Published in final edited form as:

J Virol Methods. 2011 June ; 174(1-2): 94–98. doi:10.1016/j.jviromet.2011.04.002.

Evaluation of Pooled Rapid HIV Antibody Screening of Patients Admitted to a San Diego Hospital

Sanjay R. Mehta^{*,a}, Vu T. Nguyen^{*,a}, Georgina Osorio^a, Susan Little^a, and Davey M. Smith^{a,b}

Vu T. Nguyen: yukysaky@gmail.com; Georgina Osorio: geosorio@ucsd.edu; Susan Little: slittle@ucsd.edu; Davey M. Smith: davey@ucsd.edu

^aDivision of Infectious Diseases, University of California San Diego, La Jolla, CA 92093-0711, USA

^bVeterans Affairs San Diego Healthcare System, San Diego, CA 92093, USA

Summary

Current HIV screening guidelines in the United States recommend expanding the scope of HIV screening to include routine screening in health care settings; however, this will require increased resources. Since testing of pooled samples can decrease costs, the test characteristics of pooled rapid antibody testing were determined and optimal pool sizes were estimated for populations with HIV prevalence ranging from 0.25–10%. Based on these results, pooled testing methods were evaluated for screening patients admitted to hospital in San Diego, California. Evaluation of pooled antibody testing on samples collected from individuals with known HIV infection found only a modest reduction in sensitivity. These false negative results were only found among samples with very low optical density readings (<0.125 by the ADVIA Centaur® HIV assay). These readings are considered as HIV negative by the ADVIA Centaur® HIV assay, and therefore likely correspond to samples collected during acute infection. Further evaluation of pooled testing of samples collected from individuals during recent infection, found that mini-pool testing of five samples detected HIV antibody in 86% of samples taken within 60 days of the initial infection and 92% of samples taken within 90 days of the initial infection. Based on estimations of optimal pool sizes for low prevalence populations, it was decided to evaluate mini-pools consisting of 10 samples to screen the study's hospitalized patients. During this evaluation, the HIV prevalence among hospitalized patients was 0.8%, and the 10 sample mini-pool testing had 100% sensitivity and specificity. Additionally, pooled testing resulted in an 84.5% reduction in the number of rapid HIV antibody tests needed, as compared to testing each sample individually. Even when incorporating the increased costs of technician time, mini-pooled tested would have resulted in a net savings of 8760 USD for the 523 samples tested in the study. Taken together, these results indicate that pooled rapid antibody testing may reduce substantially the costs for HIV screening in low prevalence populations without a loss in accuracy.

Keywords

HIV screening; rapid antibody testing; pooled testing; cost-effective screening

© 2011 Elsevier B.V. All rights reserved.

Corresponding author: Sanjay R. Mehta MD, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0711 USA, Tel: +1 858 822 4092, Alt tel: +1 858 822 0333, Fax: +1 858 822 5362, srmehta@ucsd.edu.

*These authors contributed equally to this work

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Despite decades of HIV testing and prevention efforts, the Centers for Disease Control and Prevention estimates that 21% of individuals infected with HIV in the United States remain unaware of their infection (Campsmith et al., 2009). More complete identification of individuals infected with HIV is likely to be important for controlling the epidemic, as persons infected with HIV often reduce risk behavior when they become aware of their infection (Eisele et al., 2009; Kalichman et al., 2005; Kamb et al., 1998; Marks et al., 2005; Sweat et al., 2000; Weinhardt et al., 1999), and earlier treatment may reduce their infectivity (Granich et al., 2009). However, universal testing continues to be impeded by high costs, testing and notification procedures and difficulties in reaching underserved populations (Sanders et al., 2005). While point-of-care rapid HIV antibody tests (Branson et al., 2006; Delaney et al., 2004; Lyamuya et al., 2009; Pinkerton et al., 2009) have addressed the problem of notification delays and decentralized testing, they are more costly than a conventional enzyme linked immunoassay (EIA).

Pooling of blood samples for nucleic acid testing has been used to reduce the cost of screening for acute HIV infection in low prevalence populations (Pilcher et al., 2005; Roth et al., 2002; Sherlock et al., 2007), as well as a method for screening for virologic failure among individuals taking antiretroviral therapy (ART) (Smith et al., 2009). Analogous to these efforts, this study evaluated sample pooling (Dorfman, 1943; Soroka et al., 2003) with point-of-care rapid antibody tests as a method to reduce the cost of HIV screening in a low prevalence population.

2. Materials and methods

The study was conducted at the University of California San Diego Medical Center, and ethical approval for the study was obtained from the local Human Research Protections Program. Testing of patient plasma samples was performed for patients aged 13–64 years admitted to a San Diego hospital and who gave verbal consent during a period of HIV testing between October 2008 and October 2009 were used for this study. Throughout the study, the technicians who constructed the pools and performed the tests were blinded to the previously ascertained HIV serostatus of each of the samples. The OraQuick® Rapid HIV Test (OraSure Technologies, Bethlehem, PA) was the point-of-care rapid antibody test used for all testing. The ADVIA Centaur® HIV 1/O/2 Enhanced Assay (Bayer HealthCare, New York, USA) was used for EIA testing and to obtain optical density (OD) values. All testing was performed according to the manufacturer's instructions, and the results were interpreted as positive, negative or invalid.

2.1. Validation of rapid HIV antibody testing on pooled samples

Five HIV positive plasma samples and 95 HIV negative samples were used to form 20 mini-pools. A mini-pool was constituted by combining blood plasma from five samples and then mixing thoroughly with a micropipette. The minipool was then sampled using the specimen collection loop provided by the rapid antibody test. This sample was then tested per manufacturer's directions and the results were documented.

2.1.1. Terminal dilution testing—Terminal dilution analysis was performed to determine the maximum dilution at which plasma samples collected from individuals with chronic HIV infection would still test positive. This would also allow for a rough estimate for a maximum possible pool size. Initially, blood plasma samples from 5 subjects infected chronically were diluted 1:10 with plasma from 5 HIV negative individuals and tested for HIV antibody. These samples were then diluted serially 1:1 with additional HIV negative

plasma and tested for HIV antibody until the diluted sample was no longer HIV antibody positive, and this level of dilution was recorded.

2.1.2. Evaluation of pooling methods that incorporate samples with low antibody titers—The test characteristics of the pooling method were also evaluated among individuals from the acute HIV testing program (Morris et al., 2010) who were identified with HIV infection but with low titers of HIV antibodies detectable by the ADVIA Centaur® HIV 1/O/2 Enhanced Assay (Bayer HealthCare, New York, USA). The ADVIA Centaur® HIV 1/O/2 Enhanced Assay (Bayer HealthCare, New York, USA) detects HIV infection on average 26.7 days post-infection with an optical density (OD) index of >1.0 . This assay considers OD values <1.0 as ‘nonreactive’. This study evaluated 40 samples collected from individuals during acute infection with OD indices of <0.5 by the ADVIA Centaur® HIV 1/O/2 Enhanced Assay. These samples were divided into four groups of 10 samples based on OD readings of: 0–0.125, 0.126–0.250, 0.251–0.375, and 0.376–0.500. To simulate a 5 sample mini-pool, each sample was then diluted 1:4 with HIV antibody negative plasma and tested using the rapid point-of-care test (OraQuick® Rapid HIV Test).

2.1.3. Detection of primary HIV infection—To evaluate further the effect of specimen pooling on HIV antibody detection during primary infection, samples collected from individuals with primary infection (Morris et al., 2010) who had longitudinal samples available were evaluated. First, 12 individuals were identified with primary HIV infection and available plasma specimens were collected at longitudinal time points in the acute phase of infection prior to seroconversion, as determined by the ADVIA Centaur® HIV assay. The estimated duration of infection (EDI) for each individual was determined using published algorithms (Morris et al., 2010), and these data were then used to estimate the duration of infection for each sample collection. Samples that were estimated to be collected within the first 90 days after infection were evaluated. To simulate pooling, these samples were diluted 1:4 with HIV negative plasma and tested for HIV antibody using the rapid antibody test, per manufacturer’s instructions. The estimated duration of infection that corresponded to the timing of the earliest positive sample was recorded.

2.2. Estimation of optimal mini-pool sizes

Based on the evaluation of the pooling test characteristics, described above, the optimal pool sizes that would be required to screen populations with different HIV prevalence rates (0.25%–10%) were estimated. These calculations considered a range of pool sizes (3–10 samples/pool) and the sensitivity and specificity of the pooling methods. Additionally, the cost savings of using pooling approaches to screen a population of 1000 individuals with varying HIV prevalence rates (0.25–10%) was determined.

2.3. Evaluation of mini-pool HIV screening among hospitalized patients

After evaluating the test characteristics of the pooling approach, as described above, pooled antibody testing was evaluated in samples from hospitalized patients. During the hospital testing program, all samples were tested individually using the OraQuick® Rapid HIV Test and positives were considered ‘preliminary’ until repeat testing was performed by: a second rapid test (Uni-Gold™ Recombigen® HIV, Trinity Biotech plc, Wicklow, Ireland), enzyme immunoassay (EIA) with immunofluorescent antibody (Bio-Rad HIV-1/HIV-2 PLUS O EIA kit [Bio-Rad Laboratories, Redmond, WA]) and HIV Western blot (WB). HIV RNA quantification (Roche AMPLICOR™ HIV-1 Monitor Test [Roche Molecular Systems, Inc., Branchburg, NJ]) was also performed on the positive samples (Urassa et al., 2002). Plasma samples from this HIV testing program were then pooled and tested in a blinded fashion. Specifically, mini-pools were created using equal parts of plasma (30μL) from 10 patients

and each pool was tested for HIV antibody using the OraQuick® Rapid HIV Test. A pool size of 10 samples was chosen on the basis of the validation experiments to maximize cost-efficacy and maintain test sensitivity. Deconvolution to determine the positive samples within a positive pool, was performed by first splitting the pool into two mini-pools of 5 samples and testing each of these smaller minipools. All individual samples were then tested individually in any minipool of 5 samples that was positive, as described previously (Westreich et al., 2008). The results of the pooling methods were compared to the testing of samples individually and the characteristics of cost, sensitivity, specificity and 'number of tests saved' were determined.

3. Results

3.1. Validation of pooled antibody testing

When previously known HIV positive and negative samples were pooled and tested blindly, pooled antibody testing identified correctly all five mini-pools containing HIV positive samples and all 15 pools that were negative. Terminal dilution testing on those five HIV antibody positive samples demonstrated that all remained assay positive down to a dilution of 1:20, while one sample remained positive at a dilution of 1:160. Next, HIV positive samples with low antibody titers were tested in mini-pools of five samples, and of the 40 low titer samples that were evaluated, all samples (n=30) with OD indices >0.125 (ADVIA Centaur® HIV 1/O/2 Enhanced Assay, Bayer HealthCare) remained positive in pooled antibody testing. Only mini-pools containing two of the ten samples with OD indices ≤0.125 were identified as falsely negative. These false negative mini-pools contained plasma samples with very low OD indices of 0.04 and 0.01, which likely represented samples collected during acute HIV infection. These OD indices would be considered as 'nonreactive' by the ADVIA Centaur® assay, and therefore would have also been considered as false negative by the ADVIA Centaur® assay.

Since low OD indices are usually associated with early stage HIV infection (Janssen et al., 1998; Killian et al., 2006), pooled antibody testing at 1:4 dilution was performed using plasma samples collected from 12 individuals with primary HIV infection who were followed longitudinally (Morris et al., 2010). In samples collected at 90 days of EDI, eleven of the twelve subjects (92%) tested positive in the pooled testing of mini-pools of five samples. For the seven subjects for whom we had samples from within 60 days of EDI, six of the seven (86%) tested positive in pooled testing. Notably, the same individual whose sample collected at 60 days of EDI did not test positive in a mini-pool was the same individual whose sample collected at 90 days of EDI did not test positive in a mini-pool, and a sample collected at 45 days of EDI had an OD index of only 0.01. Based on previous work demonstrating the increasing rate of HIV antibody response over time, these results suggest that using mini-pools of ten samples to test for antibody would be expected to increase the 'window period' by no more than 2 weeks (Janssen et al., 1998; Killian et al., 2006; Novack et al., 2006; OraSure, 2004).

3.2. Pooled HIV antibody testing in patients admitted to hospital

Based on the studies described above, an evaluation of pooled antibody testing was performed for an inpatient hospital HIV testing program held between October 2008 and October 2009. During the study period, a total of 11,398 hospital admissions occurred, and 8,488 patients were found to be eligible. Of these eligible patients: 1,480 patients declined participation, 1,799 were unable to consent for unspecified reasons, and 3,672 did not undergo HIV testing (i.e. off-peak hours for testing, recent negative test, or unknown reason). In total, 1,537 HIV tests were ordered and 1,389 tests were completed. Of these completed tests, only 523 patients consented for storage of their blood plasma for further

testing, and of these, 6 were found to test HIV positive on initial individual rapid HIV antibody testing. On confirmatory testing, two of these samples had an 'invalid' result by a second rapid antibody test and tested negative by EIA and WB testing. These individuals were considered to be HIV uninfected (Owen et al., 2008). Taken together, the study population used to evaluate the pooled antibody methods had an HIV prevalence of 0.8% and a false positive rate of 0.4% on individual rapid antibody testing. These characteristics are consistent with the screening of low prevalence populations (Soroka et al., 2003).

Samples from the eligible population (n=523) were tested for antibody in pools of 10 samples. Pools were constructed in chronologic order of sample collection, and the testers were blinded to the individual rapid antibody test results. These methods detected correctly the four positive and the 49 negative pools. The two samples that tested positive by individual rapid antibody testing, but identified by confirmatory EIA and western blot as negative, were correctly identified as negative by the pooling study. Based on these results, pooled antibody testing had both sensitivity and specificity at 100% using the EIA testing/WB as the gold standard, while the initial rapid antibody testing of the individual samples had a sensitivity of 100% and specificity of 99.6%.

In this population with a low prevalence of HIV infection, the pooling strategy of 10 sample mini-pools and stepwise deconvolution of positive pools used of 81 rapid HIV antibody tests to identify all of the HIV positive individuals. This is in contrast to the 523 tests that were required to test each sample individually. This pooling strategy represented a savings of 84.5% in the number of tests used and a cost savings of 8,760 USD when estimated extra labor costs are included (four hours at 20 USD/hr). If a strategy of five sample mini-pools was used, with a one-time pool deconvolution in which all samples in the positive pools are tested at once (figure 1a), 125 tests would have been required, i.e. 76% reduction in the number of tests used. Simulating the use of different pooling strategies with varying HIV prevalence rates, it was found that in general, pooled rapid antibody testing could provide a considerable reduction (>40%) in the number of tests used as long as HIV prevalence in the screened population was less than 10% (Figure 1b). Of course, the estimated optimal size of the mini-pools and the costs saved varied based on the prevalence of HIV in the population screened (Soroka et al., 2003; Stekler et al., 2009) (Figures 1 and 2).

4. Discussion

Pooled antibody testing using 10 sample mini-pools in a low prevalence population of hospitalized patients resulted in equal sensitivity and better specificity than standard individual HIV testing. This increase in specificity by the pooling method is likely due to the reduced sensitivity of the rapid antibody assay using a pooled specimen. Although an expected effect of sample pooling is to improve specificity while reducing sensitivity, with tests that have very high sensitivity and specificity this reduction in sensitivity may be negligible, as is most likely demonstrated in this study. Overall, these results suggest that pooling of samples will be a successful strategy when using a rapid HIV antibody test for screening lower-risk and lower-prevalence populations, rather than for use for diagnosis, where maximizing sensitivity is most important.

This pooling strategy also resulted in an 84.5% savings in the number of tests required to identify the persons who are HIV positive, as compared to testing each sample individually. This decrease in the number of tests utilized would have resulted in a significant cost savings, approximately 22994 USD or 60% of the cost of the original testing program. Although these cost estimates include pooling labor as an increase in overall costs, pooling strategies could also provide savings in labor costs. Rather than having laboratory personnel perform a rapid test at the time of ordering each test, pooled testing of samples could be

performed at the end of a pre-specified time period that would depend upon the testing volume. Resolution of the test result for each sample in a positive pool, or deconvolution of the pool, would take extra time. However, personnel could vary the strategy used to deconvolute the pool to optimize efficiency. For example, a one-time deconvolution strategy, where each sample in the pool is tested at once, could be used when pool sizes are small (e.g. 5 or less samples) or when personnel time is limited. A stepwise strategy could be used when pool sizes are larger, and the reduction in test kits used would compensate for the increased laboratory personnel time. The maximum extra laboratory personnel time required with a pooling strategy would be approximately 30 min for each positive sample for a one-time deconvolution (e.g. pool sizes of 5 or smaller) and 60 min for each positive sample using a step-wise deconvolution (e.g. pool sizes of >5). Therefore, in unscreened populations with a high HIV prevalence even a one-time deconvolution strategy could increase laboratory personnel time significantly. For example, there would be an increase of 25 hours of time for a 5% prevalence in 1000 individuals, but even at a personnel cost of 20 USD an hour (i.e. \$500 for the example), the cost savings from the reduction in kits used (i.e. 11,000 USD for the example) would far outweigh the increase in labor costs. Figure 2 demonstrates the cost savings in testing kits associated with a pooling strategy over a range of populations with different HIV prevalences. Overall, the optimal strategy will differ between screening centers and will likely depend upon: test volume, cost of the test, and the cost of lab personnel time.

As shown in Figure 1, in higher prevalence populations the optimal pool size is smaller, and if the prevalence of HIV in the screened population is greater than 10%, the benefits of pooling are much smaller (Soroka et al., 2003). This loss of benefit is because of the increase in both number of kits required and laboratory personnel time. In addition, for high incidence populations, the likelihood of missing recently HIV infected individuals is likely to be higher using pooling methods (Novack et al., 2006; Soroka et al., 2003). All of these factors should be considered when choosing an HIV screening strategy.

Finally, an important limitation of the pooled rapid antibody testing method is the potential for delay in the turnaround time for the result of the test. Many testing centers use a centralized laboratory, which can often delay the reporting of the results by at least a day. This delay is usually dependent on the proximity of the testing center to the central laboratory. Point-of-care rapid antibody tests, which are simple enough to perform that there is little risk of error and thus can be performed in Clinical Laboratory Improved Amendments (CLIA) waived laboratories, have practically solved this issue, which is the reason rapid assays were evaluated in the pooling methods. However, the benefits of point-of-care screening could be negated somewhat by these pooling methods, since the test results may be delayed. Also, the use of plasma instead of a buccal swab and the pooling and deconvolution procedures would add complexity to the testing procedures. This complexity could result in the loss of the CLIA waiver. In a high volume setting with numerous persons screened each hour, however, the turnaround time should be relatively short as the laboratory could rapidly obtain enough samples to form a pool. In low volume settings where the time between patients being screened is large, the use of pooling could delay significantly the results. A delay of greater than a day would likely negate some of the benefits of using rapid HIV testing, but a flexible approach in which the number of samples incorporated into a pool varied with the volume of subjects to be screened would perhaps overcome much of this limitation.

4.1. Conclusion

Overall, the results demonstrate that pooled antibody testing can reduce costs significantly when screening populations with a low prevalence of HIV.

Acknowledgments

We would like to thank Tari Gilbert, Paula Potter and Joanne Santangelo for their clinical assistance and Jason Young and DeeDee Pacheco for their technical assistance. This work was supported by National Institutes of Health grants MH083552, AI077304, AI69432, MH62512, AI27670, AI38858, AI43638, AI43752, AI047745, NS51132, UCSD Centers for AIDS Research Translational Virology Core (AI36214), AI36214, AI080353, AI29164, AI47745, AI047745, AI 064086, AI57167, AI087164, the James B. Pendleton Foundation, and the San Diego Veterans Affairs Healthcare System

References

- Branson BM, Handsfield HH, Lampe MA, Janssen RS, Taylor AW, Lyss SB, Clark JE. Revised recommendations for HIV testing of adults, adolescents, and pregnant women in health-care settings. *MMWR Recomm Rep*. 2006; 55:1–17. quiz CE1–4. [PubMed: 16988643]
- Campsmith ML, Rhodes PH, Hall HI, Green TA. Undiagnosed HIV prevalence among adults and adolescents in the United States at the end of 2006. *J Acquir Immune Defic Syndr*. 2009; 53:619–624. [PubMed: 19838124]
- Delaney KP, Branson BM, Uniyal A, Phillips S, Candal D, Owen SM, Kerndt PR. Evaluation of the Performance Characteristics of 6 Rapid HIV Antibody Tests. *Clin Infect Dis*. 2004; 52:257–263. [PubMed: 21288553]
- Dorfman R. The Detection of Defective Members of Large Populations. *Annals of Mathematical Statistics*. 1943; 14:436–440.
- Eisele TP, Mathews C, Chopra M, Lurie MN, Brown L, Dewing S, Kendall C. Changes in risk behavior among HIV-positive patients during their first year of antiretroviral therapy in Cape Town South Africa. *AIDS Behav*. 2009; 13:1097–1105. [PubMed: 18846418]
- Granich RM, Gilks CF, Dye C, De Cock KM, Williams BG. Universal voluntary HIV testing with immediate antiretroviral therapy as a strategy for elimination of HIV transmission: a mathematical model. *Lancet*. 2009; 373:48–57. [PubMed: 19038438]
- Janssen RS, Satten GA, Stramer SL, Rawal BD, O'Brien TR, Weiblen BJ, Hecht FM, Jack N, Cleghorn FR, Kahn JO, Chesney MA, Busch MP. New testing strategy to detect early HIV-1 infection for use in incidence estimates and for clinical and prevention purposes. *JAMA*. 1998; 280:42–48. [PubMed: 9660362]
- Kalichman SC, Rompa D, Cage M. Group intervention to reduce HIV transmission risk behavior among persons living with HIV/AIDS. *Behav Modif*. 2005; 29:256–285. [PubMed: 15657411]
- Kamb ML, Fishbein M, Douglas JM Jr, Rhodes F, Rogers J, Bolan G, Zenilman J, Hoxworth T, Malotte CK, Iatesta M, Kent C, Lentz A, Graziano S, Byers RH, Peterman TA. Efficacy of risk-reduction counseling to prevent human immunodeficiency virus and sexually transmitted diseases: a randomized controlled trial. Project RESPECT Study Group. *JAMA*. 1998; 280:1161–1167. [PubMed: 9777816]
- Killian MS, Norris PJ, Rawal BD, Lebedeva M, Hecht FM, Levy JA, Busch MP. The effects of early antiretroviral therapy and its discontinuation on the HIV-specific antibody response. *AIDS Res Hum Retroviruses*. 2006; 22:640–647. [PubMed: 16831088]
- Lyamuya EF, Aboud S, Urassa WK, Sufi J, Mbwana J, Ndugulile F, Massambu C. Evaluation of simple rapid HIV assays and development of national rapid HIV test algorithms in Dar es Salaam, Tanzania. *BMC Infect Dis*. 2009; 9:19. [PubMed: 19226452]
- Marks G, Crepaz N, Senterfitt JW, Janssen RS. Meta-analysis of high-risk sexual behavior in persons aware and unaware they are infected with HIV in the United States: implications for HIV prevention programs. *J Acquir Immune Defic Syndr*. 2005; 39:446–453. [PubMed: 16010168]
- Morris SR, Little SJ, Cunningham T, Garfein RS, Richman DD, Smith DM. Evaluation of an HIV nucleic acid testing program with automated Internet and voicemail systems to deliver results. *Ann Intern Med*. 2010; 152:778–785. [PubMed: 20547906]
- Novack L, Galai N, Yaari A, Orgel M, Shinar E, Sarov B. Use of seroconversion panels to estimate delay in detection of anti-human immunodeficiency virus antibodies by enzyme-linked immunosorbent assay of pooled compared to singleton serum samples. *J Clin Microbiol*. 2006; 44:2909–2913. [PubMed: 16891511]

- O.T. Inc., editor. OraSure. OraQuick Rapid HIV-1 Antibody Test. Bethlehem: OraSure; 2004.
- Owen SM, Yang C, Spira T, Ou CY, Pau CP, Parekh BS, Candal D, Kuehl D, Kennedy MS, Rudolph D, Luo W, Delatorre N, Masciotra S, Kalish ML, Cowart F, Barnett T, Lal R, McDougal JS. Alternative algorithms for human immunodeficiency virus infection diagnosis using tests that are licensed in the United States. *J Clin Microbiol.* 2008; 46:1588–1595. [PubMed: 18322061]
- Pilcher CD, Fiscus SA, Nguyen TQ, Foust E, Wolf L, Williams D, Ashby R, O'Dowd JO, McPherson JT, Stalzer B, Hightow L, Miller WC, Eron JJ Jr, Cohen MS, Leone PA. Detection of acute infections during HIV testing in North Carolina. *N Engl J Med.* 2005; 352:1873–1883. [PubMed: 15872202]
- Pinkerton SD, Bogart LM, Howerton D, Snyder S, Becker K, Asch SM. Cost of OraQuick oral fluid rapid HIV testing at 35 community clinics and community-based organizations in the USA. *AIDS Care.* 2009; 21:1157–1162. [PubMed: 20024775]
- Roth WK, Weber M, Buhr S, Drosten C, Weichert W, Sireis W, Hedges D, Seifried E. Yield of HCV and HIV-1 NAT after screening of 3.6 million blood donations in central Europe. *Transfusion.* 2002; 42:862–868. [PubMed: 12375658]
- Sanders GD, Bayoumi AM, Sundaram V, Bilir SP, Neukermans CP, Rydzak CE, Douglass LR, Lazzeroni LC, Holodniy M, Owens DK. Cost-effectiveness of screening for HIV in the era of highly active antiretroviral therapy. *N Engl J Med.* 2005; 352:570–585. [PubMed: 15703422]
- Sherlock M, Zetola NM, Klausner JD. Routine detection of acute HIV infection through RNA pooling: survey of current practice in the United States. *Sex Transm Dis.* 2007; 34:314–316. [PubMed: 17483725]
- Smith DM, May SJ, Perez-Santiago J, Strain MC, Ignacio CC, Haubrich RH, Richman DD, Benson CA, Little SJ. The use of pooled viral load testing to identify antiretroviral treatment failure. *AIDS.* 2009; 23:2151–2158. [PubMed: 19730348]
- Soroka SD, Granade TC, Phillips S, Parekh B. The use of simple, rapid tests to detect antibodies to human immunodeficiency virus types 1 and 2 in pooled serum specimens. *J Clin Virol.* 2003; 27:90–96. [PubMed: 12727534]
- Stekler JD, Swenson PD, Coombs RW, Dragavon J, Thomas KK, Brennan CA, Devare SG, Wood RW, Golden MR. HIV testing in a high-incidence population: is antibody testing alone good enough? *Clin Infect Dis.* 2009; 49:444–453. [PubMed: 19538088]
- Sweat M, Gregorich S, Sangiwa G, Furlonge C, Balmer D, Kamenga C, Grinstead O, Coates T. Cost-effectiveness of voluntary HIV-1 counselling and testing in reducing sexual transmission of HIV-1 in Kenya and Tanzania. *Lancet.* 2000; 356:113–121. [PubMed: 10963247]
- Urassa W, Nozohoor S, Jaffer S, Karama K, Mhalu F, Biberfeld G. Evaluation of an alternative confirmatory strategy for the diagnosis of HIV infection in Dar Es Salaam, Tanzania, based on simple rapid assays. *J Virol Methods.* 2002; 100:115–120. [PubMed: 11742658]
- Weinhardt LS, Carey MP, Johnson BT, Bickham NL. Effects of HIV counseling and testing on sexual risk behavior: a meta-analytic review of published research, 1985–1997. *Am J Public Health.* 1999; 89:1397–1405. [PubMed: 10474559]
- Westreich DJ, Hudgens MG, Fiscus SA, Pilcher CD. Optimizing screening for acute human immunodeficiency virus infection with pooled nucleic acid amplification tests. *J Clin Microbiol.* 2008; 46:1785–1792. [PubMed: 18353930]

Figure 1.

Maximum number of tests required for screening 1000 samples based on levels of HIV prevalence using pool sizes ranging from 3 to 10 samples. Figure 1a demonstrates the maximum number of tests required using step-wise pool deconvolution strategy for pools testing positive. In this strategy, two equal-sized mini-pools are created from the samples that comprised the original pool that tested positive. Both of the mini-pools are tested, and then each of the samples that comprised the mini-pool that tested positive is tested individually at one time. Figure 1b demonstrates the maximum number of tests required using a total pool deconvolution strategy, in which all of the samples that comprised a positive pool are tested individually at one time.

Figure 2.

Minimum cost savings associated with using a pooling strategy for screening 1000 samples based on a range of HIV prevalences in the population. This analysis estimated the cost of 20 USD per point-of-care rapid antibody test and does not account for labor and time costs to perform test. Calculations for a stepwise pool deconvolution method are shown in Figure 2a and for the one-time total pool deconvolution method in Figure 2b.