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Detection of Individuals with Acute HIV-1 Infection using the ARCHITECT® HIV Ag/Ab Combo Assay

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Abstract

Background—We evaluated use of the ARCHITECT® HIV Ag/Ab Combo assay (HIV Combo; Abbott Diagnostics; available for sale outside of the U.S. only) for detection of acute HIV infection.

Methods—Samples were obtained from a behavioral intervention study (EXPLORE). HIV-uninfected men who have sex with men were enrolled and tested for HIV infection every 6 months. Samples from seroconverters collected at their last seronegative visit (n=217) were tested individually using two HIV RNA assays. Samples with detectable HIV RNA were classified as acute and were tested with HIV Combo. Samples from the enrollment visit (n=83) and the time of HIV seroconversion (n=219) were tested with HIV Combo as controls.

Results—Twenty-one (9.7%) samples from the last seronegative visit had detectable HIV RNA and were classified as acute. HIV Combo was positive for 13 (61.9%) of the acute samples. Samples not detected by HIV Combo had viral loads of 724 to 15,130 copies/ml. Expected results were obtained for positive and negative controls tested with HIV Combo.

Conclusions—HIV Combo detected nearly two-thirds of acute HIV infections identified in this high-risk population by non-pooled, HIV RNA assays. HIV Combo may be useful for high-throughput screening to identify individuals with acute HIV infection.

Keywords

acute infection; HIV-1; HIV Ag/Ab Combo assay

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INTRODUCTION

Laboratory methods routinely used for diagnosis of HIV infection are based on detection of anti-HIV antibodies, which typically appear 3–5 weeks after HIV infection [1,2]. These methods do not identify individuals with acute (pre-seroconversion) HIV infection. Many individuals with acute HIV infection are unaware of their infection status [3,4], and lack the symptoms typical of acute retroviral illness [3,4]. Furthermore, acute HIV infection is often associated with a high concentration of HIV in plasma and genital secretions [5,6], which in turn is associated with increased risk of transmission [7,8]. For these reasons, acutely-infected individuals are considered to be major drivers of the global HIV epidemic. Identification of individuals with acute HIV infection provides an opportunity for counseling, which has the potential to reduce risk behaviors during the critical period of high viremia, and also offers the possibility of initiating antiretroviral treatment early in infection, if appropriate. Identification of individuals with acute HIV infection also provides important epidemiologic data. In some research settings, analysis of acute HIV infection may complement other methods for estimating HIV incidence rates.

Diagnosis of acute HIV infection relies on detection of HIV virus (typically HIV RNA or p24 antigen) in the absence of anti-HIV antibodies. The APTIMA HIV-1 RNA Qualitative Assay (Gen-Probe, Inc., San Diego, CA) has been cleared by the U.S. Food and Drug Administration for diagnosis of early HIV infection although claims are limited to individual (non-pooled) plasma specimens. Large surveillance studies have demonstrated that strategies based on pooled HIV RNA testing can be feasible and cost-effective for identifying individuals with acute HIV infection [4,8–11]. However, the use of RNA detection algorithms is logistically cumbersome, laborious and is typically associated with a substantially delayed time to results (7–14 days). In resource-limited settings, where pooled HIV RNA testing may not be feasible, an alternative strategy for identifying individuals with acute HIV infection has been described that combines rapid HIV testing with an ultrasensitive p24 assay [12,13].

We evaluated use of a combination HIV antigen-antibody detection system (fourth generation immunoassay) [14] for detecting acute HIV infection by analyzing samples collected in a longitudinal cohort study of men at risk for HIV acquisition.

METHODS

Source of specimens

We analyzed samples (plasma or serum) from men who have sex with men (MSM) who participated in a U.S. behavioral intervention study (the EXPLORE study) [15]. HIV-uninfected MSM were enrolled between 1999 and 2001 and tested for HIV infection every 6 months for at least 24 months. HIV infection was assessed at EXPLORE study sites using an enzyme immunoassay (EIA), followed by confirmation with a Western blot or an Immunofluoresence assay (IFA) [15]. Specimens were available from seroconverters at enrollment (n=83), at the last seronegative visit (n=217), with an indeterminate Western blot (n=11), and at the time of HIV seroconversion (n=219).

HIV RNA testing

Samples from the last seronegative visit were tested individually using the AMPLICOR HIV-1 MONITOR Test, v1.5 (Monitor v1.5; Roche Molecular Systems, Inc., Branchburg, NJ; lower limit of detection: 400 copies/ml (c/ml)), and the APTIMA HIV-1 RNA Qualitative Assay (Gen-Probe Inc.: analytical sensitivity: 30 c/ml).

Serologic testing

Seronegative samples positive for HIV RNA using both assays were classified as “acute” and were subsequently tested with (1) ARCHITECT® HIV Ag/Ab Combo assay (HIV Combo; Abbott Diagnostics; Wiesbaden, Germany; available for sale outside of the U.S. only); HIV Combo is a chemiluminescent magnetic microparticle-based immunoassay run on the automated random access instrument, *i*2000SR [14], (2) Genetic Systems rLAV HIV-1 EIA (Bio-Rad, Redmond, VA), (3) HIVAB HIV-1/HIV-2 (rDNA), referred to as the 3A77 EIA (Abbott Diagnostics, Abbott Park, IL), and (4) Genetic Systems HIV-1/HIV-2 Plus O EIA (Bio-Rad, Redmond, VA). Samples from other study visits were tested with HIV Combo as controls. All testing performed with HIV Combo was blinded.

Statistical Methods

We compared the distributions of viral load (VL) values for different sample sets using the Wilcoxon-Mann-Whitney test for two-samples, using the exact two-sided p-value. These analyses were conducted in SAS 9.13.

RESULTS

To identify individuals with acute (pre-seroconversion) HIV infection, samples collected approximately 6 months prior to seroconversion were tested without pooling using the Monitor v1.5 test. Twenty-one (9.7%) of the 217 samples tested positive for HIV RNA with a median VL of 129,845 c/ml (range: 724 to >750,000; Table 1). These 21 samples were designated as acute; the 21 samples were collected a median of 180 days prior to seroconversion (range: 37–390 days). Twenty of the 21 acute samples also tested positive for HIV RNA using the APTIMA HIV-1 RNA Qualitative Assay; one sample did not have sufficient serum remaining for testing.

To evaluate the performance of HIV Combo, 334 samples were tested, including: (1) 21 acute samples (HIV RNA positive/ HIV antibody negative), (2) 83 samples collected from study enrollment (negative controls), (3) 11 samples collected prior to seroconversion with indeterminate Western blots (median VL: 59,856 c/ml; range: 3,007 to >750,000), and (4) 219 HIV antibody-positive samples (positive controls, confirmed by Western blot or IFA).

HIV Combo was positive for 13 (61.9%) of the 21 acute samples. The median VL of the 13 Combo positive samples was significantly higher than the median VL for the eight acute samples that tested negative with HIV Combo [662,217 c/ml (range: 976 to >750,000) vs. 3,576 c/ml (range: 724 to 15,130), p=0.0003)]. All enrollment controls tested negative and all Western blot indeterminate and seroconversion (antibody positive) controls tested positive in HIV Combo. The median VL in the indeterminate samples (59,856 c/ml, range: 3,007 to >750,000) was lower than the median VL in HIV Combo positive acute samples (662,217 c/ml, range: 976 to >750,000, p=0.03).

To further define the serostatus of the 21 acute samples, the samples were retested with three different EIAs. All 21 samples tested negative with the GS rLAV HIV-1 EIA, a second generation assay, and all 20 samples available for testing were negative with the Genetic Systems HIV-1/HIV-1 plus O EIA (one sample had insufficient volume remaining for testing), a third generation assay. Three (14.3%) of the 21 samples tested positive with the third generation 3A77 EIA; all 3 were positive with HIV Combo.

DISCUSSION

In this study, HIV Combo detected 13 (61.9%) of 21 acute HIV infections in a high-risk population. In all but one case, the VL in the HIV Combo positive acute samples was >125,000 c/ml. One sample with 976 c/ml most likely tested positive in HIV Combo because it contained

low-level anti-HIV antibodies in addition to p24 antigen; this sample tested positive in the IgM-sensitive 3A77 EIA, but tested negative in two other EIAs. The higher median VL seen among the HIV Combo positive vs. HIV Combo negative samples is consistent with results of Fiebig et al., where the median VL in individuals with acute HIV infection was approximately 2 $\log_{10}$ higher in subjects with detectable p24 antigen than in those who were HIV RNA positive and p24 antigen negative [2]. Results obtained with antibody-negative acute specimens from EXPLORE are also consistent with previous estimates for the sensitivity of HIV Combo based on analysis of viral isolate dilutions [14]. HIV Combo also detected all 11 samples with indeterminate Western blots; the median VL in these samples was lower than the median VL in HIV Combo positive acute samples. In contrast, a previous study found that the median VL was higher in subjects with indeterminate Western blots than in subjects with p24 antigen positive acute HIV infection [2].

In the U.S., pooled HIV RNA testing is the most widely used method for identification of individuals with acute HIV infection [4,10,11]. The sensitivity of pooled HIV RNA testing depends on the sensitivity of the HIV RNA assay used, as well as the size of the initial sample pools. For example, if 96 HIV antibody negative samples are pooled and tested with an HIV RNA assay with a lower level of detection of 50 c/ml HIV RNA, the assay would detect a single acute sample with a VL of 4,800 c/ml or more. Fifteen (71.4%) of the 21 acute samples in the EXPLORE cohort had VLs >4,800 c/ml. This is similar to the portion of individuals with acute HIV infection who tested positive with HIV Combo (13/21=61.9%). Moreover, for 5 of the 8 antibody-negative acute samples not detected by HIV Combo, VL values were <4,800 c/ml (range: 724–3984 c/ml). Thus, these samples would likely have been missed by some HIV RNA pooling algorithms. Since samples are tested individually with HIV Combo, sensitivity is not influenced by the number of samples analyzed.

The capacity of HIV Combo to detect acute and chronic infections in a single step offers several advantages over traditional HIV RNA pool testing. These include, time to result, throughput and labor. HIV Combo is run on automated random access instruments. In the present study an *i*2000SR instrument was utilized. On this platform, the time to first result is 28 minutes with a throughput of 200 tests/hour. The assay is less labor-intensive than pooled HIV RNA testing, and each sample is analyzed only once. In contrast, pooled HIV RNA testing typically involves 3 or 4 stages of testing using progressively smaller sample pools, to determine which and how many of the samples in a positive initial pool are HIV RNA positive [1]. These types of algorithms result in delays in reporting results and increase the overall cost of testing. Incorporation of HIV Combo in routine testing paradigms will likely have a significant impact on cost/benefit calculations, even in high incidence settings. It should be recognized that fourth generation assays differ in HIV p24 antigen sensitivity, antibody sensitivity, specificity, and performance on genetically divergent strains of HIV-1 [16,17]. Thus, results obtained in this study for the ARCHITECT® HIV Ag/Ab Combo assay, recognized as among the most sensitive in its class [16], may not be directly applicable to other fourth generation immunoassays.

In many settings, HIV RNA testing algorithms are not feasible or are cost-prohibitive. The relative yield provided by the immunoassays on this panel of recent/acute specimens provides a compelling argument for utilization of the most sensitive immunoassays possible. On this panel of acute samples, although there was no incremental yield between the second to third generation Genetic System EIAs (both detected 0/21), 3A77 (an alternative third generation assay) detected 3/21 (14.3%) samples whereas HIV Combo detected 13/21 (61.9%) of the panel members. Thus, the fourth generation Ag/Ab combination assay provided dramatic improvement in sensitivity of detection. These data are consistent with several previous evaluations of fourth generation assays showing substantial improvement in detection sensitivity and a reduction in the window period relative to antibody only assays [16,18–21].

For samples from acutely-infected individuals, limited data is available on the relationship between HIV-1 viral load and p24 antigen sensitivity [21]. The present study, the first analysis of fourth generation assay performance on acute HIV-1 infections identified by non-pooled HIV RNA testing, provides additional perspective on the sensitivity of HIV Combo. Studies have demonstrated the benefit of testing fourth generation HIV assays in both high risk and low risk populations [21,22]. The recent recommendation in the UK National Guidelines for HIV Testing 2008 [23] to use fourth generation immunoassays for first-line testing of blood in all healthcare settings is noteworthy. Our results suggest that ARCHITECT® HIV Ag/Ab Combo may be useful for detection of acute HIV infection.

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Table 1

Analysis of samples from men with acute HIV infection.

Twenty-one samples from men with acute HIV infection were tested with the Combo assay. The raw data from the Combo assay, including relative light units (RLU), and the signal to cutoff ratio (S/CO) are shown; samples with an S/CO ratio >1 are considered to be positive in the Combo assay (results shown in bold text). Also shown are the viral load for each sample and the results of testing in the Abbott 3A77 EIA; samples with an S/CO ratio >1 are considered to be positive in the 3A77 assay (results shown in bold text).

Sample	Days prior to seroconversion	Viral load (c/ml)	Abbott Combo RLU	Abbott Combo S/CO (>1 = +)	Abbott 3A77 EIA S/CO (>1=+)
425	238	>750,000	453,722	**171.99**	**2.38***
41	71	>750,000	921,249	**349.22**	**2.59***
31	83	>750,000	69,629	**26.39**	0.15
15	182	>750,000	20,217	**7.66**	0.12
818	117	>750,000	86,166	**32.66**	0.46
707	390	675,514	40,593	**15.39**	0.16
353	69	662,217	34,746	**13.17**	0.24
472	78	377,329	30,542	**11.58**	0.99
492	374	358,470	54,606	**20.70**	0.08
282	37	176,185	17,505	**6.64**	0.12
532	180	129,845	6,571	**2.49**	0.15
249	180	125,848	9,146	**3.47**	0.33
627	194	15,130	1,877	0.71	0.12
618	188	13,476	650	0.25	0.07
542	182	8,533	1,056	0.40	0.11
597	134	3,984	1,137	0.43	0.07
136	178	3,167	738	0.28	0.06
83	202	2,917	751	0.28	0.05
295	114	976	2,780	**1.05**	**3.20***
332	187	790	382	0.14	0.07
24	141	724	510	0.19	0.08

* Negative in the Genetic Systems HIV-1/HIV-1 plus O EIA.