JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2011, p. 1642–1645 Vol. 49, No. 4
0095-1137/11/$12.00 doi:10.1128/JCM.02183-10

Evaluation of the Gen-Probe Aptima HIV-1 RNA Qualitative Assay as an Alternative to Western Blot Analysis for Confirmation of HIV Infection▽

Virginia M. Pierce,[1,2] Brandy Neide,[2] and Richard L. Hodinka[1,2]*

Division of Infectious Diseases, Department of Pediatrics,[1] and Clinical Virology Laboratory, Department of Pathology and Laboratory Medicine,[2] Children's Hospital of Philadelphia and University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

Received 28 October 2010/Returned for modification 29 November 2010/Accepted 10 February 2011

The Gen-Probe Aptima HIV-1 RNA qualitative assay was evaluated as an alternative to Western blot analysis for the confirmation of HIV infection using serum samples that were repeatedly reactive for HIV antibodies. The Aptima HIV assay readily discriminated between HIV-1-infected and -uninfected individuals and effectively reduced the number of indeterminate results relative to Western blot analysis.

For over 20 years, the standard algorithm for diagnosis of human immunodeficiency virus (HIV) infection in the United States has remained a sequential two-step process. Screening for HIV-specific antibodies is initially accomplished using an enzyme immunoassay (EIA), which is followed by the performance of a confirmatory Western blot (WB) analysis or, less commonly, an immunofluorescence assay (IFA) for all samples that are repeatedly reactive by the EIA (6). Since this algorithm was first recommended, there have been significant advances in HIV diagnostics, in terms of both improved sensitivity and specificity of available tests and the development of new assays utilizing distinct technologies (2). There have also been changes in the population for whom HIV testing is advised. In 2006, the Centers for Disease Control and Prevention (CDC) recommended expansion from an approach of targeted testing of higher-risk groups to a strategy that also incorporates routine, universal screening of all patients aged 13 to 64 years (3). Taken together, these developments necessitate a reconsideration of how the diagnostic armamentarium can best be employed to accurately discriminate between HIV-1-infected and -uninfected individuals (13, 15).

With this objective, the CDC and the Association of Public Health Laboratories (APHL) recently convened working groups of HIV diagnostic experts to generate alternative testing algorithms. Their 2009 report proposed the use of multiple, rapid point-of-care HIV antibody tests or various combinations of more complex laboratory-based immunoassays and molecular tests for detection of antibodies or antigens and nucleic acids, respectively, to augment or possibly replace the standard combination of EIA and WB analysis in various testing settings (1). Of the strategies developed for laboratory-based HIV testing, algorithm 2 within the report most closely resembles the traditional scheme generally used in clinical laboratories and comprises an EIA as a screening test for HIV-1/HIV-2 antibodies, followed by an HIV-1 WB or IFA confirmation for repeatedly reactive samples, with the option for laboratories to substitute a nucleic acid amplification test (NAAT) as the confirmatory assay. The report acknowledges the necessity of further data, including results comparing NAATs with supplemental confirmatory antibody tests, before a formal recommendation can be made to replace the traditional testing approach with this or any of the other proposed testing algorithms. With the present study, we summarize our clinical experience in utilizing the Aptima HIV-1 RNA qualitative assay (Gen-Probe, Incorporated, San Diego, CA) for confirmation of HIV infection in individuals with samples found to be repeatedly reactive for HIV antibodies by a screening EIA.

All serum samples submitted to the Clinical Virology Laboratory at the Children's Hospital of Philadelphia between September 2008 and July 2010 for HIV antibody screening were assessed for potential inclusion in this study. The work was deemed not to be human subject research and was declared to be exempt by the institutional review board at the Children's Hospital of Philadelphia. Specimens were prospectively screened for HIV by a laboratory-based EIA [HIVAB HIV-1/HIV-2 (rDNA) EIA; Abbott Laboratories, Abbott Park, IL], and repeatedly reactive samples were eligible if sufficient specimen volume remained for performance of both WB and Aptima HIV analyses. Sera were stored at 4°C from the time of arrival in the laboratory until completion of the EIA. When a sample was found to be repeatedly reactive by EIA, an aliquot was prepared and stored at −70°C for testing by the Aptima HIV assay and the remainder of the specimen was maintained at 4°C pending completion of the WB analysis. A total of 120 samples met the eligibility criteria for testing, of which 70 were determined to be from HIV-infected patients and 50 were from individuals not infected with HIV. The HIV infection status of each patient was carefully determined by a retrospective review of clinical history and an examination of all HIV-related laboratory testing ordered for individuals as part of their medical care and management, including results obtained from HIV-1 culture, qualitative HIV-1 proviral DNA

* Corresponding author. Mailing address: Clinical Virology Laboratory, Room 5NW39, Main Building, Children's Hospital of Philadelphia, 34th Street and Civic Center Boulevard, Philadelphia, PA 19104. Phone: (215) 590-2028. Fax: (215) 590-2556. E-mail: hodinka@email.chop.edu.

▽ Published ahead of print on 23 February 2011.

TABLE 1. Comparison of Aptima HIV and WB results for HIV-infected patients

Aptima HIV result	No. of samples with WB result:		
	Reactive	Nonreactive	Indeterminate
Reactive	66	0	3
Nonreactive	1	0	0

TABLE 2. Comparison of Aptima HIV and WB results for HIV-uninfected patients

Aptima HIV result	No. of samples with WB result:		
	Reactive	Nonreactive	Indeterminate
Reactive	0	0	0
Nonreactive	20	2	28

PCR analysis, quantitative measurements of HIV-1 RNA, and serial monitoring of HIV antibody over time. Patients were considered to be infected with HIV-1 if, in addition to their having positive serologic testing, the virus was grown in culture and/or nucleic acid was detected using qualitative or quantitative molecular assays. For the 70 patients infected with HIV, the mean age was 21.0 years, with a range of 3 months to 45 years; 2 of the infected patients were perinatally exposed infants diagnosed with HIV-1 infection during infancy. The population of HIV-uninfected patients was unique in that 48 of 50 individuals were HIV-exposed, antibody-positive infants <18 months of age (mean, 12 months; range, 21 days to 17 months) who were born to HIV-infected mothers and were at various stages of seroreversion (7) at the time of this study; the other 2 patients in this group were teenagers at low risk for HIV infection.

WB testing was performed with 20 μl of serum by using the Cambridge Biotech HIV-1 Western blot kit according to the instructions of the manufacturer (Maxim Biomedical, Inc., Rockville, MD) (12). Results were interpreted according to the criteria established by the Association of State and Territorial Public Health Laboratory Directors and the CDC (5). The Aptima HIV-1 RNA qualitative assay was also performed according to the manufacturer's instructions (9) by using a total of 500 μl of serum. Briefly, the Aptima HIV assay involves three main steps that take place in a single tube. These include (i) isolation of HIV-1 RNA using a specific target capture oligonucleotide and magnetic microparticles, (ii) transcription-mediated amplification of highly conserved regions of the HIV-1 long terminal repeat and polymerase genes using Moloney murine leukemia virus reverse transcriptase and T7 RNA polymerase, and (iii) detection of amplified product using single-stranded nucleic acid probes with chemiluminescent labels complementary to the specific amplicons. The signal produced by the hybridized probes is measured in a luminometer and is reported in relative light units. Assay software is used to compare these measurements to run-specific cutoffs to generate a reactive or nonreactive result for each sample (9, 10). Three positive and three negative calibrators provided in the kit and one each of external negative and low- and high-level-positive controls (Accurun control sets 803 and 315, series 300 and 500, respectively; SeraCare BBI Diagnostics, West Bridgewater, MA) were tested with each batch of patient samples. A kit internal control that can be distinguished from the HIV-specific target by using a probe with a different label and different kinetics of light emission was added as part of the target capture reagent to each specimen, calibrator, and external control to monitor the performance of the extraction, amplification, and detection steps. Since the purpose of this study was to compare methods for confirming HIV-1 infection, laboratory personnel who performed and interpreted the WB assays were blinded to the results of the Aptima HIV assay.

Table 1 shows the comparison of Aptima HIV and WB results for the 70 HIV-infected patients. The sensitivity of Aptima HIV was 98.6% (91.2% to 99.9% at a 95% confidence interval [CI]), and the specificity was 100% (90.9% to 100% at a 95% CI) when using HIV infection status as the reference. One patient designated as infected had an Aptima HIV result that was negative. Upon review of the patient's clinical information and laboratory testing history, the Aptima HIV test performed on this patient appeared to have been ordered in error by the clinician. This patient was previously known to be HIV infected, was receiving antiretroviral therapy, and had multiple quantitative measurements of HIV-1 RNA with results that were below the lower limit of detection (40 copies/ml at a 95% detection rate) in temporal proximity to submission of the serum sample tested in this study, likely indicating pharmacological achievement of an undetectable viral load.

In the WB analysis, 67 (95.7%) of 70 specimens from HIV-infected patients were reactive and none were nonreactive, while 3 specimens (4.3%) were classified as indeterminate. All three patients with indeterminate WB results were diagnosed as having recent primary infection based on risk assessment, clinical presentation, and high levels of HIV-1 RNA in their plasma.

Table 2 shows the comparison of Aptima HIV and WB results for the 50 patients determined not to be infected with HIV. Of the 50 specimens tested, 48 were from antibody-positive infants undergoing seroreversion and having reactive or indeterminate WB results and 2 were from low-risk adolescent patients with reactive EIA results that had low specimen-to-cutoff ratios and negative HIV-1 WB results, which is most suggestive of a false-positive screening test given that there was no history of risk for HIV-2 infection. All 50 specimens were shown to be negative for HIV-1 RNA using the Aptima HIV assay, while 20 (40.0%) of 50 samples were confirmed to be reactive by WB analysis and 28 (54.9%) had indeterminate WB results.

After completion of the traditional two-step EIA-WB analysis testing algorithm for specimens reactive for HIV antibody, only 69 (57.5%) of 120 samples had results that were both conclusive and reflective of their reference HIV infection status. In contrast, with a combination of EIA and Aptima HIV testing of antibody-positive specimens, 99.2% (119 of 120) of the samples had results that were concordant with the reference HIV-1 status, with discordant results observed for only one patient for whom the Aptima HIV assay was mistakenly ordered and who was known to be infected with HIV-1 and was on suppressive antiretroviral therapy with an undetectable viral load.

In laboratory algorithm 2 of the APHL/CDC status report where NAAT is proposed as an alternative method for confirmation of repeatedly reactive EIAs, it is also recommended that samples negative by NAAT be subjected to HIV-1 WB analysis or IFA as additional supplemental testing to confirm the presence or absence of HIV-1 antibodies (1). By simulating this proposed algorithm using data generated in our study, 51 samples (43%) repeatedly reactive by EIA and negative by the Aptima HIV assay would have been referred for WB testing. Except possibly in the atypical case described above, adding a supplemental WB analysis would not have provided useful diagnostic information. However, given the possibility, albeit low in the United States, that a patient may be repeatedly reactive in an HIV-1/HIV-2 screening EIA and infected with HIV-2, consideration should be given to the local prevalence of this virus and to testing for HIV-2 antibodies if warranted by an appropriate assessment of travel and a documented history of risk.

Because of the time and labor involved in WB analysis and the low number of samples referred for WB confirmation, WB testing is typically performed only once per week in our laboratory using the procedure for overnight incubation; the assay is begun in the afternoon of the first day and completed almost a full 24 h later. For repeatedly reactive samples in our study, the mean time (± standard deviation) from serum collection until the final reporting of the WB results was 5.5 (±2.6) days. The Aptima HIV assay is currently used in our laboratory for the diagnosis of HIV-1 infection in newborns and adolescents at risk for infection; it takes approximately 5 h to complete, and specimens are normally tested in scheduled batches 2 to 3 times a week depending upon the number of specimens available for testing. For both the WB and Aptima HIV analyses, the time spent on manual labor is approximately 90 min, so the major difference in total assay time for the two tests is the dissimilar lengths of incubation. Although we did not formally examine the impact that implementation of the Aptima HIV assay for routine confirmation of repeatedly reactive EIA results would have on result reporting time, we anticipate that the shorter time necessary to complete the Aptima HIV assay and the performance of multiple test runs each week should result in a faster turnaround of results than that obtained with WB analysis. If, however, all nonreactive Aptima HIV tests are referred for WB testing, as suggested by one of the newly proposed APHL/CDC algorithms, the time to result reporting for these samples may actually be longer.

At present, one limitation of using the Aptima HIV test for confirmation of repeatedly reactive EIA results in our laboratory is test cost. Given that WB confirmations were typically performed on small batches of only one to three specimens at a time over the course of this study, the cost (which included kit components, added reagents, supplies, and labor) of testing a specimen by WB analysis ranged from $76.90 (for three samples) to $176.16 (for a single sample). In comparison, the cost of testing a specimen by the Aptima HIV assay was $225.19 to $584.10, making Aptima HIV almost three times as expensive as WB analysis. Therefore, the Aptima HIV test may be more cost-effective for this indication in a laboratory with higher testing volumes. Our estimates do not take into account, however, the attendant costs of future additional testing and patient care and management that may be expected to conclusively determine a patient's HIV infection status when indeterminate WB results are reported to health care providers.

The Aptima HIV test is the first nucleic acid amplification assay approved by the U.S. Food and Drug Administration (FDA) for the diagnosis of HIV-1 infection. The test was originally licensed in October 2006 for use with plasma, and the FDA also recognized serum as a valid specimen source for this assay in January 2009. The manufacturer reports detection rates of 98.5% for 30 RNA copies/ml, 82.6% for 10 copies/ml, 42.5% for 3 copies/ml, and 19.4% for 1 copy/ml, and the assay is designed to detect all major groups (e.g., M, N, and O) and subtypes of HIV-1 (9, 10). Currently, the Aptima HIV assay is clinically employed in situations in which HIV-1 antibodies may not yet be present, including the diagnosis of acute or primary infection in symptomatic patients (6) and targeted and routine screening for defined patient populations (3, 8), and in settings in which the presence of antibodies may not reflect disease status, such as screening of newborns of infected mothers (11, 14, 16). Potential advantages of using the Aptima HIV assay instead of WB analysis to confirm HIV infection in clinical samples with repeatedly reactive EIA results include (i) less subjective result interpretation since the Aptima HIV assay generates tangible numerical values rather than a series of bands that must be visually compared to controls, (ii) a reduction in the number of indeterminate results since the Aptima HIV assay uses a technology that is distinctly different from the current antibody-based tests used for screening and confirmation, (iii) a decrease in the time to obtain results, and (iv) the comparative ease of simultaneously processing and analyzing large numbers of samples if necessary. Also, WBs detect only IgG, so a patient in early seroconversion producing only detectable IgM may be repeatedly positive by EIA and negative by WB but readily detected as positive by the Aptima HIV assay. Of interest with this assay is that whole blood can be stored for up to 72 h at ≤25°C without loss of HIV-1 RNA and that the RNA is stable in serum separated from cells for an additional 5 days at 2 to 8°C, with no adverse effect on assay performance. This feature would readily complement our laboratory practice of storing serum at 2 to 8°C for up to a week pending completion of the HIV-related antibody screening and confirmation assays and, in practice, would require no added or special handling and processing of specimens. Of note, for infants ≤18 months of age who are born to HIV-infected mothers and who are antibody positive by EIA, a single negative or positive Aptima HIV result may be helpful but is not sufficient by itself to definitively exclude or confirm the early diagnosis of HIV infection in this setting. Additional virologic testing of newly collected samples is recommended (4, 14).

In conclusion, to our knowledge, this is the first report to directly compare the performances of Aptima HIV and WB analyses as confirmatory assays for clinical serum samples repeatedly reactive by HIV EIA. Our results demonstrate that the Aptima HIV assay has excellent performance in this setting and that this FDA-approved molecular amplification test can be used in place of WB analysis while maintaining high sensitivity and providing increased specificity by reducing the proportion of samples with indeterminate results. Though the cost of the Aptima HIV test is higher than that of WB analysis, the shorter turnaround time is advantageous. Also, given the re-

cent FDA approval of the first fourth-generation screening assay to detect both HIV antigen and antibodies, the Aptima HIV assay may have extended utility as an aid in the confirmation of HIV-1 infection in persons who are antigen positive but have not yet seroconverted.

REFERENCES

1. **Bennett, B., et al.** 2009. HIV testing algorithms: a status report. Association of Public Health Laboratories, Silver Spring, MD. http://www.aphl.org/hiv/statusreport.
2. **Branson, B. M.** 2007. State of the art for diagnosis of HIV infection. Clin. Infect. Dis. **45:**S221–S225.
3. **Branson, B., et al.** 2006. Revised recommendations for HIV testing of adults, adolescents, and pregnant women in health-care settings. MMWR Recommend. Rep. **55**(RR-14)**:**1–17.
4. **CDC.** 2008. Revised surveillance case definitions for HIV infection among adults, adolescents, and children aged <18 months and for HIV infection and AIDS among children aged 18 months to <13 years—United States, 2008. MMWR Recommend. Rep. **57**(RR-10)**:**1–12.
5. **CDC.** 1989. Interpretation and use of the Western blot assay for serodiagnosis of human immunodeficiency virus type 1 infections. MMWR Morb. Mortal. Wkly. Rep. **38**(Suppl. 7)**:**1–7.
6. **CDC.** 1987. Perspectives in disease prevention and health promotion: public health service guidelines for counseling and antibody testing to prevent HIV infection and AIDS. MMWR Morb. Mortal. Wkly. Rep. **36:**509–515.
7. **Chantry, C. J., et al.** 1995. Seroreversion in human immunodeficiency virus-exposed but uninfected infants. Pediatr. Infect. Dis. J. **14:**382–387.
8. **Ethridge, S. F., et al.** 2010. Performance of the Aptima HIV-1 RNA qualitative assay with 16- and 32-member specimen pools. J. Clin. Microbiol. **48:**3343–3345.
9. **Gen-Probe, Inc.** 2009. Aptima HIV-1 RNA qualitative assay product insert. Gen-Probe, Inc., San Diego, CA. http://www.gen-probe.com/pdfs/pi/501623RevA.pdf.
10. **Giachetti, C., et al.** 2002. Highly sensitive multiplex assay for detection of human immunodeficiency virus type 1 and hepatitis C virus RNA. J. Clin. Microbiol. **40:**2408–2419.
11. **Kerr, R. J., G. Player, S. A. Fiscus, and J. A. E. Nelson.** 2009. Qualitative human immunodeficiency virus RNA analysis of dried blood spots for diagnosis of infections in infants. J. Clin. Microbiol. **47:**220–222.
12. **Maxim Biomedical, Inc.** 2009. Cambridge Biotech HIV-1 Western blot kit product insert. Maxim Biomedical, Inc., Rockville, MD. http://www.mbidiagnostic.com/images/HIV-1_SERUM_WESTERN_BLOT_KIT_PN98002_PACKAGE_INSERT_REV_06b.pdf.
13. **Owen, S. M., et al.** 2008. Alternative algorithms for human immunodeficiency virus infection diagnosis using tests that are licensed in the United States. J. Clin. Microbiol. **46:**1588–1595.
14. **Read, J. S., and the Committee on Pediatric AIDS.** 2007. Diagnosis of HIV-1 infection in children younger than 18 months in the United States. Pediatrics **120:**e1547–e1562.
15. **Ren, A., et al.** 2008. Screening and confirmation of human immunodeficiency virus type 1 infection solely by detection of RNA. J. Med. Microbiol. **57:**1228–1233.
16. **Stevens, W. S., L. Noble, L. Berrie, S. Sarang, and L. E. Scott.** 2009. Ultra-high-throughput, automated nucleic acid detection of human immunodeficiency virus (HIV) for infant infection diagnosis using the Gen-Probe Aptima HIV-1 screening assay. J. Clin. Microbiol. **47:**2465–2469.