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Use of the APTIMA® HIV-1 RNA Qualitative Assay to Monitor Patients Under Therapy for Viral Suppression

CT Nugent*, DP Kolk*, C Giachetti*, CM Magno*, DD Richman† and DJ Looney†, *Gen-Probe Incorporated, San Diego, CA, †Veterans Affairs San Diego Healthcare System (VASDHCS) and University of California San Diego.

Charles T. (Tom) Nugent IV, Ph.D.
Gen-Probe, Inc.
10210 Genetic Center Drive
San Diego, CA 92121
tomn@gen-probe.com
(858) 410-8331

Abstract

Background: *Plasma viral load (pVL) is routinely quantitated for monitoring HIV-1 patient responses to antiviral therapy, which, when successful, suppresses viral replication to levels lower than 50 copies/mL (cp/mL; below detection limits of quantitative assays). Due to the cost and complexity of quantitative testing, Gen-Probe's APTIMA HIV-1 RNA Qualitative Assay (98% limit of detection = 30 cp/mL) was evaluated as an alternative test to determine if patients' pVLs are below 50 cp/mL.*

Methods: *pVLs were quantitated in 446 HIV-1 patient samples using Roche's COBAS AMPLICOR® HIV-1 MONITOR Test, version 1.5, at VASDHCS: 285 specimens had pVLs below 50 cp/mL. Samples were then sent to Gen-Probe for testing with the APTIMA Assay. APTIMA Assay signal to cut-off (S/CO) values were analyzed to determine the optimal S/CO value to reduce sensitivity for samples with less than 50 cp/mL while retaining sensitivity for the samples with greater than 50 cp/mL. A masked study was then conducted with 115 samples using the determined S/CO value.*

Results: *Using an S/CO value of 1, samples with pVLs greater than 50 cp/mL were reactive in the APTIMA Assay. However, 140 of 285 samples with less than 50 cp/mL were also reactive (49% sensitivity). After the S/CO value was raised to 15, 79% of samples with less than 50 cp/mL were identified as below the limit of detection; sensitivity for samples with greater than 50 cp/mL was 93.1%. In the masked study, specificity was 79% and sensitivity was 97.6% using the established S/CO value of 15. One sample with greater than 50 cp/mL was missed but had a pVL of 72 cp/mL.*

Conclusions: *This study demonstrates that raising the S/CO value for reactivity of the APTIMA HIV-1 RNA Qualitative Assay can detect samples with pVL greater than 50 cp/mL with high sensitivity and is a feasible method for using a qualitative assay for patient monitoring.*

Introduction

HIV-1 plasma RNA is an extremely useful predictor of prognosis and response to therapy, and is frequently used in decisions to determine the best course of treatment and to determine treatment effectiveness.

HIV-1 patients undergoing therapy are routinely monitored for viral load every 2 to 6 months using a quantitative nucleic acid test. Patients with pVL <50 cp/mL (below the quantitative assay's limit of detection) are considered to have viral replication suppressed and will not need a change in treatment. Results from the VASDHCS laboratory indicate that 54% to 60% of all plasma samples examined for HIV-1 RNA are below the current limit of detection (<50 cp/mL). Therefore, a qualitative test that would distinguish between these virally suppressed patients and patients requiring a quantitative test (pVL > 50 cp/mL) could save resources, due to the cost and complexity of a quantitative test.

The APTIMA HIV-1 RNA Qualitative Assay (APTIMA HIV-1 Assay) was recently licensed by the FDA as an aid in the diagnosis of acute HIV-1 infection in plasma specimens and as a confirmatory test for plasma specimens repeatedly reactive for HIV-1 antibodies. Analytical sensitivity is 98.5% at 30 cp/mL (95% confidence interval: 97.3% to 99.2%) when using the standard cutoff.

We wanted to determine whether the APTIMA HIV-1 Assay could be used to differentiate samples with pVL≤ 50 cp/mL from samples with > 50cp/mL when using a different cutoff.

Materials and Methods

APTIMA HIV-1 Assay Procedure

The APTIMA HIV-1 Assay is a molecular diagnostic assay that detects HIV-1 RNA and is run on a semi automated platform. One operator can obtain 188 results in approximately 6 hours.

The three main steps of the APTIMA HIV-1 Assay are depicted in Figure 1:

- *Target Capture – virus is lysed and target sequences are captured with magnetic particles and complementary oligonucleotides.*
- *Amplification – isothermal exponential amplification is performed using Transcription-Mediated Amplification technology.*
- *Hybridization and Detection – acridinium ester labeled probes are used to detect amplicon utilizing the Hybridization Protection Assay and Dual Kinetic Assay technologies.*

An internal control RNA transcript is added to each reaction at the target capture step to control for proper capture, amplification, and detection.

Specimens

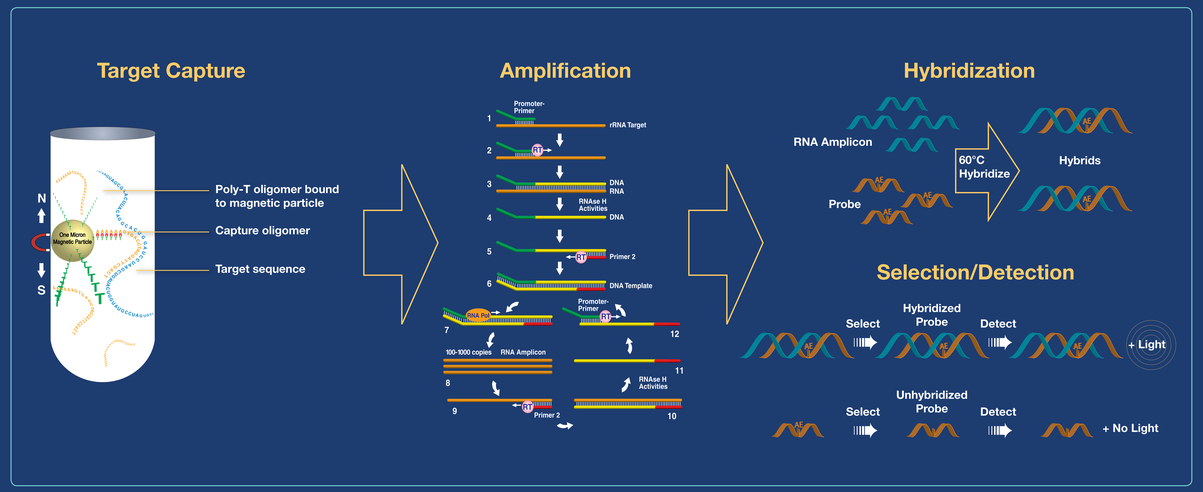
Plasma specimens were obtained from HIV-1 patients attending the VASDHCS Laboratory Medicine and Clinical Pathology Microbiology Facility. Aliquots were tested in the UCSD Center for AIDS Research Molecular Biology using the COBAS AMPLICOR HIV-1 MONITOR Test v1.5. Cryopreserved aliquots were shipped to Gen-Probe and tested with the APTIMA HIV-1 Assay.

APTIMA HIV-1 Assay and Data Analysis

The specimens were processed following the APTIMA HIV-1 package insert instructions. APTIMA software interpreted results as "Reactive", "Non Reactive", or "Invalid". A reactive result occurs when the analyte Signal/Cutoff ratio (S/CO) is equal to or greater than 1.

To determine the optimal cutoff for patient monitoring, valid results were analyzed and interpreted as reactive or non reactive using various S/CO values. The new, defined S/CO value was then used to evaluate assay performance in a masked panel consisting of HIV-1 patient specimens with various viral loads.

Figure 1: The APTIMA® Assay Steps



Preliminary Study Results

Figure 2 . Distribution of APTIMA HIV-1 Assay S/CO Results in Samples with Various HIV-1 Viral Loads

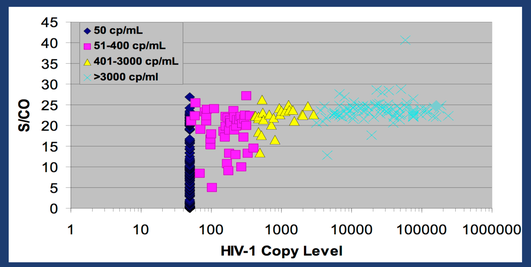


Table 1. The Effect of Increasing the S/CO Value on Detection of HIV-1 Positive Samples Grouped by Viral Load

	<50 cp/mL		551-400 cp/mL		401-3000 cp/mL		>3000 cp/mL	
	# Reactive	% Reactive	# Reactive	% Reactive	# Reactive	% Reactive	# Reactive	% Reactive
S/CO>1	140	100	45	100	29	100	85	100
S/CO>10	69	24.20	42	93.30	29	100	85	100
S/CO>15	60	21.00	36	80.00	28	96.50	84	98.82

- *A total of 446 patient samples were analyzed using the APTIMA HIV-1 Assay.*
- *Figure 2 shows the S/CO value for all samples when they are grouped by viral load.*
- *Table 1 shows the number of reactive samples when the S/CO is set to 1, 10 and 15.*
- *Results are grouped by viral load.*

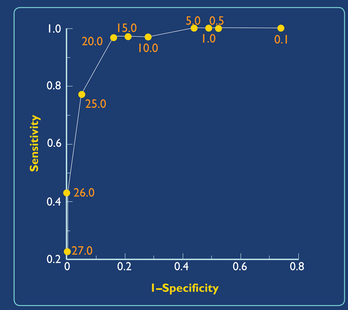
Masked Study Results

Table 2. HIV-1 Reactivity Observed in Masked Panel Samples Using the APTIMA HIV-1 Assay With an S/CO Value of 15

HIV-1 Viral Load	Number of Samples	Number Reactive	Number Non-reactive
<50 cp/mL	85	18	67
51-400 cp/mL	10	9	1*
401-3000 cp/mL	5	5	0
>3000 cp/mL	15	15	0

*Sample had a pVL of 72 cp/mL

Figure 3 . APTIMA HIV-1 Assay ROC Analysis in Masked Patient Samples



- *A total of 115 patient samples were analyzed using the APTIMA HIV-1 Assay and an S/CO value of 15.*
- *Table 2 shows number of reactive samples when the S/CO value is set to 15. Results are grouped by viral load.*
- *Figure 3 is a receiver-operator characteristics (ROC) curve generated from this data showing the impact on sensitivity for true positive results (pVL > 50) cp/mL and specificity for samples with true negative results as the S/CO value is raised from 1 to 27 as depicted by the numbers along the curve.*

Discussion

1) *Preliminary Experiment (Figure 2 and Table 1)*
   - *When the S/CO was set to 15:*
     - *79% of samples with pVL<50 cp/mL were non reactive.*
     - *80% of samples with pVL 51 to 400 cp/mL were reactive.*
     - *96.5% of samples with pVL 401 to 3000 cp/mL were reactive.*
     - *98.82% of samples with pVL > 3000 cp/mL remained reactive.*
2) *Masked Experiment (Table 2 and Figure 3)*
   - *Using an S/CO value of 15, 29 out of 30 samples with pVL >50 were detected (Table 2), as were 18 of 85 samples (21.2%) with pVL< 50 cp/mL.*
   - *One sample with a pVL of 72 cp/mL was missed, however this result would usually be clinically insignificant because it would still indicate viral suppression.*
   - *ROC analysis showed that specificity for patient samples with pVL of <50 cp/mL increased from 51% to 79% when the S/CO value was increased from 1 to 15 with minimal change to sensitivity (97.5%) for samples with true positive results (>50 cp/mL) due to the one sample with a false negative result described above.*
   - *In this particular panel, increasing the S/CO value to 20 maintained sensitivity and raised the specificity to 84%.*

Summary

1) *These results suggest that increasing the S/CO may be useful in determining whether samples from patients under therapy may need to be sent for quantitative HIV-1 testing.*
2) *In the masked study, this approach would have reduced the number of samples for quantitative testing from 115 to 57 or by 49.6%, resulting in a significant cost savings for clinics.*

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