Screening for Acute HIV Infection: Lessons Learned

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Human immunodeficiency virus (HIV) nucleic acid testing is increasingly being used by researchers and public health screening programs to identify highly infectious, HIV antibody–negative individuals with acute HIV infection. We present cases to illustrate unusual instances of acute HIV screening, which include “window period” testing and the discovery of low-level HIV RNA.

Individuals with recent HIV acquisition may be more infectious than individuals with established infection [1]. Consequently, increasing numbers of public health programs are screening for acute HIV infection with pooled HIV nucleic acid testing (NAT) [2–4], because HIV antibody testing alone may fail to identify HIV-infected individuals during the several weeks following acquisition [5, 6]. Pooled HIV NAT is performed after specimens from HIV antibody–negative individuals are combined into larger master pools, which decreases the cost and time required to screen large populations. As the use of pooled HIV NAT becomes more widespread, the knowledge and previous experiences of acute HIV research programs and existing public health departments that employ HIV NAT will be beneficial.

Neither HIV antibody testing nor HIV NAT has 100% sensitivity or specificity. Similar to antibody testing, interpretation of HIV NAT results varies with local HIV epidemiology. We report cases involving 3 individuals tested for acute HIV infection at the Public Health–Seattle & King County Sexually Transmitted Diseases (STD) Clinic and University of Washington Primary Infection Clinic (Seattle) to describe unusual situations in acute HIV testing and to illustrate the importance of risk assessment in the interpretation of HIV test results.

Case reports. Subject A and subject B presented to the Public Health–Seattle & King County STD Clinic for evaluation. They had been in a same-sex relationship for 4 months and reported unprotected anal intercourse 4 days and 3–4 weeks prior to clinical evaluation. Both also engaged in unprotected intercourse with other, unshared partners. Subject A described a 4-day history of fever, night sweats, rash, headache, pharyngitis, cervical lymphadenopathy, diarrhea, fatigue, and myalgias. Physical examination revealed pharyngeal erythema and a maculopapular rash on the face, torso, and both arms, sparing the palms. Acute HIV infection was confirmed by laboratory evaluations, which included a negative OraQuick Rapid HIV-1 Antibody Test (OraSure Technologies) result and an independently validated real-time RT-PCR amplification assay (with a lower limit of detection equal to 50 copies/mL) that recorded an HIV RNA level of 700,000 copies/mL [7]. Subject A was referred to the University of Washington Primary Infection Clinic for enrollment in an observational research protocol.

Subject B was asymptomatic at presentation to the Public Health–Seattle & King County STD Clinic. He was tested for HIV infection using a second-generation EIA (Vironostika HIV-1 Microelisa System; bioMérieux) and pooled HIV RNA testing (using the pooling algorithm, described in [2], adapted for the RT-PCR assay). The results of the HIV tests were negative. One week later (11 days following his last sexual exposure to subject A), subject B began to develop symptoms that included fever, headache, pharyngitis, fatigue, and myalgias. Two days after the onset of symptoms, subject B underwent a second round of HIV testing at the University of Washington Primary Infection Clinic and was found, on an unpoled specimen, to have an HIV RNA level of 621,000 copies/mL and a CD4+ T cell count of 219 cells/mm³. One week later (3 weeks after his likely HIV acquisition), subject B remained EIA negative, with an HIV RNA level of 2,515,000 copies/mL; his primary care provider initiated antiretroviral therapy following a discussion of the risks and potential benefits of therapy initiated during the period of acute HIV infection.

Transmission between subject A and subject B was confirmed by viral sequence analysis [8], and subject A was presumed from the clinical history to be the transmitting partner. Retrospective HIV NAT of unpoled, frozen serum specimen obtained at subject B’s initial presentation to the Public Health–Seattle & King County STD Clinic (4 days after his last exposure to subject A) showed no evidence of HIV infection.

Received 11 August 2006; accepted 27 September 2006; electronically published 20 December 2006.

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Clinical Infectious Diseases 2007;44:459–61
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1058-4838/2007/4403-0027$15.00
Subject C presented to the University of Washington Primary Infection Clinic 9 days after unprotected, receptive anal intercourse with a partner of unknown serostatus; subject C reported 6 days of symptoms consistent with acute HIV infection that included sore throat and diarrhea, as well as sneezing and nasal congestion. Laboratory evaluations revealed negative results of an HIV EIA and a Western blot assay (Genetic Systems HIV-1 Western blot [Bio-Rad]). The HIV RNA level was 923 copies/mL, and the CD4+ T cell count was 1264 cells/mm³. Although this was considered likely to be a false-positive RNA result, clinic staff counseled subject C to avoid unprotected intercourse until a second round of testing was completed. He was re-evaluated at the University of Washington Primary Infection Clinic 1 week later (15 days following exposure) and had a negative EIA result and an HIV RNA level of 1,050,000 copies/mL. After 1 additional week (3 weeks following exposure), subject C had a reactive EIA result, a negative Western blot assay result, an extremely high HIV RNA level (26,785,000 copies/mL), and a CD4+ T cell count of 336 cells/mm³.

**Discussion.** The first 2 subjects described above are examples of secondary HIV transmission from an individual with acute HIV infection (subject A) and, to our knowledge, the first documented failure of a public health HIV RNA screening program to identify an individual with acute HIV infection (subject B) who was tested during the narrow “window period” between HIV acquisition and detection of HIV viremia by HIV NAT. The third case is one in which the discovery of low-level RNA in subject C might have been considered a false-positive test result were it not for comprehensive retesting. Together, these cases illustrate situations in which routine antibody testing for HIV infection, or even antibody testing plus a standard approach to HIV NAT, would have failed to correctly diagnose these cases during the earliest phase of HIV infection. Deferral of retesting or use of only antibody and Western blot assays could have delayed or possibly missed the diagnoses for these individuals at high risk to transmit HIV to others [1]. Because of the combination of symptom awareness, clinical suspicion, and use of HIV NAT, these individuals received diagnoses in a timely manner, and no additional individuals were exposed.

HIV NAT was only recently approved for diagnosis of HIV infection and is not recommended by the Centers for Disease Control and Prevention guidelines for HIV screening of individuals [9]. However, HIV NAT has been used for years by primary HIV infection research programs to determine protocol eligibility [10–12] and, more recently, by public health programs to increase the yield of HIV screening [2–4]. The delay between HIV acquisition and the ability of testing to detect HIV infection reliably (the “window period”) can be reduced by up to 2 weeks with HIV NAT compared with third-generation EIAs [5] and even further compared with early generation EIAs that are frequently used in large HIV testing programs (table 1). However, the case of subject B illustrates that, even with HIV NAT, a “window period” still exists, and selected individuals at high risk for HIV acquisition should be educated about the need for retesting even when HIV NAT results are negative.

False-positive test results are a valid concern with use of HIV NAT for diagnostic purposes. This error occurs in 2.6%–5% of HIV NAT results, depending on the assay used and population prevalence, with false-positive test results reported for individuals with HIV RNA levels of 52–2058 copies/mL [11–13]. Consequently, experts advise caution in interpreting low-

Table 1. HIV testing assays and their “window periods.”

<table>
<thead>
<tr>
<th>HIV test</th>
<th>Assay method</th>
<th>“Window period” estimates, weeks</th>
<th>“Window period” reduction, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>First-generation EIA</td>
<td>Viral particles used to bind patient HIV Ab, detected by marker conjugated to anti-human Ab</td>
<td>−6</td>
<td>...</td>
</tr>
<tr>
<td>Second-generation EIA</td>
<td>Same as first-generation EIA except uses purified HIV Ag or recombinant virus</td>
<td>−4–6</td>
<td>10</td>
</tr>
<tr>
<td>Third-generation EIA</td>
<td>“Antigen sandwich”: synthetic peptide used to bind patient HIV Ab followed by marker conjugated to additional HIV Ag, able to detect IgM</td>
<td>−3–4</td>
<td>6</td>
</tr>
<tr>
<td>Fourth-generation EIA</td>
<td>Uses third-generation EIA methodology plus monoclonal Ab to detect patient p24 Ag</td>
<td>−2</td>
<td>5</td>
</tr>
<tr>
<td>Pooled HIV NAT</td>
<td>First combines multiple individual samples into one common pool, then uses PCR or other amplification techniques to detect patient viral nucleic acids</td>
<td>&lt;1–2</td>
<td>3</td>
</tr>
<tr>
<td>Individual HIV NAT</td>
<td>As above, except that samples are tested individually rather than diluted by pooling</td>
<td>&lt;1–2</td>
<td>3</td>
</tr>
</tbody>
</table>

**NOTE.** Data are from [5, 6, 16, 19]. Ab, antibody; Ag, Antigen; HIV NAT, HIV nucleic acid testing.

a “Window periods” listed are averages. For example, although second generation EIAs will detect HIV infection in nearly all individuals within 6 weeks of HIV acquisition, in a study of occupationally exposed health care workers, 5% of subjects did not have seroconversion until at least 6 months following the exposure [20].

b Compared with an immediate less sensitive assay, the “window period” for pooled HIV NAT is, on average, 3 days shorter than the “window period” for a fourth-generation EIA.
level HIV NAT results [11–13]. To our knowledge, only 2 individuals with low-level HIV viremia and true acute infection have been prospectively identified and described previously [12, 14], although a retrospective analysis of a small number of blood donors found intermittent HIV RNA levels <100 copies/mL in samples obtained up to 25 days before seroconversion [15]. Dilution of samples for pooled HIV NAT would reduce the frequency of detection of low-level HIV RNA, which would produce fewer false-positive test results [3] but may potentially result in false-negative test results for individuals with very recent HIV acquisition [16].

These cases illustrate continued need for risk assessment of individuals seeking HIV testing and for clinicians to understand the operating characteristics of different HIV tests, including EIAs, rapid HIV antibody testing, and HIV NAT. The importance of these issues will only increase as national recommendations for HIV screening promote testing among low-prevalence populations [17] and as HIV NAT becomes more common. Interpretation of HIV-positive test results in low-risk individuals should continue to be done with caution; risk-reduction behavior coupled with repeated testing should be conservatively recommended. When confirmatory testing for HIV NAT is performed immediately after receipt of positive results, a second round of testing should include HIV NAT, because individuals may remain EIA and Western blot negative for weeks following exposure.

These cases also demonstrate the importance of recognizing symptoms associated with acute HIV infection, because the majority of newly infected individuals have symptoms beginning a median of 10 days following acquisition [10]. Individuals at high risk for HIV acquisition, especially those in discordant partnerships or receiving postexposure prophylaxis, should be instructed to seek attention for symptoms consistent with acute HIV infection. Although HIV acquisition may be asymptomatic, and symptoms in high-risk individuals may not always represent acute HIV infection, clinicians also should become more alert to these symptoms, because individuals with acute HIV infection often seek care but do not receive an appropriate diagnosis [10, 18]. With increasing availability of HIV NAT, clinicians must become more adept at evaluating individuals’ risks for HIV acquisition, employing HIV NAT, and appropriately interpreting these results to diagnose acutely infected individuals at high risk of transmitting HIV to others.

Acknowledgments

We thank Jim Mullins and members of the Mullins laboratory for their work on viral sequencing.

Financial support. National Institutes of Health (K23 AI-65243 [to J.S.], T32 AI07044 [to M.S.C.], P30 AI-27757 [to R.W.C. and D.C.N.], P01 AI-57005, and U01 AI-38858).

Potential conflicts of interest. All authors: no conflicts.

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