Comparison of the Aptima® HCV Quant Dx Assay to the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test v2.0 and the Abbott® RealTi*m*e HCV Assay

C. Clark[1], M. Kantzanou[2], H. Papachristou[2], A. Worlock[1], A. Williams[1], M. Vi[1], J. Pham[1], A. Hatzakis[2]

[1] Hologic, 10210 Genetic Center Drive, San Diego, CA, 92121, USA ,858 410 8704 [2] Athens University, Medical School, Athens, Greece

Abstract

Background:

The Hologic Aptima HCV Quant Dx Assay is a fully automated quantitative assay being developed on the Panther system, and is based on real-time Transcription-Mediated Amplification (TMA) technology. This assay is intended for detecting and monitoring hepatitis C virus (HCV) in plasma and serum specimens.

Methods:

A cohort of 490 clinical specimens from University of Athens Medical School was tested using the Aptima HCV Quant Dx Assay (Hologic assay), the COBAS AmpliPrep/COBAS TaqMan HCV Test, v2.0 (Roche assay) and the Abbott RealTi*m*e HCV Assay (Abbott assay). The specimens included HCV genotypes 1, 2, 3, and 4, and ranged in concentration from 16IU/mL (1.20 log IU/mL) to 26.4 million IU/mL (7.42 log IU/mL) as measured by the Roche assay. Analytical sensitivity of the Aptima assay was assessed using dilutions of the 2nd WHO Standard (NIBSC 96/97) run in replicates of at least 100 per dilution on multiple Panther systems. Linearity of the Aptima assay was tested by dilution HCV 1a armored RNA from 10 to 100,000,000 IU/mL and testing in replicates of 5 on a single Panther system.

Results:

For the purposes of the clinical specimen correlation to the Roche assay, a lower limit of quantitation for the Aptima assay of 15 IU/mL (1.18 log IU/mL) was used. Four hundred and twenty-four (424) specimens gave results quantifiable for both Aptima and Roche assays. The slope was 1.07 (Aptima vs. Roche) with an intercept of -0.34 and an R^2 of 0.97.

For the purposes of the clinical specimen correlation to the Abbott assay, a lower limit of quantitation for the Aptima assay of 12 IU/mL (1.08 log IU/mL) was used. Due to specimen volume constraints only 412 specimens could be tested using the Abbott assay from which 404 gave results above 12 IU/mL (1.08 log IU/mL). The slope was 1.05 (Aptima vs Abbott) with an intercept of 0.07 and an R^2 of 0.97.

The analytical sensitivity (limit of detection) of the Aptima assay as measured by the dilutions of the WHO 2nd standard was 4.3 IU/mL for plasma and 3.9 IU/mL for serum using probit analysis and a 95% positivity rate. The Aptima assay was shown to be linear over the range of 10 to 100,000,000 IU/mL.

Conclusion:

The Aptima HCV Quant Dx Assay gave comparable viral load results when compared to the Roche COBAS AmpliPrep/COBAS TaqMan HCV Test, v2.0 and Abbott RealTime HCV Assay. The performance of the Aptima assay makes it an excellent candidate for the detection and monitoring of HCV.

Assay Procedure

The Aptima HCV Quantitative Assay Reagents and samples are loaded into the Panther System (Figure 1)

The PANTHER System processes 0.5 mL of sample through target capture, amplification, and real-time detection in the presence of an internal control

The assay has 1 negative control and two positive controls (1 low and 1 high concentration)

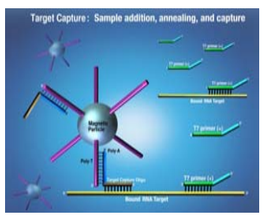
KEY FEATURES OF PANTHER SYSTEM

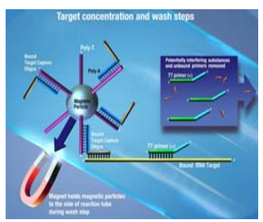
- **Full automation from sample to result with primary sample tube option**
- **Random sample loading eliminating the need for batching samples together to perform the assay**
- **Continuous access to change reagents, samples, and consumables**
- **Small instrument foot print**
- **2.5 hrs to first result: 100 tests results in 4.5 hours; 500 test results in <11 hours**

Figure 1. Panther System

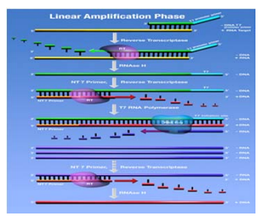
Assay Technology

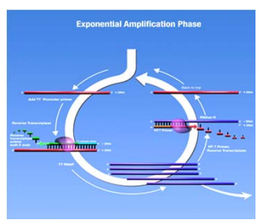
Target Capture



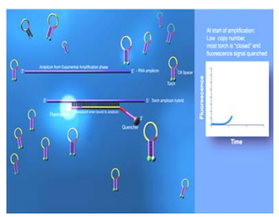


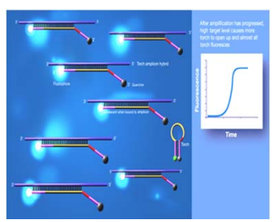
Transcription-Mediated Amplification





Real-time Signal Generation





Assay Performance

Method Comparison

A cohort of 490 clinical specimens from University of Athens Medical School was tested using the Aptima HCV Quant Dx Assay (Hologic assay), the COBAS AmpliPrep/COBAS TaqMan HCV Test, v2.0 (Roche assay) and the Abbott RealTi*m*e HCV Assay (Abbott assay)

Figure 2. Comparison of Hologic and Roche Assays (n=424)

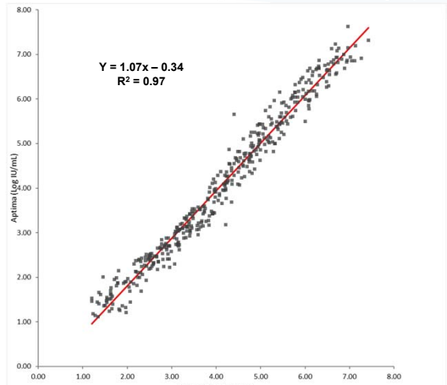
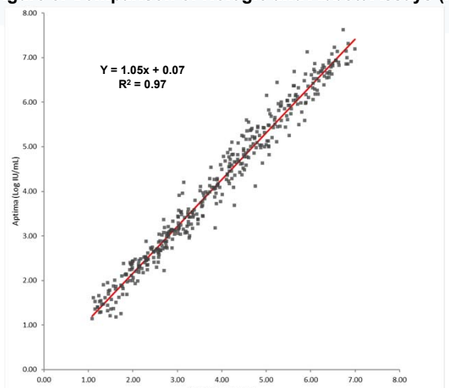


Figure 3. Comparison of Hologic and Abbott Assays (n=404)



Limit of Detection

Limit of Detection was found by testing dilutions of the WHO 2nd standard in both plasma and serum with 3 reagent lots. The limit of detection was found to be 4.3 IU/mL for plasma and 3.9 IU/mL for serum using probit analysis and a 95% positivity rate.

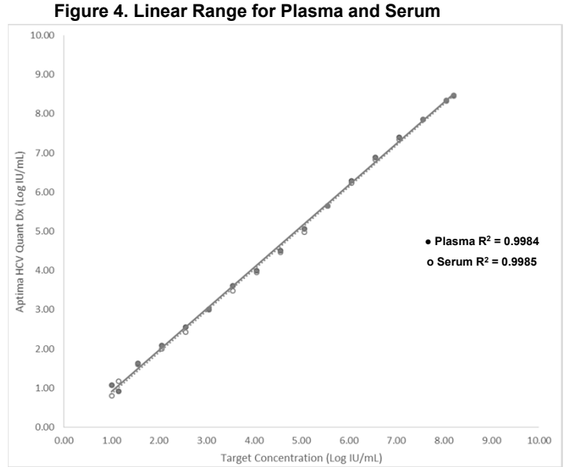
Table 1: Limit of Detection for Plasma & Serum

Predicted Detection Limit	Concentration in IU/ml (95% CI)	
	Serum	Plasma
10%	0.3	0.3
	(0.2-0.4)	(0.2-0.3)
20%	0.5	0.4
	(0.3-0.6)	(0.3-0.5)
30%	0.6	0.5
	(0.5-0.7)	(0.4-0.7)
40%	0.8	0.7
	(0.6-0.9)	(0.5-0.9)
50%	1.0	0.9
	(0.8-1.1)	(0.7-1.1)
60%	1.2	1.1
	(1.0-1.4)	(0.9-1.4)
70%	1.5	1.5
	(1.3-1.8)	(1.2-1.8)
80%	2.0	2.0
	(1.6-2.5)	(1.6-2.5)
90%	2.9	3.0
	(2.3-3.8)	(2.4-4.1)
95%	**3.9**	**4.3**
	(3.1-5.6)	(3.3-6.2)

Linear Range

The linear range was found by testing dilutions of HCV 1a armored RNA in replicates of 5. Plasma and serum dilutions were tested at concentrations from 10 IU/mL to 100,000,000 IU/mL.

Figure 4. Linear Range for Plasma and Serum



Conclusions

Based on these data, the Aptima HCV Quant Dx Assay gave comparable viral load results when compared to the Roche COBAS AmpliPrep/COBAS TaqMan HCV Test, v2.0 and Abbott RealTi*m*e HCV Assay. The performance of the Aptima assay makes it an excellent candidate for the detection and monitoring of HCV.

 Poster presented at 13th European HIV and Hepatitis Meeting 2015 , Barcelona, Spain