



Short communication

Performance of a transcription-mediated-amplification HIV-1 RNA assay in pooled specimens

Nicola M. Zetola^a, Ann Mintie^b, Sally Liska^b, Ernest Wong^b,
Joan Dunn Williams^c, Michael P. Busch^d, Jeffrey D. Klausner^{e,*}

^a Division of Infectious Diseases, University of California-San Francisco, San Francisco, CA, United States

^b San Francisco Department of Public Health, Public Health Laboratory, San Francisco, CA, United States

^c Blood Systems Laboratory, Tempe, AZ, United States

^d Blood Systems Research Institute, San Francisco, CA, United States

^e San Francisco Department of Public Health, STD Prevention and Control Services, San Francisco, CA, United States

Received 4 June 2007; accepted 11 June 2007

Abstract

Background: Very limited data exist on the comparative performance of nucleic acid amplification tests (NATs) for the screening of pooled specimens for acute human immunodeficiency virus (HIV) infection.

Study design: In this study, we compared a transcription-mediated-amplification assay (Procleix[®] HIV-1 Discriminatory Assay, [TMA]) with a branched DNA assay (Bayer Versant HIV-1 RNA 3.0 assay, [bDNA]).

Results: After re-testing 1552 samples that were negative for HIV RNA by bDNA, we found one additional positive sample with the TMA assay.

Conclusion: Our results suggest that TMA could potentially detect acute HIV infections missed by other technologies.

© 2007 Elsevier B.V. All rights reserved.

Keywords: HIV; Pooled testing; TMA; bDNA

1. Background

Public health agencies have recently recognized the value of detecting patients with acute HIV infection (Pilcher et al., 2002). Accordingly, several public health departments are routinely screening pooled human immunodeficiency virus (HIV) antibody-negative specimens with HIV nucleic acid amplification test (NAT) assays (Liska, 2005; Patel et al., 2006; Pilcher et al., 2002; Shahkolahi, 2006; Stekler et al., 2005). In this approach, all blood samples are initially screened for HIV antibodies. Seronegative specimens

are then pooled into groups and tested by NAT. A negative pool ends the testing protocol. In the event of a reactive specimen pool, that pool is deconstructed and individual blood samples are re-tested in order to identify the unique patient sample with the positive result. The identification of antibody-negative, RNA-positive patients confirms the diagnosis of acute HIV infection (Centers for Disease Control and Prevention, 1997; Coste et al., 2005; Stramer et al., 2004).

Currently, sample-pooling protocols vary significantly in terms of the size of the master pool and subsequent intermediate pools. Unfortunately, very few data exist on the comparative performance of different NAT assays when used for HIV-RNA detection by sample-pooling protocols (Busch et al., 2005). As a consequence, multiple different NAT assays are currently being used by public health agencies. In this study we evaluated the sensitivity of Procleix[®] HIV-1 Discriminatory Assay (a transcription-mediated-amplification

* Corresponding author at: STD Prevention and Control Services, San Francisco Department of Public Health, 1360 Mission Street, Suite #401, San Francisco, CA 94103, United States. Tel.: +1 415 355 2000; fax: +1 415 554 9636.

E-mail address: Jeff.Klausner@sfdph.org (J.D. Klausner).

[TMA] assay manufactured by Gen-Probe Incorporated and distributed by Chiron Corp.) (Procleix[®] HIV-1/HCV Assay; Giachetti et al., 2002). The HIV-1 Discriminatory assay is part of the Procleix[®] HIV-1/HCV assay which is routinely used for blood donor screening. This assay consists of the same formulations as the recently cleared APTIMA[®] HIV-1 RNA Qualitative Assay (Gen-Probe Incorporated, San Diego CA), an *in vitro* nucleic acid assay system for the detection of HIV-1 in human plasma which is intended for use as an aid in the diagnosis of HIV-1 infection, including acute infection. Presence of HIV-1 RNA in the plasma of patients without antibodies to HIV-1 is indicative of acute HIV-1 infection and may also be used as an additional test, when it is reactive, to confirm HIV-1 infection in an individual whose specimen is repeatedly reactive for HIV-1 antibodies (APTIMA[®] HIV-1 RNA Assay).

Since the APTIMA[®] HIV-1 RNA Qualitative Assay is the only FDA cleared assay for diagnosis of acute infection in HIV antibody-negative samples, we were interested in evaluating samples previously tested negative with the Bayer Versant HIV-1 RNA 3.0 assay (bDNA).

2. Study design

To determine the performance of the TMA HIV-1 Assay on the yield of acute HIV detection in public health practice, we re-tested with this assay all HIV antibody-negative and HIV RNA-negative samples obtained from patients seen at San Francisco City Clinic at the San Francisco Department of Public Health, between November 2003 and December 2004.

Plasma was separated from the whole blood drawn in EDTA tubes, at the San Francisco Department of Public Health Public Health Laboratory within 12 h of collection. The standard Vironostika HIV type 1 enzyme immunoassay (bioMerieux) was used for antibody screening. Remnant plasma was stored frozen at -70°C until tested for HIV RNA.

HIV antibody-negative samples were routinely tested in pools of 10 with the Bayer Versant HIV-1 RNA 3.0 assay (bDNA) following the procedures recommended by the manufacturer (Versant HIV-1 RNA 3.0 Assay, 2002–2004). HIV antibody-negative samples by Vironostika HIV type 1 enzyme immunoassay and HIV RNA-positive by bDNA assay were re-tested by Genetic SystemsTM HIV-1/HIV-2 PLUS O enzyme immunoassay (Bio-Rad Laboratories) to confirm their antibody-negative status. Specimens from patients with initially HIV RNA-positive samples were re-tested and confirmed either by repeatedly measurable HIV RNA (bDNA) or HIV-1 antibody seroconversion. As part of the study, HIV RNA-negative samples by this protocol were re-tested in pools of 8 at the Blood System Laboratories in Tempe, Arizona, using the TMA HIV-1 Assay (Procleix[®] HIV-1/HCV Assay; Giachetti et al., 2002). The University of California San Francisco Committee on Human Research approved this study and waived patient consent requirements.

3. Results

A total of 1571 antibody-negative or indeterminate plasma samples were tested in pools of 10 with the Bayer Versant HIV-1 RNA 3.0 assay (bDNA). Nineteen samples were found to be HIV RNA-positive by bDNA testing and confirmed upon re-testing. Samples of those 19 specimens were re-tested by Genetic SystemsTM HIV-1/HIV-2 PLUS O enzyme immunoassay and 9 of them were confirmed to be antibody-negative. Among those 9 patients, viral loads ranged between 1177 and $>500,000$ copies/mL. A total of 1552 HIV RNA-negative samples by the bDNA assay were re-tested in pools of 8 with the TMA HIV-1 Assay. One additional HIV RNA-positive sample was identified and verified as positive upon re-testing.

4. Discussion

Very few data exist on the performance of different NAT assays for acute HIV infection screening using sample-pooling protocols. Procleix[®] HIV-1 Discriminatory Assay, a test developed as part of the Procleix[®] HIV-1/HCV Assay, which is widely implemented in blood donor screening using pools of up to 16 donations (Stramer et al., 2004), is similar to the APTIMA[®] HIV-1 RNA Qualitative Assay cleared by the FDA for the diagnosis of HIV infection, including detection of acute antibody-negative infections and confirmation of infection when samples have test reactive by a licensed HIV antibody screening assay. With a 50% detection limit of 5.0 copies/mL (confidence interval [CI], 4.5–5.5) and a 95% detection limit of 13.1 copies/mL (CI, 12.1–14.4) (Giachetti et al., 2002; Lelie et al., 2002), this qualitative TMA HIV-1 assay uses a target capture specimen processing system that may offer the potential advantage of decreased false negative results by removing inhibitors and reduced contamination by targeting a specific sequence not included in the amplicon produced by TMA (APTIMA[®] HIV-1 RNA Assay; Kolk et al., 2002; Linnen et al., 2002). On the other hand, Bayer Versant HIV-1 RNA 3.0 bDNA assay has a 95% detection limit of 68 HIV-1 RNA copies/mL (CI, 50–73) (Versant HIV-1 RNA 3.0 Assay, 2002–2004; Collins et al., 1997). Similarly to the TMA HIV-1 Assay, the bDNA assay has shown good correlation with other NAT assays (Elbeik et al., 2000; Galli et al., 2005) and shows a high degree of precision (Kern et al., 1996). However, samples containing less than 50 HIV RNA copies/mL are read as “negative” by the bDNA assay. Whereas, the importance of detecting less than 50 copies/mL when monitoring the response to therapy of patients on antiretroviral therapy is controversial, the detection of low levels of viremia might be of critical importance when testing highly diluted pools of samples. Although the TMA HIV-1 Assay has shown better analytical sensitivity for HIV-1 RNA detection in individual and pooled samples, when compared with other NAT assays (Busch et al., 2005; Lelie et al., 2002), no studies have compared its performance

with the bDNA assay, when used for the screening of acute HIV infections by sample-pooling protocols.

Our results indicate that the TMA HIV-1 Assay could potentially detect newly HIV infected patients missed by other technologies. This likely reflects the better analytical sensitivity of the TMA assay, compared to bDNA. Although the different number of samples pooled together for testing by the two different assays could have affected the results, it seems unlikely given the rather small difference between the two protocols (pooling of 8 versus 10 samples). The relatively low incremental yield of the TMA HIV-1 Assay relative to bDNA is likely a reflection of the relatively small sample size and the high viral load of acutely infected persons detected in high risk screening settings such as STD clinics (Busch and Hecht, 2005; Stekler et al., 2005).

Differences likely exist in the performance of different NAT assays when used for the testing of pooled samples. Given the paucity of data, the technology with the highest performance in this setting remains to be determined. As HIV RNA screening of pooled patient samples becomes more common, studies comparing the performance of different assays and protocols will be required.

Acknowledgment

We thank Cristina Giachetti for her critical review and comments to this manuscript.

Financial Support: This project was supported in part by the Universitywide AIDS Research Program grant CH05-SMCHC-612 and the San Francisco Department of Public Health. Gen-Probe, Inc. supplied reagents for HIV Procleix HIV-1 Discriminatory RNA testing at no cost to the study.

References

- APTIMA® HIV-1 RNA Assay. Package insert. Gen-Probe Incorporated, San Diego, California.
- Centers for Disease Control and Prevention. Performance evaluation programs for determining HIV-1 viral loads, testing for HIV p24 antigen, and identifying Mycobacterium tuberculosis using nucleic acid amplification tests. *JAMA* 1997;277:786.
- Procleix® HIV-1/HCV Assay. Package insert. Gen-Probe Incorporated, San Diego, California.
- Versant HIV-1 RNA 3.0 Assay (bDNA) [U.S. package insert], 2002–2004. Tarrytown, NY: Bayer HealthCare.
- Busch MP, Glynn SA, Wright DJ, Hirschhorn D, Laycock ME, McAuley J, et al. Relative sensitivities of licensed nucleic acid amplification tests for detection of viremia in early human immunodeficiency virus and hepatitis C virus infection. *Transfusion* 2005;45:1853–63.
- Busch MP, Hecht FM. Nucleic acid amplification testing for diagnosis of acute HIV infection: has the time come? *AIDS* 2005;19:1317–9.
- Collins ML, Irvine B, Tyner D, Fine E, Zayati C, Chang C, et al. A branched DNA signal amplification assay for quantification of nucleic acid targets below 100 molecules/ml. *Nucleic Acids Res* 1997;25:2979–84.
- Coste J, Reesink HW, Engelfriet CP, Laperche S, Brown S, Busch MP, et al. Implementation of donor screening for infectious agents transmitted by blood by nucleic acid technology: update to 2003. *Vox Sang* 2005;88:289–303.
- Elbeik T, Charlebois E, Nassos P, Kahn J, Hecht FM, Yajko D, et al. Quantitative and cost comparison of ultrasensitive human immunodeficiency virus type 1 RNA viral load assays: Bayer bDNA quantiplex versions 3.0 and 2.0 and Roche PCR Amplicor monitor version 1.5. *J Clin Microbiol* 2000;38:1113–20.
- Galli R, Merrick L, Friesenhahn M, Ziermann R. Comprehensive comparison of the VERSANT HIV-1 RNA 3.0 (bDNA) and COBAS AMPLICOR HIV-1 MONITOR 1.5 assays on 1,000 clinical specimens. *J Clin Virol* 2005;34:245–52.
- Giachetti C, Linnen JM, Kolk DP, Dockter J, Gillotte-Taylor K, Park M, et al. Highly sensitive multiplex assay for detection of human immunodeficiency virus type 1 and hepatitis C virus RNA. *J Clin Microbiol* 2002;40:2408–19.
- Kern D, Collins M, Fultz T, Detmer J, Hamren S, Peterkin JJ, et al. An enhanced-sensitivity branched-DNA assay for quantification of human immunodeficiency virus type 1 RNA in plasma. *J Clin Microbiol* 1996;34:3196–202.
- Kolk DP, Dockter J, Linnen J, Ho-Sing-Loy M, Gillotte-Taylor K, McDonough SH, et al. Significant closure of the human immunodeficiency virus type 1 and hepatitis C virus preseroconversion detection windows with a transcription-mediated-amplification-driven assay. *J Clin Microbiol* 2002;40:1761–6.
- Lelie PN, van Drimmelen HA, Cuypers HT, Best SJ, Stramer SL, Hyland C, et al. Sensitivity of HCV RNA and HIV RNA blood screening assays. *Transfusion* 2002;42:527–36.
- Linnen JM, Gilker JM, Menez A, Vaughn A, Broulik A, Dockter J, et al. Sensitive detection of genetic variants of HIV-1 and HCV with an HIV-1/HCV assay based on transcription-mediated amplification. *J Virol Methods* 2002;102:139–55.
- Liska, S. 2005. Pooled RNA testing of antibody negative high-risk persons. San Francisco and Los Angeles.
- Patel P, Klausner JD, Bacon OM, Liska S, Taylor M, Gonzalez A, et al. Detection of acute HIV infections in high-risk patients in California. *J Acquir Immune Defic Syndr* 2006;42:75–9.
- Pilcher CD, McPherson JT, Leone PA, Smurzynski M, Owen-O'Dowd J, Peace-Brewer AL, et al. Real-time, universal screening for acute HIV infection in a routine HIV counseling and testing population. *JAMA* 2002;288:216–21.
- Shahkolahi AM. Early diagnosis of HIV infection at Whitman Walker clinic STD program using NAATs on pooled blood samples. In: 2006 National STD Prevention Conference; 2006.
- Stekler J, Swenson PD, Wood RW, Handsfield HH, Golden MR. Targeted screening for primary HIV infection through pooled HIV-RNA testing in men who have sex with men. *AIDS* 2005;19:1323–5.
- Stramer SL, Glynn SA, Kleinman SH, Strong DM, Caglioti S, Wright DJ, et al. Detection of HIV-1 and HCV infections among antibody-negative blood donors by nucleic acid-amplification testing. *N Engl J Med* 2004;351:760–8.