentiation, and his HIV viral load was again negative. An HIV DNA PCR also was checked at this time and was negative. He ultimately was deemed HIV negative and was continued on PrEP.

Case 2

The second patient is a 41-year-old transgender man who presented to establish care with a primary care clinician in Colorado in August 2023. As part of this visit, routine screening labs were collected, including an HIV Ag/Ab test, which ultimately resulted positive with a positive HIV-1 antibody differentiation. His viral load was undetectable. This news came as a shock, as the patient reported having a negative HIV test in 2021 and since then had been in a monogamous sexual relationship with a cisgender female partner. He denied any other risk factors for HIV acquisition, including injection drug use. He purchased 2 separate at-home oral HIV testing kits, which both resulted negative. He was referred to an infectious disease specialist, where he was started on bictegravir/emtricitabine/tenofovir-alafenamide (Biktarvy).

Shortly thereafter, the patient relocated to Wisconsin, where his partner lives, and established care with an infectious disease clinician in Madison. Given the lack of risk factors for HIV acquisition and negative viral load result, further studies were collected at this visit, including another HIV Ag/Ab test (run with a heterophile blocking reagent) and HIV viral load, as well as an HIV DNA PCR. The HIV Ag/Ab test returned negative, and both the HIV viral load and DNA PCR returned negative/undetectable. He was deemed to not have HIV and was instructed to stop taking his antiretroviral medication.

DISCUSSION

These cases shed light on unique scenarios in which the clinician must utilize their knowledge of the pathophysiology of HIV and the various modalities of HIV testing in order to accurately interpret the patient's HIV status. With this comes an assessment of risk of HIV acquisition, which can be somewhat arbitrary. The current CDC PrEP guidelines suggest taking into account number of sexual partners, knowledge of partners' HIV status, use of condoms, and history of bacterial sexually transmitted infections as factors that elevate one's risk for HIV acquisition.[3]

The patient in the first case may be deemed "high risk" given his history of anal receptive sex with a male partner of unknown HIV status; however, he endorsed absolute adherence to his PrEP, which, in theory, would make his risk very low. Undoubtedly, the patient in the second case should be considered low risk given his lack of sexual or environmental exposures.

We suggest taking risk of HIV acquisition into account when determining next steps to interpret a positive HIV Ag/Ab test. In any case, the HIV-1 and HIV-2 antibody differentiation test is the best first step in determining the validity of a positive HIV Ag/Ab test, followed by the HIV RNA PCR (viral load) if the antibody differentiation returns negative or indeterminate, which is the current testing algorithm supported by the CDC.[6] Importantly though, there are only 3 Food and Drug Administration (FDA)-approved HIV RNA PCR tests for diagnosis of HIV infection, one being the Cobas HIV-1/HIV-2 Qualitative test. The other two have a dual claim for diagnosis (qualitative nucleic acid testing [NAT]) and management (quantitative NAT) – these are the Aptima HIV-1 Quant Dx Assay and the Alinity m HIV-1 Assay.[7] Other HIV RNA PCR tests may be used more commonly depending on institutional availability, though non-FDA-approved tests may come with a higher risk of false positive results.[8]

Considering this testing strategy of an initial HIV Ag/Ab test with Ab differentiation followed by an FDA-approved HIV RNA PCR test, one also must consider the natural history of an HIV infection. Though the HIV RNA PCR is a highly sensitive and specific test, there exists an "eclipse" period – typically spanning 8 to 10 days following the initial acquisition of HIV – where the level of HIV RNA is too low to be detected.[9] This is again complicated in patients taking PrEP, where exposure to such antiretroviral therapy may result in low level viremia that is below the threshold of detection.[5] It is also important to consider the wide variability in PrEP dosing and adherence when interpreting these results. A patient's adherence to PrEP may be spotty for several reasons, including lack of adequate education regarding the medication, concerns about side effects, social and financial factors, or by choice – in fact, some patients are opting for "on-demand" or "2-1-1" PrEP. This refers to taking PrEP around the timing of sexual encounters as opposed to daily, and while it is technically an off-label use of PrEP, this method has been shown to be effective

in the prevention of HIV in men who have sex with men.[10] For all of these reasons, we recommend taking a thorough patient history regarding risk factors, utilization of PrEP, and timeline of potential exposures in order to accurately interpret the aforementioned tests.

One important consideration that manifested itself in our cases was the matter of heterophile antibody interference. Heterophile antibodies are weak antibodies that are produced in response to exposure to an external antigen (oftentimes animal products[11]) and are usually weakly reactive to many antigens and antibodies. This becomes clinically significant as they have the potential to interfere with immunoassays by nonspecifically binding to the assays' recombinant proteins and capture antibodies.[12] This may be overcome with the utilization of a heterophile blocking reagent during laboratory processing, as demonstrated in the second case.

Another rare but important consideration when interpreting ambiguous HIV tests is that of elite controllers. While exact definitions vary, the term "elite controller" refers to people living with HIV who can maintain a level of control over viral replication without antiretroviral therapy.[13] This becomes clinically significant in that an elite controller may present with a positive HIV Ag/Ab test and a positive HIV-1 or HIV-2 Ab and a negative/undetectable viral load. In contrast to our second case, in the case of an elite controller, these results could indicate a true infection as opposed to a false positive.

With all of these potential confounding factors, a tool the clinician may utilize to determine the validity of a positive HIV test is the HIV DNA PCR test. This test measures the integrated HIV DNA that persists in infected CD4 cells despite elite controller status or the use of antiretroviral medications.[14] Though this test is most often used to investigate perinatal transmission of HIV in infants of mothers living with HIV, it was used in the cases presented as a means to more definitively determine the patients' HIV status in the context of ambiguous results. It is important to note that while in rare circumstances it may be clinically useful, this is not an FDA-approved indication of this test; and albeit a highly sensitive and specific test, there is no perfect test that effectively rules out HIV infection, meaning clinical context and judgment must be utilized. It is also important to note that timing of infection and presentation to care would impact the interpretation of the HIV RNA test, which may eliminate the need to pursue HIV DNA testing. If the patient had presented with a distinct recent exposure (barring potential interference of PrEP therapy as previously discussed) or symptoms of acute HIV infection, an initial high level of viremia is expected—even in the case of elite controllers—as it takes time for the immune system of an elite controller to successfully suppress the virus.

Table 1. False Positive Results

Reason	Type of Test Affected	Explanation	Possible Intervention
Heterophile antibody	HIV antibody/antigen test	Weakly reactive antibodies that may interact with various immunoassays	Repeat test with utilization of heterophile blocking reagent
Lab error	HIV antibody/antigen or HIV RNA test	Mislabeling of samples/breakdown in sample ID	Repeat test

Table 2. False Negative Results

Reason	Type of Test Affected	Explanation	Possible Intervention
PrEP	HIV antibody/antigen or HIV RNA test	Exposure to incomplete antiretroviral regimen may delay antibody formation or lead to low level viremia below limit of detection	If suspecting false negative antigen/antibody test, check FDA-approved HIV RNA test; if suspecting false negative HIV/RNA test, repeat test while off PrEP for approximately 2 months[15]
Poor antibody response	HIV antibody/antigen test	May not mount antibody response due to severely immunocompromised state due to advanced HIV or other condition)	Check FDA-approved HIV RNA test
Elite controller	HIV RNA test	Innate nature of elite controller status allows for viral suppression without use of antiretroviral medication	Consider HIV DNA test
Heterophile antibody	HIV antibody/antigen test	Weakly reactive antibodies that may interact with various immunoassays	Repeat test with utilization of heterophile blocking reagent

CONCLUSIONS

While HIV testing initially may seem formulaic, there are many intricacies to consider when interpreting these studies—especially in the current age of PrEP. Knowledge of potential causes of false positive and negative results, the effects of PrEP on result interpretation, and various tools available to elucidate ambiguous results are essential. Bearing in mind these concepts, in conjunction with a thorough history and physical, allows the clinician to be able to best counsel patients on their HIV status. And while complicated situations may arise, the more clinicians are comfortable prescribing PrEP and diagnosing HIV, the sooner the HIV epidemic will end. We present Tables 1 and 2 and the National Clinician Consultation Center PrEP hotline phone number (855.448.7737) as resources for navigating complex clinical scenarios.

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