Performance Characterization of the Second Generation COBAS® AmpliPrep/COBAS® TaqMan® HCV, v2.0 Quantitative Test Incorporating A Novel Dual-Probe Assay Design

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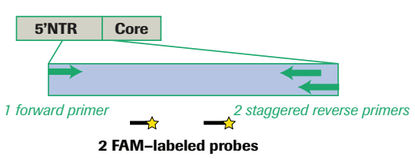
1. Background

HCV RNA viral load (VL) monitoring has been well established as a diagnostic tool for management of chronic hepatitis C patients. HCV RNA VL results are used to guide treatment decisions with the goal of antiviral therapy to achieve undetectable VL results. Therefore, a sensitive assay with high specificity in detecting and accurately quantifying HCV RNA across genotypes is critical.

In this study, we evaluated the performance characteristics of a second generation real-time PCR assay, the COBAS® AmpliPrep/COBAS® TaqMan® HCV Quantitative Test, version 2.0 (COBAS® HCV v2 test), designed with a novel dual-probe approach. The COBAS® HCV v2 test was designed to quantify challenging HCV genotype 4 specimens, show improved HCV mismatch tolerance and contains a redundancy design for potential new polymorphisms.

Figure 1: Dual probe approach



2. Results

Limit of Detection

The Limit of Detection (LOD) / Analytical Sensitivity as determined by PROBIT analysis to obtain a 95% Hit Rate is 11 IU/mL in EDTA Plasma and 12 IU/mL in Serum (data not shown). The 95% confidence limits are 10–13 IU/mL in plasma and 10–14 IU/mL in serum.

Table 1: Limit of detection in EDTA Plasma

All Kit-Lots combined, EDTA Plasma, 3 days			
Input Titer (HCV RNA IU/mL)	Number of Valid Replicates	Number of Positives	Hit Rate in %
50	251	251	100
25	251	250	100
15	251	246	98
10	252	236	94
5	252	280	71
2.5	251	121	48
0	250	0	0
LOD by PROBIT at 95% Hit Rate	11 IU/mL 95% confidence range: 10–13 IU/mL		
LOD by Hit Rate	15 IU/mL		

The verified Limit of Detection (LOD) for Genotypes as determined by ≥ 95 % Hit Rate analysis is 15 IU/mL in EDTA Plasma and Serum for all Genotypes (GT1a, GT1b, GT2a, GT2b, GT3, GT4, GT5 and GT6). For each Genotype (1a, 1b, 2a, 2b, 3, 4, 5, and 6) the absolute deviation from Linearity was ≤ 0.2 Log10 for all concentration levels tested (in EDTA Plasma).

Table 2: Detailed Results for LOD Genotypes in EDTA Plasma and Serum

Genotype	15 IU/mL (plasma)			15 IU/mL (serum)		
	No. Valid Replicates	No. Positives	Hit Rate (%)	No. Valid Replicates	No. Positives	Hit Rate (%)
1a	63	63	100	62	62	100
1b	63	62	98	63	63	100
2a	63	61	97	61	60	98
2b	62	62	100	63	61	97
3	63	63	100	63	63	100
4	63	62	98	63	62	98
5	62	62	100	61	60	98
6	63	62	98	70	69	99

Correlation with CAP/CTM HCV test

Correlation to the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test (version 1) was good among the clinical samples explored (n=412 genotype 1-6 samples, R^2=0.88; R^2=0.94 without n=104 genotype 4 samples).

In sub-analysis of genotype 1a and 1b clinical samples (n=150), the mean difference in measurements was 0.28 log_{10}.

Bland-Atman analysis of genotype samples shows improved performance of the COBAS® HCV v2 by an average of 0.53 log_{10}.

Figure 2: Deming regression analysis of CAP/CTM HCV Test, v2.0 versus CAP/CTM HCV Test (including all genotypes)

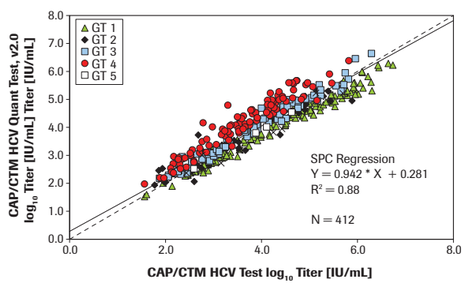
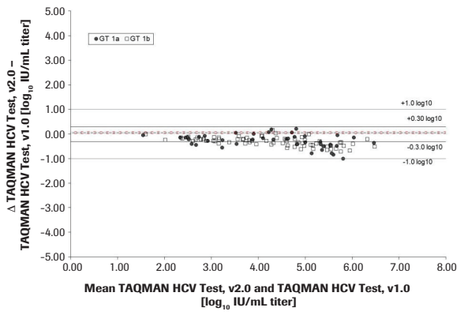


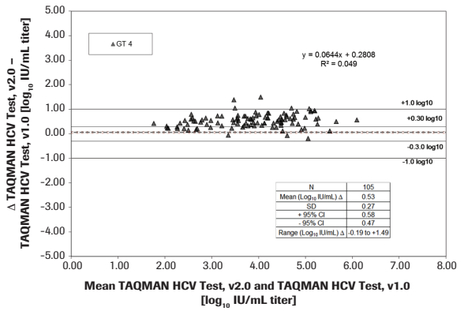
Figure 3: Bland-Altman analysis of CAP/CTM HCV Test, v2.0 versus CAP/CTM HCV Test in 150 HCV genotype 1a and 1b clinical samples



	Average Δ (v2.0 – v1.0)	Number
gt1a	-0.28	45
gt1b	-0.31	71
gt 1*	-0.21	34
Total	**-0.28**	**150**

* Subtype information not available.

Figure 4: Bland-Altman analysis of CAP/CTM HCV Test, v2.0 versus CAP/CTM HCV Test in 105 HCV genotype 4 clinical samples



Clinical Utility

Samples for testing in this study were from subjects who had received interferon alfa-2a/2b plus ribavirin treatment for chronic HCV infection. A total of 328 subjects with at least one available sample at (a) Baseline and (b) Week 4 or Week 12 were included in this study.

Of the 328 subjects, most were male (61.6%), and ≥40 years of age (69.5%). Out of these 328 subjects, 80.2% subjects were infected with genotype 1 HCV. Viral load at Week 0 was greater than 400,000 IU/mL for 86.6% of subjects.

For this study, the definition of RVR required a value for HCV RNA < LLoQ (the lower limit of quantitation of the assay) at Week 4 of antiviral therapy.

Table 6 presents the NPV, PPV, and corresponding OR at Week 4 for all genotypes combined and stratified by genotype 1 and non-genotype 1. These results demonstrate a high PPV (0.90) for all subjects at Week 4 when analyzed independent of genotype. The PPV is higher for both genotypes combined or for genotype 1 alone than for genotype non-1. The NPV for not achieving SVR is 0.54 for all subgroups and is less useful for predicting No SVR, especially in the non-1 genotype population.

Table 3: NPV and PPV at Week 4 (RVR) and Corresponding OR

Genotype	Prediction Rule	Negative Predictive Value (NPV)		Positive Predictive Value (PPV)		Odds Ratio (OR)
		Estimate (95% CI)	N	Estimate (95% CI)	N	Estimate (95% CI)
All	<LLoQ	0.54 (0.46, 0.61)	96/178	0.90 (0.81, 0.96)	72/80	10.5 (4.7, 26.6)
1	<LLoQ	0.54 (0.46, 0.62)	89/164	0.91 (0.79, 0.98)	42/46	12.5 (4.2, 49.5)
Non-1	<LLoQ	0.50 (0.23, 0.77)	7/14	0.88 (0.73, 0.97)	30/34	7.5 (1.4, 43.5)

Note: <LLoQ is equal to <1.5E+01 IU/mL (Lower Limit of Quantitation of the assay).
NPV: The denominator is the number of patients with no RVR at 4 weeks; the numerator is the number of patients who did not achieve SVR among patients with no RVR at 4 weeks.
PPV: The denominator is the number of patients with RVR at 4 weeks; the numerator is the number of patients who achieved SVR among patients with RVR.
OR: The measure of association between virologic response and SVR that is equal to (NPV*PPV)/((1 NPV)*(1-PPV)).

Comparison with Qualitative Tests

The performance of the CAP/CTM HCV Test, v2.0 was compared to two FDA-approved qualitative HCV RNA tests. The primary comparison was to the VERSANT® HCV RNA Qualitative Assay, a commercially available highly sensitive qualitative test (LOD = 7.5 IU/mL). The secondary comparison was to the COBAS® AMPLICOR HCV Test, v2.0 (LOD = 50 IU/mL).

Table 4: Agreement of the CAP/CTM HCV Test, v2.0 with the Composite (Reference) Comparator

CAP/CTM HCV Test, v2.0	HCV Composite Comparator			Total
	Positive	Negative	Indeterminate*	
Positive	166	2	0	168
Negative	0	108	1	109
Total	166	110	1	277
Positive Percent Agreement (95% exact CI)	100.0% (97.7%, 100.0%)			
Negative Percent Agreement (95% exact CI)		98.2% (93.7%, 99.5%)		
Overall Percent Agreement (95% exact CI)				99.3% (97.4%, 99.8%)

Note: Subjects contributing valid CAP/CTM HCV Test, v2.0; COBAS AMPLICOR HCV Test, v2.0; and VERSANT HCV RNA Qualitative Assay results are included in this summary table.
*An indeterminate result occurred when VERSANT HCV RNA Qualitative Assay and COBAS AMPLICOR HCV Test, v2.0 tests were not concordant, ie, one test was positive and the other negative or the reverse.
CI = confidence interval.

The CAP/CTM HCV Test, v2.0 is highly concordant with the qualitative FDA-approved VERSANT® HCV RNA Qualitative Assay and COBAS® AMPLICOR HCV Test, v2.0.

5. Conclusions

In conclusion, the COBAS® HCV v2 test demonstrated excellent performance and sensitivity across all HCV genotypes. The test demonstrated clinical utility in a treatment patient cohort and high concordance with qualitative assays due to the expanded linear range. The COBAS® HCV v2 test is well suited for the management of HCV patients in today's treatment environment. Performance specifications are pre-commercialization and subject to regulatory approval. The CAP CTM HCV Quantitative Test, v2.0 is currently in development and not yet commercially available.

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