

False-Positive Result of a Confirmatory Human Immunodeficiency Virus Line Immuno Assay in an Apparently Healthy Individual – A Case Report

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ABSTRACT

*A case of a false-positive result of human immunodeficiency virus (HIV) confirmatory immunoblot-based assay is described. Repeatedly borderline reactive anti-HIV screening enzyme immunoassay result obtained in a local hospital resulted in directing the sample to the Slovenian HIV/AIDS Reference Laboratory. In the Reference Laboratory, both anti-HIV screening assays and confirmatory Western blot were negative, while a confirmatory test INNO-LIA HIV I/II Score (Innogenetics, Ghent, Belgium) was anti-HIV-1 positive due to *sgp120* and *gp41* reactivity. The results of serological testing of the second sample obtained three weeks later were completely identical, while in the third sample obtained 5 months later, seroreversion was observed. Due to a negative dynamics in anti-HIV serological profile and repeatedly negative results of the molecular tests for HIV-1 and HIV-2, HIV infection was excluded and the results of test INNO-LIA HIV I/II Score were finally interpreted as false positive.*

Key words: HIV-1, diagnosis, false-positive result, confirmatory assays, line immuno assay

Introduction

Current routine laboratory diagnosis of human immunodeficiency virus (HIV) infection is mainly based on the detection of specific anti-HIV antibodies^{1,2}. All samples are first screened for anti-HIV using basic screening serological methods such as enzyme immuno assays or agglutination assays. Since the specificity of screening tests is limited, all anti-HIV reactive screening test results have to be confirmed, using confirmatory tests. Western blot (WB) and immunoblot (IB) tests are most widely used supplemental or confirmatory tests for the detection of anti-HIV antibodies in Europe^{1,2}. Both tests are highly specific, but, in comparison with screening tests, more laborious and costly. Their high specificity is mainly based on the fact that they allow the determination of reactivity of anti-HIV antibodies with particular HIV proteins. In WB, electrophoretically separated natural HIV proteins derived from whole virus lysates are transferred (blotted) to a solid membrane. In contrast, in

IB recombinant or synthetic HIV proteins mechanically applied onto the solid membrane are used.

False-positive results of anti-HIV confirmatory tests are extremely uncommon, but they can occur³⁻⁷. In the appropriate clinical setting, a follow-up and the use of other, mainly molecular laboratory tests may help to identify such cases^{2,7}. According to the literature data, only false-positive results of WB-based HIV confirmatory tests have been described previously²⁻⁷. Here, we report, to the best of our knowledge, the first case of a false-positive result of a IB-based HIV confirmatory test in an apparently healthy individual.

Case Report

A 33-year old heterosexual man visited the outpatient clinic for sexually transmitted diseases, complaining of

TABLE 1
RESULTS OF TESTS PERFORMED TO RESOLVE HIV INFECTION STATUS OF A 33-YEAR OLD HEALTHY INDIVIDUAL

| Assay | 1 st sample (July 2004) | 2 nd sample (August 2004) | 3 rd sample (January 2005) |
|--------------------------------------|--------------------------------------------|--------------------------------------------|--------------------------------------------------|
| AxSYM HIV 1+2 gO Assay | borderline reactive (S/CO=0.93) | ND | ND |
| Enzygnost anti-HIV 1/2 Plus | nonreactive | ND | ND |
| Vitros Anti-HIV 1+2 | nonreactive | nonreactive | nonreactive |
| VIDAS HIV DUO Ultra | nonreactive | nonreactive | nonreactive |
| HIV BLOT 2.2 | negative | negative | negative |
| INNO-LIA HIV I/II Score | HIV-1 positive sgp120 (1+) gp41 (2+) | HIV-1 positive sgp120 (1+) gp41 (2+) | HIV-1 indeterminate sgp120 (+/-) gp41 (1+) |
| COBAS AMPLICOR HIV-1 MONITOR v1.5 | ND | negative | negative |
| AMPLICOR HIV-1 Test v1.5 | ND | negative | negative |
| HIV-2 PCR | ND | negative | negative |

ND – not done

scanty, watery, non-purulent urethral discharge, without disuria, that had lasted for one week. There was no significant past medical history, especially no previous episodes of urethral discharge. Despite the patient's complaints about urethral discharge, no signs of it or any other sexually transmitted disease were noted at physical examination. He denied any risk behavior regarding sexually transmitted diseases. Urethral swabs were taken and tested by smear and culture for *Neisseria gonorrhoeae*, by culture for *Ureaplasma urealyticum*, *Mycoplasma genitalium* and yeast and by direct immunofluorescence (DIF) for *Chlamydia trachomatis*. Clean-catch urine was taken for quantitative urine culture. All tests were performed using standard microbiological procedures. All cultures as well as DIF were negative. Serologic testing for syphilis, hepatitis B and C viruses were negative. Serologic testing for HIV infection was also offered to the patient.

Screening for anti-HIV was performed in a hospital laboratory using two 3rd generation enzyme immunoassays: AxSYM HIV 1+2 gO Assay (Abbott Diagnostic Division, Wiesbaden, Germany) and Enzygnost anti-HIV 1/2 Plus (Dade Boehringer, Marburg, Germany). Since Abbott assay was repeatedly borderline reactive (S/CO=0.93) and Enzygnost clearly nonreactive, the patient's serum sample was sent to the Slovenian HIV/AIDS Reference Laboratory for confirmatory testing on July 27, 2004. As shown in Table 1, in the Reference Laboratory, both screening assays, Vitros Anti-HIV 1+2 (Ortho Clinical Diagnostics, Amersham, UK) and VIDAS HIV DUO Ultra (bioMerieux, Marcy-l'Etoile, France), were non-reactive, and WB-based confirmatory test HIV BLOT 2.2 (Genelabs Diagnostics, Singapore) was negative, while

the IB-based confirmatory assay INNO-LIA HIV I/II Score (Innogenetics, Ghent, Belgium) tested HIV-1 positive. As shown in Fig. 1 (sample A), reactivity with two different HIV-1 specific proteins sgp120 (1+) and gp41 (2+) was observed, fulfilling the manufacturer's criteria for anti-HIV-1 positivity. The intensity of HIV specific bands on IB-membrane was determined using automatic

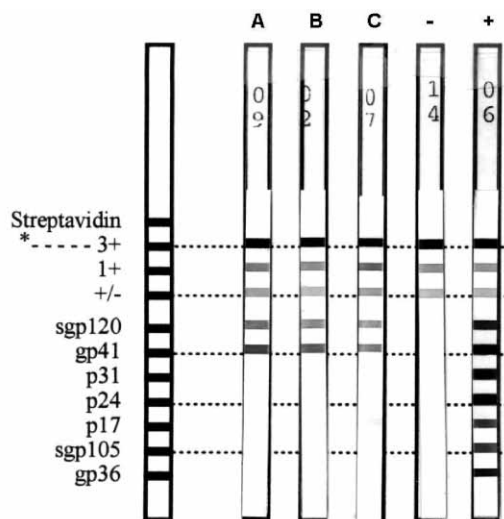


Fig. 1. The results of anti-HIV testing using immunoblot-based confirmatory assay INNO-LIA HIV I/II Score (Innogenetics, Ghent, Belgium) in consecutive serum samples obtained from a 33-year old apparently healthy man on July 27, 2004 (sample A), August 19, 2004 (sample B) and January 20, 2005 (sample C) and in a negative (-) and a positive (+) control sample.

LiRAS Line Reader and Analysis Software (Innogenetics). The patient was contacted immediately and carefully questioned about possible risk for HIV infection. He denied any risk behavior during the last 3 months. A follow-up sample was taken 3 weeks later, on August 19, 2004, and tested in the Slovenian HIV/AIDS Reference Laboratory. The results of anti-HIV testing of the second sample were completely identical with that of the initial sample, including the intensity of both HIV-1 specific proteins *sgp120* and *gp41* (Table 1, Fig. 1, sample B). To resolve the infection status of a patient with this very unusual anti-HIV serological profile, his plasma and peripheral blood mononuclear cell samples were tested by commercial polymerase chain reaction (PCR)-based assays COBAS AmpliPrep/COBAS Amplicor HIV-1 Monitor Test v1.5 (Roche Molecular Systems, Branchburg, NJ) and Amplicor HIV-1 Test v1.5 (Roche Molecular Systems) for the presence of HIV-1 RNA and HIV-1 proviral DNA, respectively. Additionally, the patient's peripheral blood mononuclear cells were tested for the presence of HIV-2 proviral DNA using an in-house real-time PCR-based assay, as described earlier⁸. As shown in Table 1, all PCR-based tests were clearly negative. The antibody profiles detected by serological testing for Epstein-Barr virus (EBV) infection and human cytomegalovirus (CMV) infection were characteristic for a past infection with both viruses. The patient was tested for the third time 5 months later, on January 20, 2005. As shown in Table 1 and Fig. 1 (sample C), the results of anti-HIV testing of the third sample were identical with that of both earlier samples, with the exception of INNO-LIA HIV I/II Score which was HIV-1 indeterminate due to lower intensity of both HIV-1 specific proteins *sgp120* and *gp41* (Table 1, Fig. 1). Since the negative dynamics in anti-HIV serological profile was recognized (seroreversion) and molecular tests for HIV-1 and HIV-2 were repeatedly negative, HIV infection was finally excluded in the particular patient and the results of IB-based HIV confirmatory assay INNO-LIA HIV I/II Score in all three samples were inter-

preted as false positive. Although we have twice invited the patient in written form to follow-up anti-HIV testing, he didn't respond.

Discussion

According to the literature data, the described case is quite unusual in some aspects. At first, only false-positive results of WB-based HIV confirmatory tests have been described previously in peer reviewed journals²⁻⁷. Additionally, in all reported cases of false-positive HIV confirmatory test results, the serum samples tested strongly reactive in at least one anti-HIV screening test. However, similarly to our case, in all but one patient with false-positivity of anti-HIV confirmatory tests reported previously, minimal, but sufficient WB reactivity was observed (e.g. *p24* and *gp160* only, or *gp41* and *gp160* only). The only exception was a 16-year old Hungarian female with seven reactive bands in WB which lasted more than one year⁴. This exceptional case of WB false-positivity was a consequence of an EBV induced proliferation of anti-HIV antibody producing B cell clones due to acute EBV infection⁴.

It should be pointed out that, if the patient had been tested in our laboratory at the very beginning, he would have been simply diagnosed as anti-HIV negative, due to nonreactivity of our both anti-HIV screening tests. However, acting as the national HIV/AIDS reference laboratory, we are mandated to perform confirmatory anti-HIV testing regardless of the result of our screening assays, when a local laboratory reports anti-HIV reactivity in a certain serum sample.

In conclusion, in the present report we described the first case of a false-positive result of IB-based HIV confirmatory test in an apparently healthy individual. Proper and close follow-up and the use of supplementary molecular tests is highly recommended in any case of unusual anti-HIV reactivity.

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LAŽNO-POZITIVAN REZULTAT LINE IMMUNO ASSAY POTVRDNOG TESTA ZA VIRUS LJUDSKE IMUNODEFICIJENCIJE U ZDRAVOG ISPITANIKA – PRIKAZ SLUČAJA

S A Ž E T A K

U radu je prikazan slučaj lažno pozitivnog rezultata potvrdnog imunoblot testa za dokazivanje protutijela protiv virusa ljudske imunodeficijencije u zdravog ispitanika. Zbog opetovano granično reaktivnih rezultata pretražnog anti-HIV enzimsko imunskog testa dobivenih u lokalnoj bolnici, uzorak je upućen u Referentni laboratorij za HIV/AIDS Republike Slovenije. U referentnom laboratoriju su oba anti-HIV pretražna testa i potvrdni Western blot test dali negativan rezultat, dok je potvrdni test INNO-LIA HIV I/II Score (Innogenetics, Ghent, Belgija) zbog reaktivnosti na sgp120 i gp41 dao pozitivan rezultat. Rezultati ponovljenog serološkog testiranja u drugom uzorku oduzetom tri tjedna kasnije, bili su u potpunosti jednaki, dok je u trećem uzorku oduzetom 5 mjeseci kasnije zabilježena postupna seroreverzija. Zbog negativne dinamike anti-HIV serološkog profila i ponovljenih negativnih rezultata molekularnih testova za HIV-1 i HIV-2, u ispitanika je isključena HIV zaraza, a rezultati potvrdnog testa INNO-LIA HIV I/II Score interpretirani su kao lažno pozitivni.