

# Feasible Primary HIV Infection Screening: The North Carolina Experience

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THERE IS NO SHORTAGE OF DATA SUGGESTING THAT PEOPLE WITH PRIMARY HIV infection (PHI) are, unknowingly, significant contributors to the spread of HIV. In turn, public health initiatives surrounding PHI—whether its aggressive testing and counseling of acutely infected individuals, stepped-up contact tracing efforts, or the use of HAART—need to be considered carefully in the larger context of HIV/AIDS prevention efforts.

Unfortunately, only a handful of the 40,000 or so new HIV infections occurring every year in the United States are diagnosed during PHI. A likely reason for this is the fact that there are few public health systems in place to facilitate PHI diagnosis. As explained by Dr. Christopher Pilcher, recent work completed at the University of North Carolina at Chapel Hill suggests that it is possible to implement a real-time universal HIV screening program that incorporates both antibody- and RNA-based assays, that is both medically and economically advantageous. To discuss this work—and its potential as a component of public health initiatives to quickly and accurately diagnose HIV infection in its earliest stages—Dr. Pilcher returned to PRN earlier this year to share the results of what appears to be fruitful research and a new statewide public-health program that is undoubtedly being eyed carefully by departments of health in other states.

## The Public Health Consequences of Late HIV Diagnosis

THE PAST, PRESENT, AND FUTURE PREVALENCE OF HIV INFECTION CAN BE understood more clearly by utilizing the concept of the reproductive number of an infectious disease agent—dubbed  $R_0$ . This relatively simple epidemiological concept describes, in a single value, the epidemic potential of an infectious agent. When, on average, one infected person infects more than one person, the  $R_0$  is greater than 1 ( $>1$ ), indicating epidemic spread of HIV. However, when, on average, one infected person infects no more than one other person, the  $R_0$  is either equal to or less than 1 ( $\leq 1$ ), indicating that HIV will either disappear or maintain itself in the population with zero or limited growth and become endemic.

According to Roy Anderson and Robert May, authors of *Infectious Diseases of Humans: Dynamics and Control*,  $R_0$  is a function of  $\beta * c * D$ , with  $\beta$  representing the infectiousness of an individual,  $c$  representing the number of sexual partners, and  $D$  representing the duration of individual infectiousness (Anderson, 1992). Many biological characteristics may influence factor  $\beta$ , including the infected individual's viral load in peripheral blood and genital secretions, as well as the presence/absence of other sexually transmitted diseases (STDs). In undiagnosed and untreated PHI, viral load is often extremely high, an observation that correlates well with increased infectiousness. "Frequently overlooked is factor  $D$ ," explained Dr. Pilcher. "There is a sizeable percentage of HIV-positive individuals who are not diagnosed until they present with an opportunistic infection, frequently with CD4+ cell counts well below 200. This means that they have unknowingly been infected for several years and have been a risk to others. Public health efforts are going to be limited in their effectiveness to the degree that people are frequently diagnosed

late. Getting HIV-positive people diagnosed, into care, and counseling, as early as possible, needs to be a priority."

Moving on to some case-clustering data, Dr. Pilcher reviewed the results of a Swiss HIV Cohort study, reported by Dr. Sabine Yerly and her colleagues in AIDS (Yerly, 2001). The study included all individuals with documented PHI identified in six AIDS centers of university hospitals in Switzerland and two AIDS centers of a hospital close to Geneva. Among the 197 individuals infected between January 1996 and January 2000, PHI was documented by evolving HIV-antibody response and/or symptoms consistent with acute retroviral syndrome within three months in 70% of individuals and by seroconversion within 12 months of presentation in 30%. Sequence analyses were performed on plasma samples where plasma had been collected before the initiation of HAART (available for 193 of 197 subjects); a phylogenetic tree was constructed using a neighbor-joining method on available reverse transcriptase sequences.

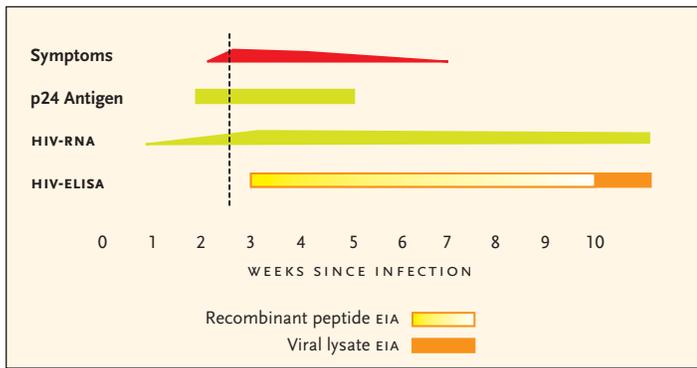
The phylogenetic analyses revealed significant clustering for 56/193 (29%) individuals, indicating that the viruses from patients in each cluster were genetically related. The eighteen clusters in this study ranged in size from two to 11 PHI individuals per cluster and involved intravenous drug use, homosexual, and heterosexual modes of transmission. Retrospective contact tracing firmly established the chain of transmission to explain 17 of the 56 clustered infections.

"What was so incredibly striking about case clustering in patients who are newly diagnosed is that one might predict that they should not have related viruses, given the tremendous size of the pool of potential transmitters out there," Dr. Pilcher commented. "To understand why we saw genetically related viruses in the Swiss study, we need to resort to a couple of explanations. The first one is the obvious one, indicating that there is hyper-infectiousness in acute infection, resulting in 'leapfrogging' of HIV from one acute infected patient to the next. Another possibility is that we have core transmitter groups. There are efficient disseminators out there—individuals who are more likely to transmit HIV to others during the acute stages of infection, perhaps because of an STD."

How does public health prepare to battle this type of epidemic, when core disseminators are involved? "We need to use a model that has been widely used for a long time in public health circles," Dr. Pilcher said. "And that model is syphilis elimination. Going after core transmitters of syphilis, it's possible to eliminate an outbreak in a community. We're now looking to see if we can apply this same approach to HIV elimination."

## The Malawi Acute HIV Infection Study

IS IT POSSIBLE TO INTERVENE IN SEXUAL NETWORKS WITH ACTIVE HIV TRANSMISSION based on detection of PHI? To help answer this pivotal question, Dr. Pilcher and his colleagues—under the direction of Irving Hoffman, PA, MPH, Director of International Affairs for the University of North Carolina, Chapel Hill, Division of Infectious Diseases—have been conducting a seminal HIV incidence and prevalence study in Lilongwe, Malawi.



**Figure 1. Diagnosing PHI: Timing of symptoms and testing**  
 Diagnosing primary HIV infection (PHI) requires heightened awareness of the timing of symptoms and the use of available diagnostic technologies. The time from exposure to the onset of symptoms is usually two to four weeks. Fever and rash may be the symptoms most strongly associated with PHI. Myalgias, arthralgias, night sweats, oral ulcers, weight loss, and loss of appetite are other possible symptoms. EIA testing is the least sensitive for diagnosing PHI, although recombinant-peptide assays are generally considered to be sensitive enough to detect HIV infection in as little as three weeks post-exposure (viral-lysate assays become reliable within 10 to 12 weeks post-exposure). Diagnostic testing involving p24 antigen is less sensitive than HIV-RNA tests. It is likely to be most sensitive within the first two to five weeks of infection. PCR or bDNA tests are highly sensitive PHI diagnostic tools, but have a lower specificity than p24 antigen tests.

Source: Christopher Pilcher, MD

Malawi is one many victims in the sub-Saharan AIDS apocalypse. It is one of the smallest and poorest counties on the troubled continent, ranking among the bottom worldwide for key indicators such as infant mortality and education. Malawi's life expectancy is 36.6 years of age and falling rapidly. Annual per capita income is \$200 and is projected to drop to \$166 by the end of this year.

At the end of 2001, UNAIDS estimated that approximately 850,000 adults and children were living with HIV/AIDS in Malawi. AIDS has orphaned approximately 470,000 Malawian children, and roughly 80,000 adults and children died of complications related to HIV in 2001.

With the epidemic quickly approaching the tipping point in Malawi, Dr. Pilcher's group set out to examine the prevalence of antibody-negative HIV infection detectable by HIV-RNA testing in clinical populations. "We also want to examine the risk associations with acute HIV infection in this setting," Dr. Pilcher explained.

The cross-sectional study discussed by Dr. Pilcher has thus far enrolled 928 consecutive male STD and 432 dermatology outpatients seen at Lilongwe Central Hospital. The HIV-antibody screening employed the Capillus LA-based rapid assay and Genetic Systems' HIV-1/2 Peptide EIA confirmation. Samples that were HIV-antibody negative were then manually pooled according to a pyramid-type pooling scheme modified from a protocol described by Dr. Thomas Quinn and his colleagues (Quinn, 2000). As illustrated in Figure 2, 200 mL aliquots from each of ten seronegative specimens were combined to create intermediate pools (A through E). From there, 200 mL aliquots from five intermediate pools were combined to create a master pool representing 50 specimens. All master pools were then screened for HIV-RNA using either the Roche Monitor version 1.5 or the NucliSens assay. Specimens from HIV-RNA-negative master pools received no additional testing. If a master pool yielded positive results—which required that at least 1000 copies of HIV-

RNA be present in one individual specimen from that pool to be detected—the intermediate pools used to make up that master pool were then tested using PCR. Only those specimens from the positive intermediate pools were tested individually.

Acute HIV infection was defined as an HIV-RNA titer of greater than 10,000 copies/mL and either a negative or indeterminate HIV-antibody test result on retesting the individual specimen. Established HIV infection was defined as either an initial antibody-positive test result or an initial antibody-negative test result that was later found to be positive upon repeat EIA/Western blot testing. Antibody-negative specimens with HIV-RNA titers below 10,000 copies/mL were classified as indeterminate.

Of the 1361 specimens analyzed by Dr. Pilcher's group, 553 (41%) were found to be HIV-antibody positive. Twenty-eight of the 774 (5%) antibody-negative samples were HIV-RNA positive. Of these, 24 were confirmed to be acute HIV infections (HIV-RNA >10,000 copies/mL), two were established HIV infections missed by the original screening antibody test (EIA positive/Western blot positive), and two were indeterminate (HIV-RNA <10,000 copies/mL).

Not surprisingly, there was a dramatic difference in the median HIV-RNA levels among the patients with established HIV infections and PHI. Among the 58 evaluable patients with established HIV infection, the median viral load at the time of diagnosis was 26,302 copies/mL. Among the 24 patients with acute HIV infection, the median viral load at the time of diagnosis was 1,258,925 copies/mL.

Twenty-three of 24 patients with acute HIV infection were from the STD clinic. The single acutely infected subject from the dermatology clinic had occult trichomoniasis. Eleven (46%) of the acutely infected patients had clinical findings suggesting an acute retroviral syndrome that was not attributable to another STD. An additional five (21%) patients had inguinal adenopathy in conjunction with genital ulcer disease (GUD). In fact, inguinal adenopathy, GUD, and non-STD symptoms were all factors that were significantly associated with acute HIV infection in the STD clinic.

In summarizing the results of this study, Dr. Pilcher explained that 2.5% of men with acute STDs in Malawi had antibody-negative acute HIV infection. More specifically, 4.5% of all antibody-negative men had acute HIV infection and 5% of all detectable HIV infections involved men in the acute stages of disease. Those with acute HIV infection presented with extremely high viral loads, up to 2.4 billion copies/mL. "And while most acute infections were clinically unapparent, genital ulceration and inguinal adenopathy proved to be strong predictors of acute HIV infection in the STD clinic," Dr. Pilcher said.

"The bottom line here is that up to 5% of HIV infections can be missed using standard EIA testing among at-risk patients in a high-prevalence area," Dr. Pilcher added. There really is a public health threat associated with missing acute infections. Additionally, delivering false-negative results at the time of counseling may actually increase risky behavior, which is another very troubling result. Clearly, we need to find ways to implement the detection of acute HIV infection."

## The North Carolina STAT Program

THE HIV/STD PREVENTION AND CONTROL PROGRAM IN NORTH CAROLINA—a politically conservative state—has developed an extremely proactive approach to HIV control in recent years. "Everybody has opinions about politics in North Carolina, some of them justified" Dr. Pilcher confessed. "Still, the course the state has taken in tackling its growing HIV epidemic can be viewed as very forward thinking." Currently, North Carolina's public testing sites only offer confidential testing for HIV and other STDs, not anonymous testing. Additionally, the reporting of positive HIV and

## Figure 2. Schema for pooling and testing samples for primary HIV infection

A representation of the schema used for pooling HIV-antibody-negative specimens and for resolution testing of HIV-RNA-positive master pools. **(A)** After initial antibody testing, HIV-EIA-nonreactive specimens were submitted for pooling. Two hundred-microliter aliquots from each of 10 specimens were combined to create intermediate pools (A-E). Two hundred-microliter aliquots from five intermediate pools were then combined to create a master pool representing 50 specimens. **(B)** All master pools were screened for HIV-RNA by RT-PCR. Specimens from HIV-RNA-negative master pools received no additional testing. Intermediate pools from the positive master pools were individually tested using RT-PCR. Only those specimens from positive intermediate pools were tested individually. In this example, 10 specimens from master pool I, intermediate pool D, were tested in the final round, identifying sample D4 as the positive specimen. This figure illustrates the schema used in the Malawi acute HIV infection study, which employed a 50:5:1 pyramid-pooling algorithm. The North Carolina STAT pilot study employed a 90:9:1 pyramid-pooling algorithm; the current North Carolina STAT program has been utilizing a 100:10:1 algorithm.

Source: Based on data and a similar figure reported in: Pilcher CD, McPherson JT, Leone PA, et al. Real-time, universal screening for acute HIV infection in a routine HIV counseling and testing population. *JAMA* 288:216-21, 2002.

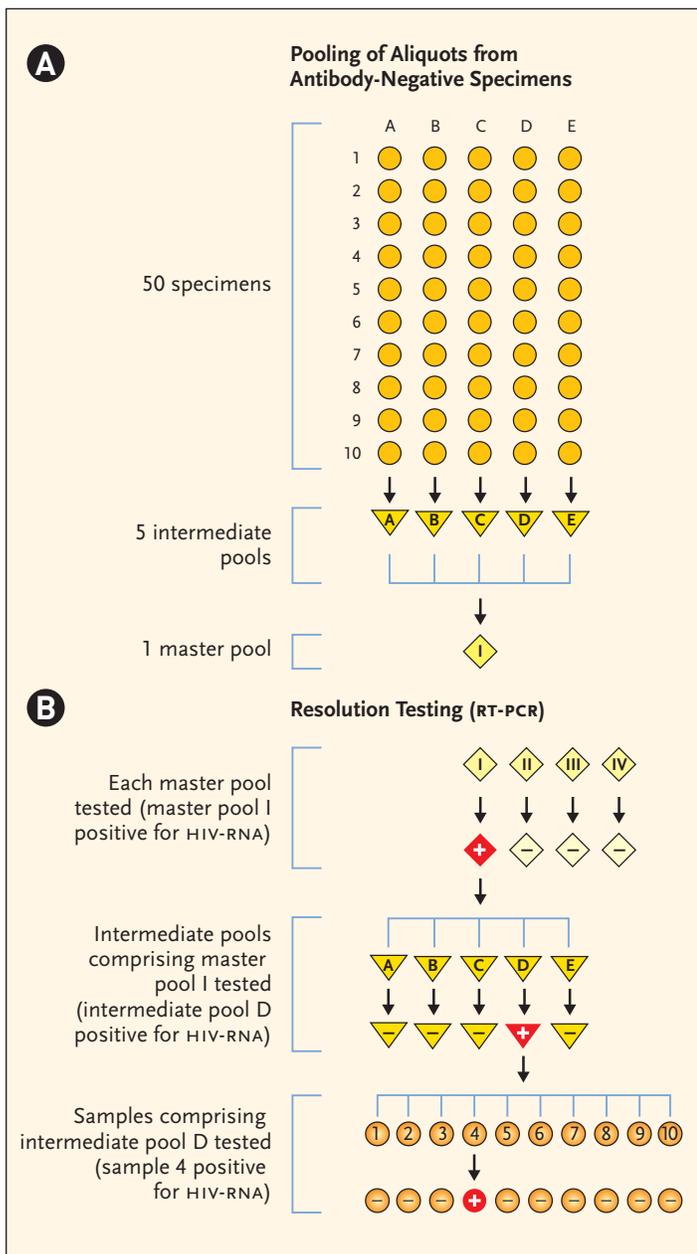
Even more impressive is the Screening and Tracing Active Transmission (STAT) program that is now in place to screen all plasma specimens submitted to the North Carolina State Laboratory of Public Health for both acute and established HIV infection. “The STAT protocol is similar to the protocol we’re employing in Malawi,” Dr. Pilcher said. “We’ve currently tested more than 50,000 people in North Carolina and we sincerely hope that other states will see the value in this protocol in assessing their own prevention needs.”

The STAT program originated as a pilot study to assess the feasibility of using specimen pooling through high-volume laboratories to screen for acute and long-term HIV infection in a routine HIV testing population (Pilcher, 2002). The study evaluated samples collected from 8505 consecutive individuals who presented for routine HIV counseling and testing during one of 20 business days between August and December 2001 (to simulate a single month of testing) at any of 110 public testing sites in North Carolina.

Similar to the pooling strategy used in the Malawi study, samples were first tested using ELISA and, if antibody-positive, Western blot to confirm. Samples negative for HIV antibodies were combined into intermediate pools and then master pools consisting of 90 samples (as opposed to 50 samples used in the Malawi study). Master pools yielding a positive HIV-RNA result led to testing of each intermediate pool and, finally, the individual samples making up an intermediate pool found to contain HIV.

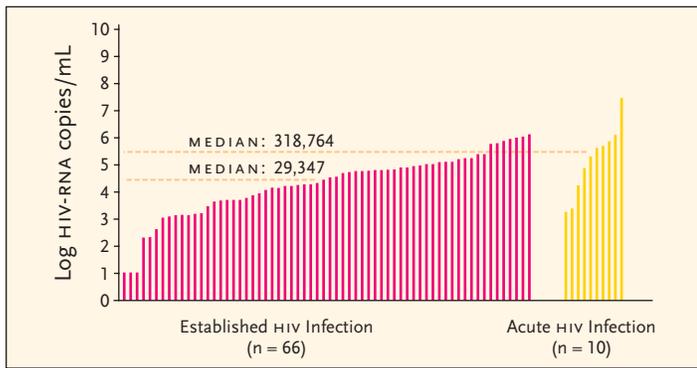
Of the 8505 individuals screened, 8194 had not previously tested HIV positive and had sufficient serum to complete the testing protocol. Of those, 39 had established HIV infection (47.6 per 10,000 at-risk persons). Of the 8155 at-risk individuals whose antibody tests were negative, five were HIV-RNA positive. Four of those had true-positive acute infection (4.9 per 10,000 at-risk persons). All four were women; two 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 99.99%. The estimated additional cost per specimen was found to be \$2.01.

Based on these data, Dr. Pilcher’s group concluded that potentially 10% of all HIV infections in a routine testing population are missed by routine HIV-antibody testing. With multistage pooling, HIV-RNA testing can effi-



STD results is mandatory in North Carolina, which is both laboratory and clinic based. Public health statutes also require mandatory HIV notification and mandatory partner notification. “The partner notification can be done by the patient or by state health workers, who we call disease intervention specialists (DIS),” he explained. “The DIS have a 98% successful interview rate within a couple of months of a new HIV report. In essence, this means that only 2% of patients in North Carolina fail to get their HIV test results; nationwide, the CDC estimates that only about a third of HIV-positive patients receive these results.”

The DIS staff in North Carolina is pivotal to the success and acceptance of the overall statewide HIV/STD prevention program. “The job of these DIS professionals is to protect confidentiality and they do an excellent job of this,” Dr Pilcher said. “We find that patients’ reactions to DIS are exactly the opposite of what many think the reaction would be. The patients are actually very receptive to DIS. They appreciate the professionalism and understand that their confidentiality is protected. In fact, the vast majority of patients prefer DIS to approach their partners, so they don’t have to approach their partners themselves. We’re extremely proud of the DIS program in North Carolina.”



**Figure 3. Viral loads: North Carolina**

Data from the North Carolina STAT program has revealed that there are significant differences in the median HIV-RNA levels among patients with established HIV infection and patients with primary HIV infection. Among 66 evaluable patients with established HIV infection, the median viral load at the time of diagnosis was 29,347 copies/mL. Among 10 evaluable patients with acute HIV infection, the median viral load at the time of diagnosis was 318,764 copies/mL.

Source: Christopher Pilcher, MD.

ciently diagnose acute infection with good positive predictive value in low prevalence populations. “The bottom line is that it is feasible for laboratories with high testing volume, to perform widespread screening for acute infection,” Dr. Pilcher said.

With the completion of the pilot study and evidence to suggest that multistage pooling could be both clinically useful and cost efficient, all publicly funded health-care delivery programs offering voluntary counseling and testing in North Carolina are now participating in the STAT program. The pooling of samples is completely automated using robotics, with a 14-day turnaround for samples submitted for HIV-RNA testing. Dr. Pilcher estimates that approximately 120,000 samples will be tested a year and, of these, between 20 and 160 will yield evidence of acute HIV infections. “We have 14 senior DIS staff members to follow up with patients screened through the STAT program,” he said. “Post-test counseling will be provided by DIS staff within 72 hours of a positive HIV-RNA test being reported. Confirmatory testing is recommended at weeks two, four, and 12 following an acute infection diagnosis. Partners of acutely infected individuals will be interviewed if the contact occurred less than eight weeks prior to the diagnosis. Once seroconversion occurs, DIS will notify all of the patients’ contacts over the previous six months. Sexual contacts beyond six months prior to seroconversion are not notified.”

An update on the STAT program was provided by Dr. Pilcher at the 2nd International AIDS Society Conference on HIV Pathogenesis and Treatment, held in July in Paris (Pilcher, 2003). Of 48,521 clients at risk of HIV infection tested by the program, 256 have been found to be HIV-antibody positive. “This number bodes well with the continued low seroprevalence in North Carolina,” Dr. Pilcher added. With respect to acute HIV infections, a total of 13 individuals were identified through routine testing, with an additional four patients who tested HIV-RNA positive and HIV-antibody negative after being referred for testing by community health-care providers. All 17 acutely infected patients were promptly notified, counseled, and are currently receiving care—many in clinical trials. Contact investigation identified source patients (active transmitters) for six of the 17 patients.

Similar to observations in the Malawi study, there were significant differences in the median HIV-RNA levels among patients with established HIV

infections and PH1 (see Figure 3). Among 66 evaluable patients with established HIV infection, the median viral load at the time of diagnosis was 29,347 copies/mL. Among 10 evaluable patients with acute HIV infection, the median viral load at the time of diagnosis was 318,764 copies/mL.

In terms of patient characteristics, Dr. Pilcher reviewed data pertaining to the 13 patients found to have acute HIV infection through routine testing. The median age of these patients was 34 years, with a range of 21 to 45 years of age. “A number of these patients were young college students, which has been very interesting and has sparked something of an outbreak investigation by the state,” Dr. Pilcher said. Six of the 13 patients were male, five of whom were men who have sex with men. Seven were female—one was 29 weeks pregnant—all of whom were heterosexual with partners at risk for HIV infection. Three of the patients were symptomatic at the time of testing; ten were asymptomatic at the time of testing (three went on to experience symptoms after specimen collection). “We haven’t had any acute infection diagnoses in family practice, prenatal OB/GYN program, TB programs, or hospital clinic sites,” Dr. Pilcher explained. “They’ve all been concentrated in HIV testing sites which are both traditional and nontraditional testing sites. We’ve had some detected through mobile testing vans set up out in rural North Carolina. We’ve also had patients with acute infection being tested in STD clinics and in the jails.”

## Conclusion

IN SUMMARIZING HIS TALK, DR. PILCHER ATTEMPTED TO ANSWER FOUR basic questions surrounding acute HIV infection as a matter of public health. First, is it common for patients to present for testing during acute HIV infection and have the diagnosis missed? According to the STAT program experience, Dr. Pilcher estimated that approximately 3% of new HIV cases in North Carolina may present for HIV testing during their acute HIV infection, “a significant number that would have been given negative results if it wasn’t for HIV-RNA testing.” Second, under what conditions do many acutely infected patients present for testing? “We’ve seen acute HIV infection among patients presenting with STD-related symptoms, STD contacts, and patients concerned about a partner’s risk.” Third, is it possible for patients to be rapidly and reliably diagnosed with acute infection? “Yes, but the labs need to have high throughput capabilities. What’s more, the populations really do need further definition and targeting. The development of targeted screening criteria based on the data that we’re collecting is going to be an incredibly important feature of what we do. Because this is not without cost. When I said two dollars a specimen, this contrasts with approximately 50 cents or less for an HIV ELISA. So, in actuality, we’re talking about a relatively expensive testing procedure.” Finally, is it possible to intervene in sexual networks with active HIV transmission, based on detection of acute HIV infections? The unequivocal answer is yes. “I believe that our preliminary moves, in North Carolina, will open up the possibility for active real-time public-health surveillance. It’s really kind of exciting.” 

## References

- Anderson R, May R. *Infectious Diseases of Humans: Dynamics and Control*. New York, NY: Oxford Science Publications; 1992.
- Pilcher CD, Foust E, McPherson JT, et al. **Screening and tracing active transmission: STAT. A North Carolina program targeting acute HIV infection** [Abstract LB-52]. 2nd IAS Conference on HIV Pathogenesis and Treatment, Paris, 2003.
- Pilcher CD, McPherson JT, Leone PA, et al. **Real-time, universal screening for acute HIV infection in a routine HIV counseling and testing population**. *JAMA* 288:216-21, 2002.
- Quinn T, Brookmeyer R, Kline R, et al. **Feasibility of pooling sera for HIV-1 viral RNA to diagnose acute primary HIV-1 infection and estimate HIV incidence**. *AIDS* 14:2751-7, 2000.