

Implications of an indeterminate HIV antibody result - a case series

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Abstract

In Sri Lanka Immunoassays using either viral lysate (Western Blot) or recombinant/synthetic antigen (Line Immunoassay) for anti-HIV capture are still the preferred methods to confirm HIV infection. Three patients infected with HIV-1 presented with Acquired Immunodeficiency Syndrome (AIDS) defining illnesses. Laboratory tests were performed using the routine commercial kits on multiple sera of one patient and same sera from the other two. All patients were strongly positive on the Enzyme Linked Immunosorbent Assay (ELISA) test. Yet, HIV-1 infection could not be confirmed using the routine Line Immunoassay. Eventually HIV infection was confirmed in two patients using the Western blot assay but in one patient that was also indeterminate. We were unable to test further due to non-availability of other tests such as nucleic acid testing (NAT) assays to confirm the presence of HIV RNA. This highlights the fact that in resource poor countries, indeterminate results may delay the diagnosis of HIV, if only Line Immunoassays are available. Some end stage HIV/AIDS patients may not produce antibodies to specific HIV antigens and may give indeterminate or negative results.

Introduction

Diagnostic testing for human immunodeficiency virus (HIV) has come a long way since the virus was first discovered as the cause of Acquired Immunodeficiency Syndrome (AIDS) in the mid 1980s¹. Most diagnostic laboratories use a combination of Microtitre plate-based or automated bead-based enzyme immunoassays (EIAs) which test for the presence of HIV antibody (anti-HIV) and antigen (HIV p24 Ag) for their initial screening of samples for HIV infection. Confirmation of positive

results on such initial screening assays is usually obtained by using one or more assays of an alternative format, which use a variety of purified, recombinant or whole virus proteins to confirm the presence of anti-HIV antibodies². Further confirmation requires testing the original sample from which the test aliquot was taken, as well as a follow-up sample. In addition, resource-rich countries have the option of using nucleic acid testing (NAT) assays to confirm the presence of HIV RNA³.

With an estimated 3500 people living with HIV and an estimated adult prevalence <0.1%, Sri Lanka has been designated as a “low HIV prevalence” area by the World Health Organization⁴. Most diagnostic laboratories in Sri Lanka can perform initial screening tests, but final confirmation of HIV infection usually requires samples to be sent to the National Reference Laboratory of the National STD AIDS Control program, Department of Health, where confirmation testing takes place using ELISA, Line Immunoassay and if necessary, Western Blot³.

We report a case series of three patients with clinical AIDS, who were strongly positive for all HIV screening ELISAs, but indeterminate on Line Immunoassay. Of these, two became positive later on Western Blot assay, whereas one patient remained indeterminate on initial Western Blot, but later became positive for Line Immunoassay.

Case history 1

A 32 year male presented to the Kalubowila STD clinic for HIV screening. He complained of fever, loss of weight and cough for 23 days, shortness of breath and exertional dyspnoea for 14 days. He had been successfully treated for pulmonary tuberculosis 5 years ago. After repeated counseling, he admitted

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to unprotected sex with his former girlfriend. On examination, he was emaciated, febrile and dyspnoeic. He had oral candidiasis, his respiratory rate was 22 per minute and there were bilateral crepitations in his lungs. His Chest X-ray and CT scan showed bilateral ground glass appearance which is suggestive of Pneumocystis Carini Pneumonia (PCP). The rapid test, ELISA and Particle Agglutination Test (PAT) for HIV antibodies were positive, but the Line immunoassay was indeterminate on two occasions. The Western Blot was also indeterminate. The CD4 count was 82 on admission and decreased to 43 within three weeks. Following the ELISA report, treatment for PCP and oral candidiasis was commenced. Within 2-3 days patient showed a marked clinical improvement. After counseling he was started on anti retroviral drugs, Stavudine, Lamuvudine and Efevirenze. Line Immunoassay became positive following six months of treatment.

Case history 2

A 44 year old male was referred to the Kalubowila STD clinic from the Colombo South Teaching Hospital for HIV screening. He presented with a history of fever, cough and shortness of breath of 2 weeks. He was treated for transverse myelitis in 2005 without an identifiable cause, investigated for dysphagia in 2007 and an abscess was removed in the right buttock in the same year. He admitted to having unprotected sexual exposures with several female partners while abroad. On examination, he was dyspnoeic, wasted and had bilateral lung crepitations. The Chest X-ray revealed peri-hilar interstitial shadows extending to the periphery. ELISA for HIV antibodies was

Details of investigations are shown in Table 1.

Table 1

Case	ELISA	Line Immunoassay	Western Blot
1	Positive	Indeterminate gp120(<+/-), gp41(+3), P24(+/-)	Indeterminate P24, gp120
2	Positive	Indeterminate gp41(+/-), p31(+/-)	Positive gp120, gp160
3	Positive	Indeterminate Gp120(+/-), gp41(+3)	Positive gp120, gp160

positive but the Line Immunoassay was indeterminate. The Western Blot test carried out from the same sample was positive. He was started on treatment for PCP but died after 5 days of treatment in the Intensive care unit.

Case history 3

A 38 year old married male was referred to the National STD AIDS Control Program from the National Hospital of Sri Lanka for HIV screening. He had progressive shortness of breath and fever for 3 days and a chronic cough for 3 months. He was treated for oral candidiasis 3 months ago. On examination, he was cyanosed, had bilateral crepitations in the lungs. Chest X-ray revealed bilateral peri-hilar patchy shadows. ELISA test for HIV antibodies was positive and the Line Immunoassay was indeterminate. Western Blot test carried out from the same blood sample was positive. He was started on treatment for PCP but died after 5 days of ventilation.

We were unable to perform any further tests such as NAT assay as they were not available in the National reference laboratory at the time.

Discussion

In a county with <10% HIV prevalence, UNAIDS and WHO recommends to follow strategy II for HIV testing for diagnostic purposes, in individuals with clinical signs and symptoms of HIV infection³. In strategy II, all samples are first tested with one ELISA or rapid/ simple assay. Any sample found reactive in the first assay is tested with a second ELISA or rapid/

simple assay based on a different antigen preparation. If both tests are positive, the sample is considered as positive for HIV³. A modified method is used in Sri Lanka, where samples are tested initially by the ELISA method. The positive samples are tested again, using a different methodology and target proteins (usually a PAT). Then a third assay mostly Western Blot or Line Immunoassay is used for further confirmation. It was surprising to find that all 3 cases discussed here were indeterminate on Line Immunoassay as one would expect a clear positive result in a clinical scenario like this. When Western Blot test was done, it was positive in Cases 2 & 3 and indeterminate in Case 1.

It is well known that HIV specific screening tests have a higher sensitivity but lower specificity than either Western Blot or Line Immunoassay, which is why the latter are used for the confirmation of any positive test result from the screening EIAs³.

One possible explanation for the indeterminate results is that specific anti-HIV antibodies may have been lost in patients with end-stage AIDS, whereas less HIV specific antibodies that cross react with host antigens may persist⁵. Since allo-antigens are also present on Western Blot strips, such antibodies may be detected in Western Blot assays. Line Immunoassay has only a few HIV specific bands and need optimal threshold of antibodies to become positive³.

Other options in diagnostic testing are the NAT assay and the viral culture. In Sri Lanka currently NAT assay is only available in the private sector and is not accessible to the patients attending the government sector.

Rarely in some end-stage AIDS patients, who have lost their ability to produce specific anti-HIV antibodies due to immune dysfunction, the specific anti-HIV antibodies may be genuinely absent¹. This may be why the sample in the first case gave an indeterminate result in the Western Blot. Serum samples that meet the criteria of stages III and IV of HIV infection may yield indeterminate results due to a fall in antibody levels. In such cases serum need

not be Re-tested³. It is recommended that a positive result is issued in an indeterminate case only if the EIA is strongly positive, p24 antigenemia is present and to repeat Western Blot as necessary considering the clinical assessment of the patient and the CD4 count^{5,6,7}. In the first case history, the Line Immunoassay became positive after treatment with antiretroviral drugs. This may be because as the immunity is improved there is a rise in the antibody levels⁵.

Conclusions

The diagnostic value of standard HIV tests in advance AIDS is influenced, by the changing antibody levels and inherent limitations of select HIV confirmation strategies and test kits. In dealing with an indeterminate result multidisciplinary approach with communication among the clinicians and laboratory is vital.

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Case reports

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