



Evaluation of the Gen-Probe APTIMA HIV-1 RNA Qualitative Assay for the Detection of HIV-1 in Plasma

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Abstract*

Background: The APTIMA HIV-1 RNA Qualitative Assay (Gen-Probe, Inc., San Diego, CA) is the first nucleic acid amplification test specifically approved by the Food and Drug Administration (FDA) for the detection of human immunodeficiency virus type 1 (HIV-1) RNA in human plasma. Indications for use of this assay include (a) screening of high-risk populations, (b) testing in the setting of known occupational exposure, (c) testing patients with acute HIV-1 symptoms and known exposure, and (d) screening newborn babies born to infected mothers.

Objective: To evaluate the technical and operational performance of the Gen-Probe APTIMA HIV-1 RNA Qualitative Assay.

Methods: A series of previously characterized plasma specimens known to be positive or negative for HIV-1 were blindly tested using the Gen-Probe APTIMA HIV-1 RNA Qualitative Assay. These included a 10 member HIV-1 linearity panel, a 10 member HIV-1 genotype performance panel representing group M subtypes A to H and circulating recombinant forms (CRFs), a 5 member HIV-1 group O panel, and 271 plasma specimens from different patient groups. The groups included patients known to be negative for HIV-1 and HIV-1 infected patients with viral RNA loads <75 copies/ml, between 75 to 10,000 copies/ml and 10,000 to 50,000 copies/ml, and >50,000 copies/ml. A defined set of serum samples was also used to examine the performance of the assay for the confirmation of HIV-1 infection in individuals who were repeatedly reactive for HIV antibodies. Lastly, semi-automated SB-100 Dry Heat Bath/Vortexers were compared to circulating water baths and multi-tube vortex mixers for use during the temperature-controlled incubation and mixing steps of the assay.

Results: HIV-1 RNA was detected in all plasma samples (118/118) from patients with viral loads >75 copies/ml when using the Gen-Probe APTIMA assay, while negative results were obtained in all patients (50/50) who were not infected with HIV-1. For patients with viral loads <75 copies/ml, HIV-1 RNA was detected in 63% (65/103) of the specimens tested, reflecting the extreme sensitivity of the test (e.g., documented detection rate of 98.5% for 30 RNA copies/ml). Subtypes A to H and the CRFs of group M and the 5 strains of group O were readily detected, as were all members of the linearity panel from 10^6 to 10^1 copies/ml. HIV-1 infection was confirmed in 21 of 21 sera from patients with HIV antibodies, and in 0 of 7 sera from patients with indeterminate Western blots and no HIV-1 infection. The assay worked equally well when using either individual water baths and multi-tube vortex mixers (as approved by the FDA) or the SB-100 Dry Heat Bath/Vortexers during the desired incubations and mixing steps, with some differences observed for specimens with viral loads <75 copies/ml.

Conclusions: The Gen-Probe APTIMA HIV-1 RNA Qualitative Assay is highly sensitive and specific and relatively simple to perform. It serves as a convenient aid in the diagnosis of HIV-1 infection, including acute or primary infection, and can be used to confirm HIV-1 infection in plasma or sera of patients with antibodies to HIV-1.

*Abstract has been modified from original

Introduction

The APTIMA HIV-1 RNA Qualitative Assay (Gen-Probe, Inc., San Diego, CA) is the first qualitative nucleic acid amplification test specifically approved by the Food and Drug Administration (FDA) for the diagnosis of human immunodeficiency virus type 1 (HIV-1) infection, including acute or primary infection.

The assay may also be used as an additional test, when reactive, to confirm HIV-1 infection in an individual whose specimen is repeatedly reactive for HIV-1 antibodies.

With this study, we describe an evaluation of the technical and operational performance of the Gen-Probe APTIMA HIV-1 RNA Qualitative Assay.

Materials and Methods

Specimens:

Approved specimens for the Gen-Probe APTIMA HIV-1 RNA Qualitative Assay include PLASMA collected in K₂EDTA, K₃EDTA, acid citrate dextrose (ACD), sodium citrate or the Becton-Dickinson EDTA Plasma Preparation Tubes (PPT) and SERUM.

All specimens used for this study were blindly tested and included a series of 296 previously characterized plasma samples known to be positive or negative for HIV-1 RNA, and a set of 28 serum samples used to examine the performance of the assay for the confirmation of HIV-1 infection in individuals who were repeatedly reactive for HIV antibodies.

Table 1. Types and Total Numbers of Specimens Examined

Specimen Type (Total No. Tested)	Source
PLASMA (296)	10 Member HIV-1 RNA Linearity Panel (PRD801)* representing a dilution series equivalent to 10^6 to 10^1 copies/ml of HIV-1 RNA
	10 Member HIV-1 RNA Genotype Performance Panel (PRD202)* representing group M subtypes A to H and circulating recombinant forms (CRFs)
	5 Member HIV-1 Group O Performance Panel (PRD301)*
	271 Clinical Plasma Specimens from Different Patient Groups <ul style="list-style-type: none"> 50 not infected with HIV-1 103 with HIV-1 viral loads <75 copies/ml 49 with HIV-1 viral loads between 75 to 10,000 copies/ml 50 with HIV-1 viral loads between 10,000 to 50,000 copies/ml 19 with HIV-1 viral loads >50,000 copies/ml
SERUM (28)	Clinical Serum Specimens from Patients with Repeatedly Reactive Antibody Test Results

*Obtained from BBI Diagnostics, a Division of SeraCare Life Sciences, Milford, MA

Methods:

The Gen-Probe APTIMA HIV-1 RNA Qualitative Assay involves three main steps which take place in a single tube. These include (1) isolation of HIV-1 and internal control RNA from specimens using specific target capture oligonucleotides and magnetic microparticles, (2) transcription-mediated amplification (TMA) of each target, and (3) detection of amplified products through a dual kinetic hybridization protection assay that uses single-stranded nucleic acid probes with chemiluminescent labels that are complementary to the specific amplicons. The chemiluminescent signal produced by the hybridized probes is measured in a luminometer and is reported as relative light units (RLU).

Semi-automated SB-100 Dry Heat Bath/Vortexers from Gen-Probe were compared to the individual circulating water baths and multi-tube vortex mixers that are recommended for use during the temperature-controlled incubation and mixing steps of the assay.

Results

Table 2. Results on 10 Member HIV-1 RNA Linearity Panel PRD801

Panel Member ID #	HIV-1 RNA copies/ml ^a	Gen-Probe APTIMA HIV-1 RNA Qualitative Assay Result	
		EXPECTED ^b	ACTUAL
01	2.9 x 10 ⁶	Reactive	Reactive
02	7.8 x 10 ⁵	Reactive	Reactive
03	4.4 x 10 ⁵	Reactive	Reactive
04	1.7 x 10 ⁵	Reactive	Reactive
05	1.4 x 10 ⁴	Reactive	Reactive
06	1.7 x 10 ³	Reactive	Reactive
07	3.0 x 10 ²	Reactive	Reactive
08	1.8 x 10 ²	Reactive	Reactive
09	8 x 10 ¹	Reactive	Reactive
10	<50	Non-Reactive	Non-Reactive

^aAs determined by vendor using Roche COBAS AMPLICOR HIV MONITOR v1.5 and v1.5 (Ultra) Assays

^bAs determined by vendor using Chiron/GenProbe Procleix HIV-1 RNA Discriminatory Qualitative Assay

All positive members of the linearity panel from 10^6 to 10^1 copies/ml were readily detected by the Gen-Probe APTIMA HIV-1 RNA Qualitative Assay.

Table 3. Results on 10 Member HIV-1 RNA Genotype Performance Panel PRD202

Panel Member ID #	HIV Subtype*	Country of Origin	Gen-Probe APTIMA HIV-1 RNA Qualitative Assay Result
01	A	Uganda	Reactive
02	B	USA	Reactive
03	C	Djibouti	Reactive
04	D	Uganda	Reactive
05	CRF01_AE	Indonesia	Reactive
06	F	Romania	Reactive
07	CRF01_AG	Liberia	Reactive
08	G	Kenya	Reactive
09	H	Zaire	Reactive
10	---	---	Non-Reactive

*Panel member subtypes were confirmed by vendor using sequencing of pol and RT regions with BLAST analysis

Subtypes A to H and the CRFs of group M were readily detected by the Gen-Probe APTIMA HIV-1 RNA Qualitative Assay.

Table 4. Results on 5 Member HIV-1 Group O Performance Panel PRD301

Panel Member ID #	HIV Group	Gen-Probe APTIMA HIV-1 RNA Qualitative Assay Result	
		EXPECTED*	ACTUAL
01	O	Reactive	Reactive
02	O	Reactive	Reactive
03	O	Reactive	Reactive
04	O	Reactive	Reactive
05	---	Non-Reactive	Non-Reactive

*As determined by vendor using Chiron/GenProbe HIV-1/CV TMA Assay

The 5 strains of group O were readily detected by the Gen-Probe APTIMA HIV-1 RNA Qualitative Assay

Table 5. Qualitative Detection of HIV-1 RNA Using Gen-Probe APTIMA HIV-1 RNA Assay on 271 Previously Characterized Clinical Plasma Specimens from Different Patient Groups

HIV Status	No. Tested	Gen-Probe APTIMA HIV-1 RNA Qualitative Assay Result	
		POSITIVE	NEGATIVE
No HIV-1 Infection ^a	50	0	50
HIV-1 +, VL <75 cps/ml ^b	103	65	38
HIV-1 +, VL 75 - 10,000 cps/ml	49	49	0
HIV-1 +, VL 10,000 - 50,000 cps/ml	50	50	0
HIV-1 +, VL >50,000 cps/ml	19	19	0

^aAs determined by serology and real-time PCR

^bAs determined by the Siemens VERSANT HIV-1 RNA 3.0 Assay (bDNA)

HIV-1 RNA was detected in all plasma samples (118/118) from patients with HIV-1 viral loads >75 copies/ml when using the Gen-Probe APTIMA assay, while negative results were obtained in all patients (50/50) who were not infected with HIV-1. For patients with viral loads <75 copies/ml, HIV-1 RNA was detected in 63% (65/103) of the specimens tested, reflecting the enhanced sensitivity of the test (e.g., documented 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).

Table 6. Comparison of Semi-Automated SB-100 Dry Bath/Vortexers to Circulating Water Baths and Multi-Tube Vortex Mixers for Use with the Gen-Probe APTIMA HIV-1 RNA Assay

Individual Circulating Water Baths and Multi-Tube Vortex Mixers	Semi-Automated SB-100 Dry Bath/Vortexers			
	+/+	+/-	-/+	-/-
No HIV-1 Infection ^a	0	0	0	50/50
HIV-1 +, VL <75 cps/ml ^b	65/103	15/103	9/103	38/103
HIV-1 +, VL 75 - 10,000 cps/ml	49/49	0	0	0
HIV-1 +, VL 10,000 - 50,000 cps/ml	50/50	0	0	0
HIV-1 +, VL >50,000 cps/ml	19/19	0	0	0

^aAs determined by serology and real-time PCR

^bAs determined by the Siemens VERSANT HIV-1 RNA 3.0 Assay (bDNA)

The Gen-Probe APTIMA HIV-1 RNA Assay worked equally well when using either individual water baths and multi-tube vortex mixers during the desired incubations and mixing steps (as approved by the FDA) or the more automated SB-100 Dry Baths/Vortexers that combine water bath and vortex operations into a single system. Some differences were observed for specimens from HIV-1-infected patients with viral loads <75 copies/ml. A total of 271 previously characterized clinical plasma specimens from different patient groups were examined.

Table 7. Qualitative Detection of HIV-1 RNA Using Gen-Probe APTIMA HIV-1 RNA Assay on Serum Specimens from Patients That Are Repeatedly Reactive for HIV Antibodies

Specimen ID #	HIV EIA Result			Western blot Result	Gen-Probe APTIMA HIV-1 RNA Qualitative Assay Result	
	INT	RPT	RPT		R/NR	Analyte RLU
01	>2.000	>2.000	>2.000	R	R	1,119,202
02	>2.000	>2.000	>2.000	R	R	1,054,140
03	>2.000	>2.000	>2.000	R	R	754,115
04	>2.000	>2.000	>2.000	R	R	1,115,131
05	0.296	0.258	0.292	I	NR	2,905
06	0.117	0.112	0.113	I	NR	2,314
07	0.253	0.277	0.296	I	NR	3,920
08	>2.000	>2.000	>2.000	R	R	1,062,868
09	>2.000	>2.000	>2.000	R	R	1,090,567
10	0.377	0.346	0.347	I	NR	5,338
11	>2.000	>2.000	>2.000	R	R	1,362,010
12	>2.000	>2.000	>2.000	R	R	1,041,559
13	0.388	0.362	0.347	I	NR	2,497
14	>2.000	>2.000	>2.000	R	R	1,149,453
15	>2.000	>2.000	>2.000	R	R	1,105,398
16	>2.000	1.858	1.826	R	NR	5,021
17	>2.000	>2.000	>2.000	R	R	1,086,104
18	>2.000	>2.000	>2.000	R	R	1,097,360
19	>2.000	>2.000	>2.000	R	R	1,100,015
20a	0.364	0.394	0.380	R	R	1,028,540
20b	0.723	0.672	0.696	R	R	1,029,179
21	>2.000	>2.000	>2.000	R	R	1,015,590
22	0.437	0.459	0.444	I	NR	5,472
23	1.535	1.511	1.473	R	R	783,979
24	>2.000	>2.000	>2.000	R	R	1,016,491
25	0.176	0.166	0.190	I	R	1,096,893
26	>2.000	>2.000	>2.000	I	R	1,088,167
27	>2.000	>2.000	>2.000	R	R	1,060,674

Abbreviations: INT, Initial; RPT, Repeat; R/NR, Reactive/Nonreactive; RLU, Relative Light Units

HIV-1 infection was confirmed in 21 of 21 sera from patients with HIV antibodies, including three patients with recent acute primary infection (□). No HIV-1 RNA was detected in 7 sera from patients with indeterminate Western blots and no HIV-1 infection.

Conclusions

The Gen-Probe APTIMA HIV-1 RNA Qualitative Assay is highly sensitive and specific for the detection of HIV-1 RNA in plasma and can readily detect major groups and subtypes of the virus, including group O.

The assay is robust and relatively simple to perform, and demonstrates excellent discrimination between positive and negative samples.

A typical test run can be completed in 4-4.5 hours, and an internal control is added to all samples, calibrators, and controls to monitor the specimen processing, amplification and detection steps.

The test works nicely on serum specimens and can be used to confirm HIV-1 infection in an individual whose serum is repeatedly reactive for HIV-1 antibodies. As of 12 January 2009, the FDA has approved serum as a valid specimen source for the Gen-Probe APTIMA HIV-1 RNA Qualitative Assay.

The semi-automated Gen-Probe SB-100 Dry Heat Bath/Vortexers that combine water bath and vortex operations into a single system can easily replace the more cumbersome individual water baths and multi-tube vortex mixers that are recommended for use during the desired incubations and mixing steps.

Indications for use of this assay may include (a) screening of high-risk populations, (b) testing in the setting of known occupational exposure, (c) testing patients with acute HIV-1 symptoms and known exposure, and (d) screening newborn babies born to infected mothers.