Abstract

Background: An individual is considered HIV positive when a confirmatory HIV-1/HIV-2 differentiation test returns positive following an initial reactive antigen/antibody combination screen. Falsely reactive HIV screens have been reported in patients with various concomitant infectious and autoimmune conditions. Falsely positive confirmatory HIV differentiation assays are seen less frequently, but have been observed in cases of pregnancy, pulmonary embolism, and malaria.

Case presentation: A healthy 27 year-old man was referred after a reactive ADVIA Centaur® HIV Ag/Ab screen and positive Bio-Rad Geenius™ HIV 1/2 Confirmatory assay, suggesting HIV-1 infection. The patient’s HIV viral load was undetectable prior to initiation of antiretroviral therapy, and remained undetectable on subsequent testing after initiation of antiretroviral therapy. Both Centaur® and Geenius™ tests were repeated and returned reactive. As this patient was believed to be at low risk of acquiring HIV infection, samples were additionally run on Genscreen™ HIV-1 Ag assay and Fujirebio Inno-LIA™ HIV-1/2 score, with both returning non-reactive. For confirmation, the patient’s proviral HIV DNA testing was negative, confirming the initial results as being falsely positive. The patient disclosed that he had been using a variety of anabolic steroids before and during the time of HIV testing.

Discussion and conclusions: The erroneous diagnosis of HIV can result in decreased quality of life and adverse effects of antiretroviral therapy if initiated, hence the importance of interpreting the results of HIV testing in the context of an individual patient. This reports suggests a potential association between the use of anabolic steroids and falsely-reactive HIV testing.

Keywords: False positive HIV test, Bio-rad Geenius, False reactive HIV screen, Anabolic steroids
confirmation by Bio-Rad Geenius™, which led to unness-
sary antiretroviral therapy (ART) for months.

Case presentation
A 27 year-old man with no past medical history presented
in the fall of 2017 with urinary hesitancy and dysuria to
his primary care provider, and was found to be positive
for gonorrhea on Hologic Altima Combo 2™ NAAT assay.
He had HIV testing done with ADVIA Centaur® at the
same time, which was negative. His only HIV risk factor
was heterosexual contact with three female partners in the
6 months prior to this visit. He had been tested and found
to be negative for HIV with the same platform in 2014,
2016, and earlier in 2017.

Repeat HIV testing was performed in January 2018,
which was indeterminate by ADVIA Centaur®. Confirma-
tory Geenius™ testing was negative at that time. Patient
returned for repeat testing in February of 2018, and the
ADVIA Centaur® screen became reactive. When the sam-
ple was subsequently run on Geenius™, gp140, p31, and
gp41 bands were present, confirming HIV-1 infection. He
was then referred to our tertiary clinic and seen 15 days
after his positive test results. Upon physical examination,
no abnormal findings were identified, and his history did
not suggest recent acute HIV seroconversion.

The patient wished to begin antiretroviral therapy (ART) immediately, motivated primarily by his desire to
decrease the risk of transmission to his HIV-negative fe-
male partner. Elvitegravir/cobicistat/emtricitabine/tenofo-
vir alafenamide was started the same day he was seen in
clinic, after initial laboratory investigations including his
HIV viral load were drawn. His baseline results showed a
CD4 count was 835 (46%) cells/cubic millimeter and an
undetectable HIV viral load. His HIV viral load was
repeated 22 days later with the same result, although by
this time he had been taking ART for over 3 weeks. Repeat
HIV testing in March and April of 2018 once again
returned reactive on ADVIA Centaur® and was confirmed
by Geenius™. HIV viral load testing was performed again
in May 2018, and returned undetectable.

Based on the unusual constellation of laboratory find-
ings and an otherwise low perceived risk of acquiring HIV
infection, further questioning and investigations were
pursued. The patient revealed that he had been using a variety
of oral and injectable supplements for bodybuilding begin-
inning in July of 2017 under the supervision of his trainer,
including testosterone, exemestane, and trenbolone
enanthate. All of these supplements were purchased from
various locations accessed on the Internet as suggested by
his trainer and fellow bodybuilders. The patient clarifie
that he always used sterile equipment and technique for
injections, and never shared injection paraphernalia with
others at any time.

Further investigations were performed in collaboration
with colleagues from the National Microbiology Labora-
tory (NML) in Winnipeg, Manitoba, Canada. Heterophi-
lic antibody interference is a phenomenon that has
previously been reported with both Centaur® and
ARCHITECT® HIV assays by the NML [4]. Our patient’s
samples were treated with a blocking agent to reduce
the likelihood of incorrect results due to heterophilic
interference, but despite this remained positive on both
Centaur® and ARCHITECT® platforms. Samples were
then run on Genscreen™ HIV-1 Ag assay and Fujirebio
Inno-LIA™ HIV-1/2 score, and both were negative. Pro-
viral HIV DNA testing performed on a dedicated whole
blood sample drawn from the patient in July 2018 was
negative, confirming that initial results were falsely posi-
tive. One day after proviral HIV DNA testing was
complete, the patient was informed of his HIV-negative
status and ART was discontinued. The patient received
a total of 133 days of ART, and did not experience any
adverse effects or tolerability-related concerns due to the
medication. He remained with the same female partner
he had at the time he initiated ART after being told his
results were incorrect.

Discussion and conclusions
False positive fourth-generation HIV screening tests have
been reported in association with a number of inflamma-
tory and infectious comorbidities, such as acute malaria
[2], schistosomiasis [5], Epstein-Barr Virus (EBV) infection
[6], malignancy, tuberculosis, and autoimmune diseases
[7]. These false positive results are hypothesized to be
mediated by cross-reactivity of the antibodies produced by
lymphoproliferation associated with these conditions.

Similarly, falsely reactive Geenius™ results have been
reported in the setting of pulmonary embolism, malaria, and
pregnancy [8]. Additionally, there has been a case of an in-
determinate Geenius™ result with HIV pre-exposure prophy-
laxis use, the mechanism of which remains unclear [9].

Our patient did not have any history of infectious or
inflammatory conditions that could account for his re-
peatedly false-positive results. It is impossible to deter-
mine whether his use of anabolic steroids was of
significance, although the timeline of events is at least
suggestive of a plausible association.

While false positive HIV tests occasionally occur, both
the ARCHITECT® and ADVIA Centaur® Ag/Ab are robust
screening assays and have reported specificity of over 99%
[10–12]. The Bio-Rad Geenius™ confirmatory assay has
been evaluated for HIV-1 and HIV-2 confirmatory testing
and discrimination in a variety of populations [8, 13–16].
The manufacturer’s instructions provided with the test
claim over 99% specificity, however the results of studies
in target populations vary with specificity reported to be
between 93 and 99% [8, 13–16].
Two Canadian studies evaluated Geenius™ as a confirmatory assay following a fourth generation screening test. Malloch et al. reported specificity of 96.3% (95% CI of 90.2–98.8) when Geenius™ was used in a Canadian population [13]. Similarly, Serhir et al. reported specificity of 93%, with 7% of all HIV-negative samples used in this study read as indeterminate by Geenius™ [8].

Although the above studies suggest that Geenius™ may be less specific than a fourth generation immunoassay, the cumulative specificity of the two assays used together is higher than that of Geenius™ alone. Assuming 99.4% specificity for ADVIA Centaur® and 93.0% for Geenius™, this still yields a cumulative specificity of 100% [17].

Geenius™ tests plasma reactivity to four HIV-1 antigens, namely gp160, p41, pol p31, and gag p24 [18]. Our patient’s plasma was repeatedly reactive to p41 and p31, as well as HIV-2 gp140 antigen on the Geenius™ assay, however the sample was not reactive when retested on the INNO-LIA™ platform. INNO-LIA™ detects antibodies against the same HIV-1 antigens, excluding gp160, and additionally detects antibodies against p120 and p17 [19]. Both tests require a positive response to a minimum of two antigens, with at least one Env antigen. Hence, the falsely reactive result obtained with Geenius™ is not due to cross reactive antibodies against specific antigens.

In summary, HIV test results should be interpreted in the context of multiple factors, including the estimated HIV prevalence in the screened population, the pre-test probability of HIV infection, and an individual’s unique risk profile. Any patient with a negative viral load pre-treatment should be investigated for a false positive HIV result before being considered an elite suppressor. When needed, both HIV viral load testing and additional screening using alternative assays, as was done in our case, can be helpful in distinguishing true HIV infection from a false positive result. Clinicians should be aware of a potential association between false-positive HIV testing results and the use of anabolic steroids.

Availability of data and materials
All data and test results that have been used in this report are included in the manuscript.

Ethics approval and consent to participate
Need for ethics consent is routinely waived for single case reports at our institution (Regina General Hospital).

Consent for publication
Written informed consent to publish this case was obtained from the patient and is available upon request.

Competing interests
The authors declare that they have no competing interests.

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