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Combination Assay Detecting both Human Immunodeficiency Virus (HIV) p24 Antigen and Anti-HIV Antibodies Opens a Second Diagnostic Window

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Fourth-generation human immunodeficiency virus (HIV) screening immunoassays reduce the diagnostic window between infection and diagnosis by the inclusion of HIV p24 antigen detection together with HIV antibody detection in the same test. We compared third- and fourth-generation HIV immunoassays and a dedicated HIV p24 antigen test for detection of a case of HIV seroconversion. This demonstrated a second diagnostic window using the fourth-generation assay due to a decline of HIV p24 antigen prior to the detection of HIV antibody. However, HIV p24 antigen was detected in the same sample by the dedicated HIV p24 antigen test, as was HIV proviral DNA. Although it is likely to be rare, this phenomenon has also been reported for other fourth-generation HIV immunoassays and has implications for the reported diagnostic windows of these assays.

CASE REPORT

A 53-year-old man was treated for gonococcal urethritis after an episode of unprotected heterosexual intercourse in Thailand, but he refused human immunodeficiency virus (HIV) testing. Eleven days later, he re-presented with symptoms of 4 days of malaise, high fever, headache, diarrhea, and a progressive maculopapular rash involving the trunk, face, and scalp. The fever subsequently settled, but the rash progressed to become confluent over the face and scalp before gradually resolving over 2 to 3 weeks. Physical examination at this time revealed marked oropharyngeal erythema and bilaterally enlarged cervical lymph nodes. There was a marked lymphopenia, at 0.34×10^9 cells/liter, and moderate thrombocytopenia. A provisional diagnosis of acute HIV seroconversion illness was made, and blood was sent for HIV serology and further diagnostic testing.

Microbiological studies. The results of diagnostic testing are presented in Fig. 1. Further testing during the seroconversion period beyond the results reported was not possible due to sample depletion. All commercial assays were performed according to the manufacturer's instructions. The in-house proviral HIV DNA PCR was performed using three nested primer pairs, specific for one *gag* (4) and two *pol* (1, 4) gene targets and optimized for local reagents. PCR was regarded as positive if all three primer sets gave positive reactions.

Four days after the patient first developed symptoms, strong reactivity was found in the serum by using the AxSYM HIV Ag/Ab Combo assay (Abbott Laboratories, Abbott Park, Ill.) (sample rate to cutoff rate [S/CO], 25.05), but the AxSYM HIV-1/2 Antibody gO assay (Abbott Laboratories, Abbott Park, Ill.) was nonreactive (S/CO, 0.36). According to the AxSYM HIV Ag/Ab Combo assay manufacturer's instructions, initial S/CO values of >1.00 are reactive and those from 0.90 to <1.00 are considered grayzone values, and both of these situations should be retested. A result of <0.90 is considered negative, and no retesting is required. An HIV p24 antigen (Ag) enzyme-linked immunoassay (EIA) (Vironostika HIV-1 antigen EIA; bioMerieux bv, Boxtel, The Netherlands) was performed on this sample and was also reactive (S/CO, 29.45). However, 9 days after the onset of illness, a second serum sample was nonreactive in both the AxSYM HIV Ag/Ab Combo assay (S/CO, 0.85; repeat value, 0.78) and the AxSYM HIV-1/2 Antibody gO assay (S/CO, 0.72). The HIV p24 Ag EIA remained reactive at a low level (S/CO, 2.11), as confirmed by neutralization (Vironostika HIV-1 antigen neutralization system; bioMerieux bv, Boxtel, The Netherlands), and the in-house proviral HIV DNA PCR detected HIV DNA in whole blood. Sixteen days after the onset of illness, a third serum sample was used, and the AxSYM HIV Ag/Ab Combo assay had again become reactive at a low level (S/CO ratio, 2.94), as had the AxSYM HIV-1/2 Antibody gO assay (S/CO ratio, 4.86), but the HIV p24 Ag EIA was nonreactive. An HIV viral load (Amplicor HIV-1 monitor; Roche Diagnostics GmbH, Mannheim, Germany) assay performed at this time detected >100,000 copies/ml, and a Western blot (HIV BLOT 2.2; Genelabs Diagnostics, Geneva, Switzerland) was indeterminate (with p17, p24, and gp160 bands) according to the criteria of the National Serology Reference Laboratory of Australia. A fourth sample collected 30 days after disease onset showed that the AxSYM HIV Ag/Ab Combo assay was more reactive (S/CO, 4.71), as was the AxSYM HIV-1/2 Antibody

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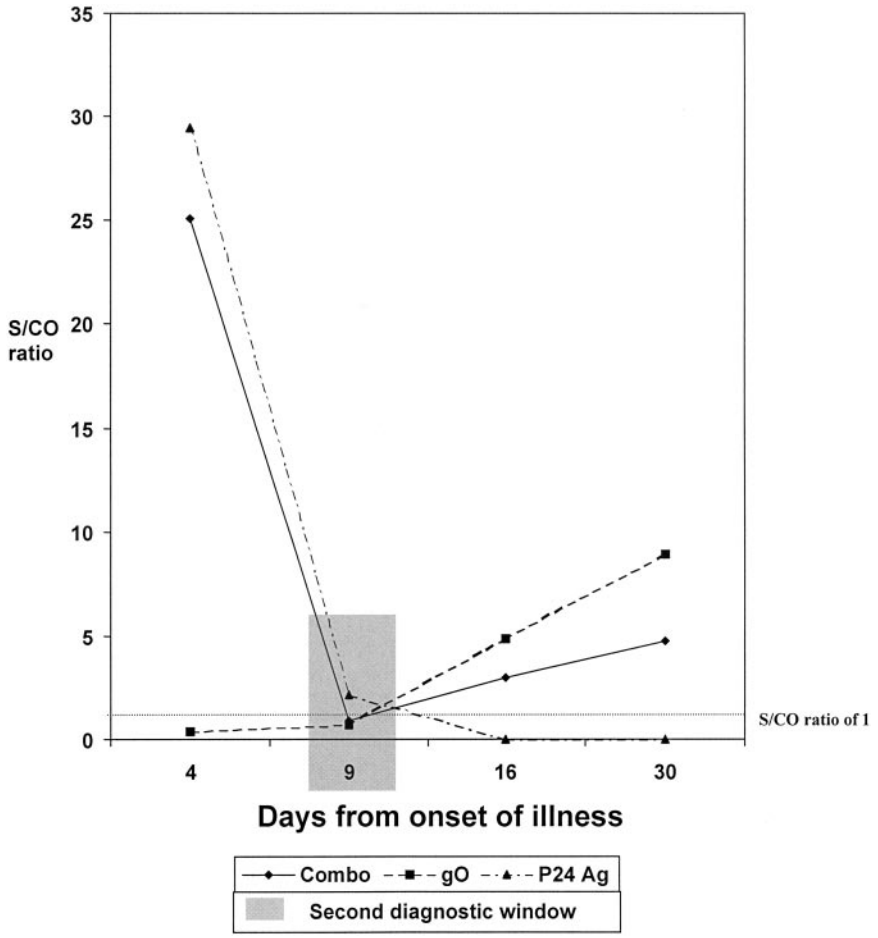

FIG. 1. Results of HIV Ag/Ab Combo assay, HIV Ab (gO) assay, and p24 antigen assay performed during HIV seroconversion.

gO assay (S/CO, 8.93). The HIV p24 Ag EIA remained nonreactive, and the Western blot was positive for HIV type 1 (HIV-1) (with p17, p24, p66, and gp160 bands). Subsequent HIV subtyping using the Stanford reverse transcriptase and protease database (http://hivdb.stanford.edu) and verified with the National Center for Biotechnology Information (NCBI) program (http://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi) confirmed an HIV-1 group M subtype CRF01_AE infection consistent with acquisition in Thailand.

The introduction of combined HIV Ag and antibody (Ab) screening assays such as the AxSYM HIV Ag/Ab Combo assay has reduced the diagnostic window period compared to that of third-generation antibody immunoassays (5). This is due to the detection of HIV core protein (p24) that appears transiently in the blood prior to a detectable humoral immune response to early HIV infection. Using HIV seroconversion panels, the times to the first reactive sample have been compared between various third-generation and fourth-generation assays. These studies have shown that the diagnostic window period is reduced by several days to as much as 2 weeks depending on the tests used. In one study (7), the AxSYM HIV Ag/Ab Combo assay became reactive a mean of 6.15 days before the AxSYM HIV-1/2 Antibody gO assay.

A concern with fourth-generation assays has been a reduction in sensitivity for HIV Ab compared to third-generation tests, which may create a second diagnostic window. In fact, this second diagnostic window has been demonstrated previously with the earlier fourth-generation assay Enzymun-Test HIV Combi (3). A second diagnostic window has also been reported for the Enzygnost HIV Integral (Dade Behring Marburg GmbH) and Vidas HIV DUO (bioMerieux) fourth-generation immunoassays (6) and the newer Cobas Core HIV Combi EIA (9).

The sensitivities of the Ab and Ag detection formats for the AxSYM HIV Ag/Ab Combo assay have not been extensively compromised by the combination. The sensitivity of Ab detection is equivalent to or better than that of the AxSYM HIV-1/2 Antibody gO assay (7). The sensitivity of HIV p24 Ag detection has been reported as comparable to that of dedicated HIV p24 Ag assays, with lower detection limits of between 4.4 and 10.2 pg/ml for a number of HIV subtypes (5). The lower limits of detection for eight CRF01_AE subtypes from Thailand were between 5.4 and 8.5 pg/ml, which are slightly less sensitive than those of the fourth-generation Murex HIV Ag/Ab Combo assay (4.3 to 6.5 pg/ml). The analytical sensitivity of the Vironostika HIV-1 antigen EIA reported by the manufacturer, using a DuPont standard, is 5.9 pg/ml.

This case suggests a slightly lower sensitivity of the AxSYM

HIV Ag/Ab Combo assay than that of the Vironostika HIV-1 antigen EIA for HIV p24 Ag. With samples from seroconversion panels containing HIV p24 Ag but no detectable Ab, the AxSYM HIV Ag/Ab Combo assay was reactive one bleed later than another antigen test (7), supporting a slightly reduced sensitivity. A transient reduction in the reactivity of the AxSYM HIV Ag/Ab Combo assay, but not a second diagnostic window period, has been reported (2), most likely due to the transition between falling free HIV p24 Ag levels and increasing HIV antibody levels. This case report is the first, to our knowledge, to demonstrate such a second diagnostic window during early HIV infection with the AxSYM HIV Ag/Ab Combo assay during which the third-generation AxSYM HIV-1/2 Antibody gO assay was similarly nonreactive. This second diagnostic window may be due to undetectable levels of both HIV p24 Ag and HIV Ab in the serum or to the formation of HIV p24 Ag-Ab complexes that cannot be detected by the HIV p24 Ag assay format (8). During the second diagnostic window, HIV proviral DNA was detected, as was HIV p24 Ag using a dedicated HIV p24 Ag EIA, suggesting a sensitivity issue with this fourth-generation immunoassay. The window period was likely to be very transient, but it is not possible to know the exact duration from the samples collected.

The appearance of reactivity in third-generation immunoassays before that in fourth-generation assays has been reported for one seroconversion panel (BCP 9017) (5). This was thought to be due to the formation of HIV immunoglobulin M (IgM) antibody prior to detectable p24 Ag. This would not explain our findings, as the AxSYM HIV-1/2 Antibody gO assay was also nonreactive at this time.

With a different seroconversion panel (PRB 952) used in the fourth-generation VIDAS HIV DUO assay (bioMerieux, Marcy l'Etoile, France), an initial reactivity on day 10 with subsequent nonreactive results on days 14 and 17 has been reported (5). Third-generation immunoassays (Genscreen HIV v. 2 [Bio-Rad, France] and Ortho HIV-1/2 Ab Capture ELISA [Ortho Clinical Diagnostics]) gave opposite results, being nonreactive on day 10 but then reactive on days 14 and 17. The authors considered this serological window to be due to the transition from anti-HIV IgM to IgG antibodies. Again, this would not explain our findings, as the AxSYM HIV-1/2 Antibody gO assay was also nonreactive at the same time as the AxSYM HIV Ag/Ab Combo assay.

Although this phenomenon is likely to be rare, the demonstration of a second diagnostic window could impair the accuracy of these assays and has implications for high-volume HIV screening such as that done for blood transfusion services. It is important that producers and users of such tests be mindful of this limitation. Newer testing modalities such as NAT are becoming widespread in resource-rich countries but are unlikely to impact HIV diagnosis in developing countries for some time. Therefore, provision of the most accurate, but less expensive, fourth-generation immunoassays is important for the diagnosis of HIV infection.

This case illustrates a sensitivity issue with a fourth-generation HIV immunoassay, emphasizing the importance of additional testing and follow-up sampling for patients with clinically suspected early HIV infection and negative fourth-generation immunoassay results.

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