Real-time, Universal Screening for Acute HIV Infection in a Routine HIV Counseling and Testing Population

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An estimated 40,000 persons are infected with human immunodeficiency virus (HIV) each year in the United States. Although the potential individual and public health benefits of recognizing acute HIV infection have been discussed, the diagnosis is rarely made in practice. There are no public health systems in place to facilitate acute HIV infection diagnosis. Proposed sensitive/less sensitive antibody testing algorithms distinguish recent from more long-standing infections, but only after seroconversion. Real-time diagnosis of acute HIV infection requires a positive HIV p24 antigen or HIV nucleic acid test result, together with a negative, equivocal, or evolving HIV antibody test result. Because specimen pooling and nucleic acid amplification of HIV antibody-negative specimens can permit accurate, low-cost blood product HIV and hepatitis screening and can be used for estimation of HIV incidence, we hypothesized that a nucleic acid–based screening strategy incorporating multistage pooling could make screening for acute infection feasible in settings with presumably low disease prevalence but high testing volume, such as public health virology and serology laboratories.

Context Acute human immunodeficiency virus (HIV) infection cannot be diagnosed by routine antibody tests and is rarely diagnosed in clinical practice. However, HIV nucleic acid–based testing is widely used to screen for antibody-negative acute infection among low-risk blood donors.

Objective To assess the feasibility of screening in high-volume laboratories for acute and long-term HIV infection in a routine HIV testing population, in which HIV infection prevalence is low, using specimen pooling and HIV RNA reverse transcriptase-polymerase chain reaction (RT-PCR) tests.

Design and Setting Clinical diagnostic performance evaluation at a state-funded public health virology and serology laboratory.

Participants A total of 8505 consecutive individuals presenting for routine HIV counseling and testing during a total of 20 business days to simulate a month of testing in August and December 2001 at 110 publicly funded testing sites in North Carolina.

Main Outcome Measures Prevalence of acute and long-term HIV infection. Serum specimens negative by HIV enzyme immunoassay (EIA) were screened in pools by an ultrasensitive HIV RNA RT-PCR test. Results for individual HIV RNA–positive specimens were reclassified as true or false according to results of confirmatory testing.

Results Of the 8505 individuals screened, 8194 had not previously tested HIV positive and had sufficient serum to complete the testing protocol. Of those, 39 had long-term HIV infection (prevalence, 47.6 per 10,000 at-risk persons [95% confidence interval, 33.8-65.0 per 10,000]). Of the 8155 at-risk individuals whose antibody tests were negative, 5 were HIV RNA positive. Four of those had true-positive acute infection (prevalence, 4.9 per 10,000 [95% confidence interval, 1.3-12.5 per 10,000]). All 4 were women; 2 developed symptoms consistent with an acute retroviral syndrome in the week after testing. Screening all specimens required 147 HIV RNA tests. Overall specificity of the strategy was 0.9999.

Conclusions These findings suggest the widespread diagnosis of acute HIV infections in a routine testing population is not only possible but feasible using specimen pooling and nucleic acid testing. These additional procedures may increase diagnostic yield by approximately 10% compared with conventional HIV antibody testing.

JAMA. 2002;288(2):216-221
large commercial and state public health laboratories.

METHODS

Study Design
We conducted a clinical diagnostic performance evaluation, comparing standard of care HIV antibody testing with a protocol including both HIV antibody testing and HIV RNA reverse transcriptase-polymerase chain reaction (RT-PCR) on pooled HIV-seronegative specimens. Specimens positive for HIV RNA by RT-PCR testing were confirmed by HIV seroconversion. The protocol was approved by the University of North Carolina at Chapel Hill Committee on the Protection of the Rights of Human Subjects. Because of the nature of the study, a waiver for requirement of informed consent was approved by the committee. Individuals had previously provided written consent for HIV testing and a confidentiality policy was already in place for HIV testing in the health departments.

Population
Consecutive serum samples submitted to the North Carolina State Laboratory of Public Health for routine HIV testing from 110 publicly funded testing centers were tested according to the study protocol. Testing centers include sexually transmitted disease (STD), antenatal, and other publicly funded clinics, as well as nontraditional (eg, mobile) HIV counseling and testing sites. A pilot phase examined 2013 specimens submitted over 4 days in August 2001. When follow-up was complete for individuals in the pilot phase, including evaluation of pilot protocols, additional specimens (n = 6492) were collected on 16 consecutive business days in December 2001 to simulate 1 month of continuous testing.

Clinical Information
Self-reported testing history and demographic and lifetime risk information were obtained at the time of specimen collection. Linked data, such as risk, demographic, and testing site information, were abstracted for all individuals from the Counseling and Testing Services Database created by the US Centers for Disease Control and Prevention and maintained for the North Carolina HIV/STD Prevention and Care Branch by the State Laboratory of Public Health. Contact information was maintained separately by the state laboratory of public health for confidentiality protection. Where multiple risk factors existed for individuals, risk categories were resolved by a standard hierarchical structure proposed by the US Department of Health and Human Services.15

Definitions
Individuals were considered at risk for HIV infection if they had no prior positive HIV test result by self-report or on record with the North Carolina Department of Health and Human Services. Infection with HIV was defined by a repeatedly reactive HIV enzyme immunoassay (EIA) result at either initial or confirmatory testing together with a positive HIV Western blot result. Specimens were considered HIV antibody negative on initial testing that were EIA negative (no repeat EIA or testing by Western blot), EIA reactive once but not repeatedly reactive (no testing by Western blot), or EIA repeatedly reactive but with a negative or indeterminate Western blot result. Following pooling and RT-PCR testing on antibody-negative specimens, patients with an antibody-negative specimen who were HIV RNA positive and were subsequently confirmed to be HIV infected (with the confirmatory protocol described below), were classified as having acute HIV infection.

Initial Confirmatory Testing Protocol
Following initial EIA testing, pooling, and initial RT-PCR and confirmatory RT-PCR testing, antibody-negative, HIV RNA–positive specimens were submitted to confirmatory antibody testing. To account for differing sensitivities of EIA kits in the setting of acute HIV infection,16 these specimens were retested using the viral lysate EIA and tested using HIVAB HIV-1/2 ELISA (enzyme-linked immunosorbent assay), a more sensitive recombinant peptide-based third-generation EIA (Abbott Diagnostics, Branchburg, NJ; an absolute lower limit of detection of 20 copies/mL permits detection of individual specimens with >1800 copies/mL in a master pool with 1:90 dilution). Resolution testing of HIV RNA–positive master pools used the standard Roche Monitor kit (absolute lower limit of detection, 200 copies/mL), with the exception of the 50-specimen intermediate pools tested by Ultrasensitive in the project’s pilot phase. Confirmatory RT-PCR was performed on all HIV RNA–positive specimens before they were reported as being HIV positive.

Follow-up Confirmatory Testing
Patients were notified of their result by North Carolina Department of Health
and Human Services HIV/STD Prevention and Care Branch disease intervention specialists, interviewed about possible symptoms of acute infection, and encouraged to return for a repeat blood draw and follow-up confirmatory testing. The viral lysate EIA and Western blot were run on these confirmatory samples.

**RESULTS**

In total, 8505 specimens were received during the study period; pilot and continuation phase populations were similar regarding sex, age, and risk category (C.D.P., unpublished data, April 2002). The disposition of specimens is given in Figure 2. Specimens from 299 patients had insufficient sample volume to complete the testing protocol and were not analyzed; they were also similar to those of the overall population (C.D.P., unpublished data, April 2002). Twelve specimens from patients who were diagnosed previously as having HIV were not eligible for pooling. Of the 8194 remaining specimens, 39 were HIV antibody positive and were also not submitted for pooling. We used the 8194 specimens eligible for pooling as the denominator for estimation of acute infection prevalence to allow for prevalence of both acute and long-term HIV infection to be assessed and compared relative to the overall pool of at-risk subjects. The 8155 HIV antibody–negative specimens were pooled and screened for HIV RNA.

A total of 147 HIV RNA determinations were required to complete all testing, equivalent to 1 HIV RNA determination per 55 specimens initially submitted. Manual pooling and HIV RNA detection procedures were completed within 14 days of specimen receipt during the project’s continuation phase.

Five specimens were HIV RNA positive. The details of confirmatory testing are given in the Table. Patient A had an initial viral load of 1771 copies/mL. The HIV RNA positivity was confirmed on repeat testing of the same specimen. However, the patient had no evidence of seropositivity on either repeat testing of the initial sample or at the follow-up confirmatory blood draw 2 months later. The HIV RNA RT-PCR test was performed to validate the negative antibody results at follow-up and results were also negative. Thus, patient A was classified as false-positive. The remaining 4 HIV RNA–positive individuals, patients B through E, were each confirmed to be HIV infected and each were classified as acute. Patients B and C were initially negative by all antibody testing and later seroconverted. Patient D was negative by viral ly-
sate EIA, was weakly positive by the more sensitive EIA, and was negative by Western blot. Patient E was initially positive by the more sensitive EIA but at an unusually low optical densitometry value consistent with low titer of antibody. This patient refused a confirmatory draw. Final classification of patient E as acute was based on the common presumption[^11] that low HIV antibody titers indicate recent or acute HIV infection.

Thus, HIV RNA screening yielded 4 additional true-positive and 1 false-positive results. The positive predictive value (PPV) of HIV RNA RT-PCR testing for diagnosing acute infection with antibody-negative specimens in this study was 0.80 (95% confidence interval [CI], 0.26-1.00) and the estimated specificity, 0.9999 (95% CI, 0.9993-1.000). The overall PPV of the serial antibody and HIV RNA RT-PCR testing strategy for either acute or long-term HIV infection was 0.98 (95% CI, 0.88-1.00) with specificity of 0.9994 (95% CI, 0.9986-0.9998). Because confirmatory testing results are lacking for HIV antibody–negative patients who tested negative for HIV RNA, the true sensitivity cannot be estimated, and it is possible that overall specificity may have been slightly overestimated.

**Figure 2.** Disposition of Specimens Screened for HIV RNA and Antibodies From Publicly Funded Testing Sites During a Total of 20 Business Days in North Carolina

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**Table. Results of Tests Used for Reclassification of Human Immunodeficiency Virus (HIV) RNA–Positive, HIV Antibody–Negative Results**

<table>
<thead>
<tr>
<th>Patient Identification</th>
<th>HIV RNA, Copies/mL*</th>
<th>Initial Confirmatory Test</th>
<th>Follow-up Confirmatory Test</th>
<th>Clinical Features</th>
<th>Reclassified Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1771</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>False+ HIV uninfected</td>
</tr>
<tr>
<td>462,259</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>True+ Acute infection</td>
</tr>
<tr>
<td>796,500</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>True+ Acute infection</td>
</tr>
<tr>
<td>34,788,030</td>
<td>–</td>
<td>+§</td>
<td>–</td>
<td>–</td>
<td>True+ Acute infection</td>
</tr>
<tr>
<td>18,484</td>
<td>–</td>
<td>+§</td>
<td>–</td>
<td>–</td>
<td>True+ Acute infection</td>
</tr>
</tbody>
</table>

*At the conclusion of resolution testing, after individual HIV RNA–positive specimens had been identified by reverse transcriptase-polymerase chain reaction (RT-PCR) test, positivity of each specimen was confirmed by repeat RT-PCR. The HIV RNA levels reported in the Table were the first result obtained for each specimen rather than the confirmatory result. For cases C and D, the initial RT-PCR test yielded an HIV RNA level estimate greater than the limit of quantitation for the assay, and the specimen was rerun with a dilution step to obtain a more reliable estimate. The first and second RT-PCR results for patients were within the range of laboratory assay variation.

†These data represent the Vironostika enzyme immunoassay (EIA) conducted as part of the confirmatory protocol (see “Methods” section) to rule out laboratory error. The first Vironostika EIA was negative for all the patients.

‡HIVAB HIV-1/2 is an enzyme-linked immunosorbent assay (ELISA) (Abbott Diagnostics, Abbott Park, Ill).

§Weakly positive results with optical densitometry value of less than 1.5 times the calculated cutoff for positivity.

[^11]:[^2]^
Acute vs Long-term HIV Infection

The 4 patients with confirmed HIV antibody–negative acute infections detected by the study protocol represented a 10.3% increase in diagnostic yield, above the 39 new HIV infections diagnosed with antibody testing alone during the study period. Overall acute HIV infection prevalence was estimated at 4 per 8194 or 4.9 per 10,000 persons at risk (95% CI, 1.3–12.5 per 10,000). The overall long-term HIV infection prevalence estimate of 47.6 per 10,000 persons (95% CI, 33.8–69.0 per 10,000) was consistent with North Carolina’s annual prevalence figures.17

All 4 acute infections were in women presenting for testing at STD clinics. One woman had painful genital ulceration at the time of testing; mucocutaneous ulceration of the genitals can be a presenting feature of the acute retroviral syndrome (http://hivatis.org/trigldns.html#.Adult). A genital ulcer viral culture was negative for herpes simplex virus. Three women had no symptoms to suggest acute retroviral syndrome at testing (1 was tested after presenting for evaluation of chronic vaginitis, and 2 were tested because of concern with risky sexual exposures.) Two women developed symptoms consistent with an acute retroviral syndrome in the week following testing; 1 presented to an emergency department and was diagnosed as having acute urinary tract infection.

COMMENT

This first clinical evaluation of real-time universal acute HIV infection screening showed that in a routine HIV-testing population, a number of prevalent HIV infections may be missed by routine antibody testing. The observed prevalence of acute HIV infection in at-risk persons undergoing routine HIV testing in North Carolina (4.9 cases per 10,000) was similar to estimates derived from retrospective studies of routine testing populations in Europe16,20 and is about 1000-fold higher than that found in low-risk blood donors with similar testing procedures.14

Time, Cost, and Accuracy

All continuation-phase testing was completed within 14 days of specimen receipt, at an estimated additional cost of US $2.01 per specimen or $4109 per additional case diagnosed. Resolution testing of only positive pools markedly improves both the approximate overall specificity (0.9999) and PPV (0.80) of nucleic acid–based testing, which may otherwise be prone to an unacceptable rate of false-positive results.13 The imperfect sensitivity of nucleic acid testing for early acute infection can be further reduced by pooling, and it is imperative that patients with recent exposures still be counseled to have follow-up testing if all initial testing results are negative.

Potential Implications for Public Health

The ability to identify patients with acute HIV infection in real time could potentially provide clinical benefits to the individuals identified since early initiation of antiretroviral therapy may improve long-term prognosis.2,3 This information could present a unique public health opportunity to influence the risk of sexual transmission occurring at a time of particular contagiousness.4,5,7,12,12–20 Sexual partners of individuals with acute HIV infection may be candidates for postexposure prophylaxis. Tracing both source patients and exposed partners could facilitate direct interventions targeting networks with active HIV transmission.6,7

The strategy presented herein is limited in that the pooling that makes this strategy medically and economically feasible is possible only in laboratories that have large testing volume, such as public health and commercial laboratories. The relative cost advantage of pooling over individual testing may be lost in settings where acute infection prevalence may be high. Additionally, regulatory concerns could delay implementation of commercial attempts to adapt blood-screening procedures to the clinical setting.

However, this study suggests that HIV nucleic acid–based screening could make early diagnosis, entry into care, and partner screening possible for persons with acute HIV infection who would otherwise be missed.

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Financial Disclosures: Dr Pilcher has received research grants or funding, honoraria including for continuing medical education (CME) programs, lecture sponsorships, assay kits or reagents, or government grants or research funding from or is a consultant to Beckman-Coulter, Biomerieux, GlaxoSmithKline, Immunogenetics, and Roche; Dr Hicks has research grants or funding, honoraria including for CME, or government grants or research funding from or is a consultant to Abbott, Agouron, Bristol-Myers Squibb, Boehringer Ingelheim, DuPont, Merck, Roche, Triangle, and ViroLogic; Dr Eron is a principal investigator for University of North Carolina research contracts from Abbott, Merck, Roche/Trimeris, and Pharmasset; consultant to, receives research grant funding or honoraria for ad hoc consulting, sponsored talks, or CME programs from Abbott, Boehringer Ingelheim, Bristol-Myers Squibb, GlaxoSmithKline, Merck, NIH-NIAID, Substance Abuse and Mental Health Services Administration, Triangle Pharmaceuticals, Trimeris, ViroLogic, and Tibotec-Virco; Dr Fiscus has received research grants or funding, assay kits or reagents, or government grants or research funding from or is a consultant for BioMerieux, Centers for Disease Control and Prevention, CONRAD, GlaxoSmithKline, National Institutes of Health, PerkinElmer LifeSciences, Roche Molecular Systems, and Visible Genetics.

Author Contributions: Dr Pilcher had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Obtained funding: Pilcher, Hicks, Eron.

Administrative, technical, or material support: Pilcher, McPherson, Leone, Owen-O’Dowd, Peace-Brewer, Harris, Hicks, Eron, Fiscus.

Study supervision: Pilcher, Leone, Eron, Fiscus.

Funding/Support: This work was funded in part by UNC Center for AIDS Research grants NICHD/NIAID 9-P30-AI50410-04 from the National Institute of Child Health and Human Development and National Institute of Allergy and Infectious Diseases, grants N01DK-R0149381, AI-07001, K23AI01781-01, and K24AI07668-01 from the National Institutes of Health. Boehringer Ingelheim supported a poster presentation but had no input into its content.
Universal Screening for Acute HIV Infection

Previous Presentation: This work was presented in part at the 9th Conference on Retroviruses and Opportunistic Infections, Seattle, Wash, February 2002. Abstract 359-M.

Acknowledgment: We thank Lou Turner, PhD, and the staff of the Laboratory of Public Health Serology/VIrology who did the antibody testing and pooling. Regina Lee, BS, provided assistance with data collection and management. Ada Cachafeiro, BS, Mark Turner, BS, Amy James, BS, Paul Alabanza, BS, and Priya Joshi, BS, of the UNC Center for AIDS Research Retrovirology Core Laboratory pooled specimens for the pilot phase; Melissa Kerkau, BS, performed the virologic testing throughout. Confirmatory antibody testing was done by the staff of the McLendon Clinical Laboratories at UNC-Chapel Hill. We especially thank the Disease Intervention Specialists of the HIV/STD Prevention and Care Branch of the NC Department of Health and Human Services for contacting and interviewing patients, and Del Williams, PhD, for assistance with the HIV Prevention and Care Database. Sonnia Napravnik, MPH, and William Miller, MD, PhD, and Myron Cohen, MD, MPH, provided critical review of the manuscript.

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