CPAL
Central **P**ennsylvania **A**lliance **L**aboratory

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cobas® 6800 HCV Viral Load Assay
- New Platform -

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Ordering Information and Suggested Codes:

Mnemonic	**HCV VL**
Test Name	HCV Viral Load (PCR)
Test Number	3500190
Specimen	Preferred: EDTA Plasma, Alternative: Serum Requested volume 3 mL, Minimum volume accepted 1.5 mL. Preferably no false-bottom tubes.
Stability	Upon plasma/serum separation: **up to 6 days refrigerated at 2°C to 8°C,** up to 12 weeks frozen at ≤ -18°C. **Note: separate plasma/serum from cells within 24 hours of collection.**
LOINC Codes	11011-4
CPT Codes	87522

Effective Date: Testing offered beginning on Monday, June 5th, 2017.

Performed: Monday through Friday, dayshift

Reference Range:

Age Range	
All	Not Detected

Background:

Detection of antibodies to HCV (anti-HCV) indicates prior exposure to hepatitis C but does not distinguish between cleared or active infection (i.e. where the virus is still replicating). Detection of HCV RNA with the detection of anti-HCV identifies an active hepatitis C infection. The results of HCV RNA testing together with other biochemical and clinical information, may be used to confirm an active HCV infection, measure the level of virus in the blood and assist in HCV prevention counseling, medical care, and treatment decision making.

Quantitation of HCV RNA for measuring baseline viral loads and for on-treatment viral loads have been well established in demonstrating the efficacy of antiviral response to pegylated interferon plus ribavirin (pegIFN/RBV) combination therapy. More recently direct acting antiviral combination therapies are prescribed, consisting of a nucleotide analogue viral polymerase inhibitor (NS5B) and a viral protease (NS3) or viral replicase inhibitor (NS5A) agent and lists of preferred first-line anti-HCV therapies per HCV genotype have been established. Current guidelines for the management and treatment of HCV recommend quantitative testing for HCV RNA before the start of antiviral therapy, and at 12 weeks or later, following the end of treatment. Additional time points may be recommended per therapy type, see current guidelines.

An HCV RNA level below 25 IU/mL, 12 weeks after the end of treatment, is the goal of treatment and indicates that a sustained virologic response (SVR) has been achieved

Principle of Test:

Roche Molecular Systems, Inc. **cobas®** HCV is a quantitative test performed on the cobas® 6800 System and **cobas®** 8800 System. **cobas®** HCV enables the detection and quantitation of HCV RNA in EDTA plasma or serum of infected patients. Dual probes are used to detect and quantify, but not discriminate genotypes 1-6. **cobas®** HCV is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection.

Validation Data:

Precision Studies:

Within-run and between-run precision were evaluated at two levels, low and high. Patient samples with low-level viral loads were used for the low pool (target 3 to 4 $\log_{10}$ IU/mL), and patient samples with high-level viral loads were used for the high pool (target >5 $\log_{10}$ IU/mL).

For within run precision, ten aliquots of each of the two pools were tested on a single run. For between run precision, five aliquots of each pool were tested on two subsequent runs. Results were combined with the results from within-run precision (total of 20 replicates for each pool). The SD for low and high pools fall within manufacturer's claim of 0.10 $\log_{10}$ IU/mL (see Table 1).

Table 1: Precision Summary

	Within Run				Between Run			
	Sample Number	Mean (log10)	SD	CV	Sample Number	Mean (log10)	SD	CV
Low Pool	10	4.62	0.054	1.16%	20	4.62	0.050	1.09%
High Pool	10	7.10	0.054	0.76%	20	7.08	0.068	0.96%

Accuracy, Reportable Range, AMR, and Linearity Verification Studies:

The purpose of these studies was to verify that the performance of the **cobas®** HCV assay is capable of producing linear and accurate results spanning the assay's analytical measurement range. To accomplish this, two bottles each of seven HCV panel specimens from Exact Diagnostics were individually pooled. The results of each pool ranged from 2.00-7.48 $\log_{10}$ IU/mL. To reach the low end of the analytical measurement range, an additional sample was prepared by diluting the remaining pooled level 1 panel specimen with tested negative filler plasma. Additional panel materials were also provided from Exact for further linearity studies, and per Roche and Exact, at least 3 replicates should be run and the results averaged prior to performing linearity and AMR analysis. Four replicates were obtained and averaged prior to assessment in EP Evaluator. Results are shown in Figure 1 below. Results passed calibration verification, accuracy, linearity, and reportable range passed across the full range tested.

Figure 1: Accuracy, Reportable Range, AMR, and Linearity Studies Summary

Accuracy, Reportable Range, and Linearity

	Assigned	N	Accuracy & Recovery			Linearity	Rpt Range
			Mean	% Rec	Status		
HCV 1	2.00	1	2.2200	111.0	Pass	Pass	Pass
HCV 2	2.48	1	2.6450	106.7	Pass	Pass	--
HCV 3	3.48	1	3.5860	103.0	Pass	Pass	--
HCV 4	4.48	1	4.7910	106.9	Pass	Pass	--
HCV 5	5.48	1	5.7100	104.2	Pass	Pass	--
HCV 6	6.48	1	6.7040	103.5	Pass	Pass	--
HCV 7	7.48	1	7.5750	101.3	Pass	Pass	Pass

See User's Specifications for Pass/Fail criteria.

Linearity Summary

	Overall
Slope	1.015
Intercept	0.1359
Obs Err	0.0545 log IU/mL (conc) or 2.3%
N	7

LINEAR within SEa of 0.24 log IU/mL (conc) or 10.0%

Experimental Results

HCV 1	2.220
HCV 2	2.645
HCV 3	3.586
HCV 4	4.791
HCV 5	5.710
HCV 6	6.704
HCV 7	7.575

X: Excluded from calculations

User's Specifications

Allowable Total Error	0.24 log IU/mL (conc) or 10.0%
Systematic Error Budget	100%
Allowable Systematic Error	0.24 log IU/mL (conc) or 10.0%
Reportable Range	1.18 to 8.00 log IU/mL
RR-Low Range	0.000 to 2.360 log IU/mL
RR-High Range	6.800 to 9.200 log IU/mL

Calibration Verification

	Assigned	N	Accuracy & Recovery			Linearity	Rpt Range
			Mean	% Rec	Status		
HCV 1	2.00	1	2.2200	111.0	Pass	--	Pass
HCV 2	2.48	1	2.6450	106.7	Pass	--	--
HCV 3	3.48	1	3.5860	103.0	Pass	--	--
HCV 4	4.48	1	4.7910	106.9	Pass	--	--
HCV 5	5.48	1	5.7100	104.2	Pass	--	--
HCV 6	6.48	1	6.7040	103.5	Pass	--	--
HCV 7	7.48	1	7.5750	101.3	Pass	--	Pass

See User's Specifications for Pass/Fail criteria.

Linearity Summary

	Reg. Regression
Slope	0.995 ± 0.017
Intercept	0.2146 ± 0.0816
SEE	0.0829
N	7

Experimental Results

HCV 1	2.220
HCV 2	2.645
HCV 3	3.586
HCV 4	4.791
HCV 5	5.710
HCV 6	6.704
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Reportable Range	1.18 to 8.00 log IU/mL
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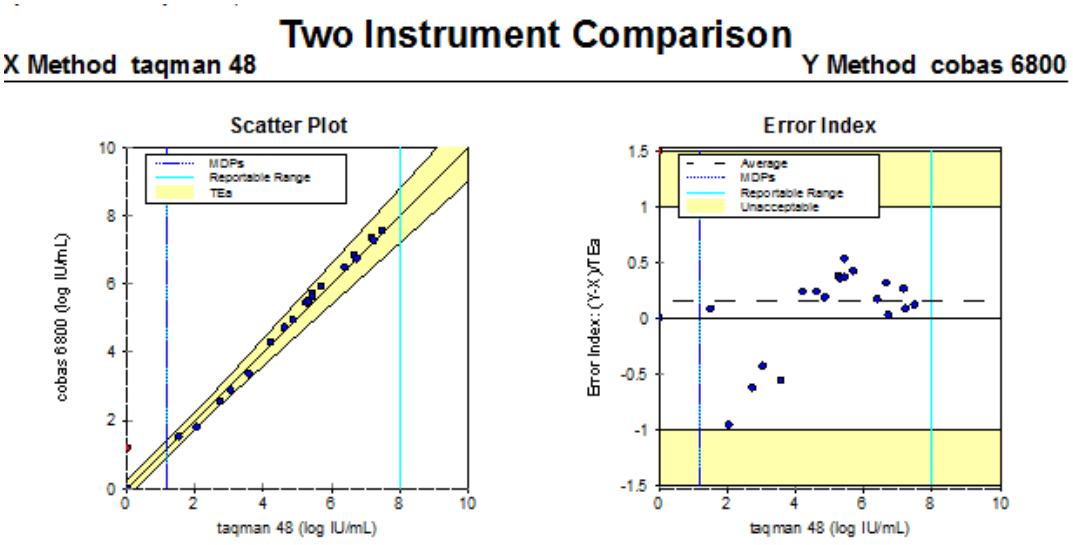
RR-High Range	6.800 to 9.200 log IU/mL

Method Comparison:

The purpose of this study was to verify that the performance of the HCV Viral Load assay on the **cobas®** 6800 System is capable of providing comparable results when specimens previously tested on the **cobas®** Ampliprep/TaqMan Systems are re-assayed on the **cobas®** 6800 System. Forty specimens were compared over a range of 0.00-7.47 $\log_{10}$ IU/mL. Analysis in EP Evaluator was performed using both Two Instrument Comparison and Alternate Method Comparison modules. The tests passed within allowable error limits, see Figures 2 and 3. One sample with an acceptable result of <15 IU/mL (<1.18 log IU/mL) was excluded, as EP Evaluator is unable to perform statistics on < values.

Figure 2: Method Comparison: cobas® Ampliprep/Taqman System vs. cobas® 6800 (Two Instrument Comparison)



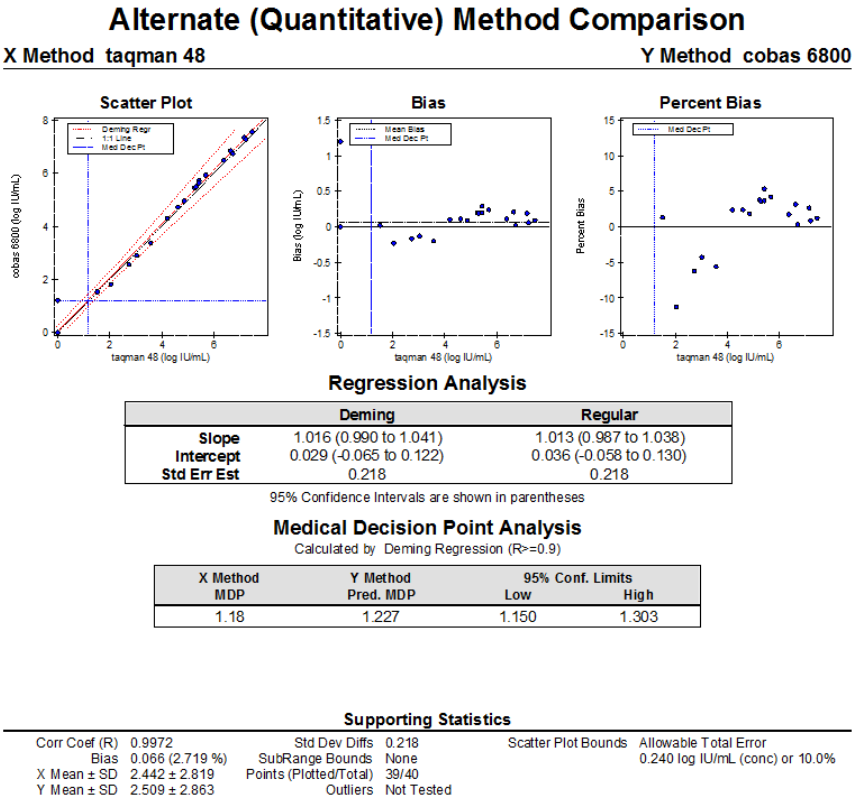
Evaluation of Results

HCV VL was analyzed by methods taqman 48 and cobas 6800 to determine whether the methods are equivalent within AllowableTotal Error of 0.24 log IU/mL (conc) or 10%. 39 specimens were compared over a range of 0.00 to 7.47 log IU/mL. The test Passed. The difference between the two methods was within allowable error for 38 of 39 specimens (97.4%). The average Error Index (Y-X)/TEa was 0.16, with a range of -0.96 to 5.00. The largest Error Index occured at a concentration of 0.00 log IU/mL.

Key Statistics	
Average Error Index	0.16
Error Index Range	-0.96 to 5.00
Coverage Ratio	92%
Evaluation Criteria	
Allowable Total Error	0.24 log IU/mL (conc) or 10%
Reportable Range	1.18 to 8.00 log IU/mL

Deming Regression Statistics	
Y = Slope * X + Intercept	
Correlation Coeff (R)	0.9972
Slope	1.016 (0.990 to 1.041)
Intercept	0.029 (-0.065 to 0.122)
Std Error Estimate	0.218
N	39 of 40

Figure 3: Method Comparison: cobas® Ampliprep/Taqman System vs. cobas® 6800 (Alternate Method Comparison)



Limitations:

1. **cobas®** HCV has been evaluated only for use in combination with the **cobas®** HBV/HCV/HIV-1 Control Kit, **cobas®** NHP Negative Control Kit, cobas omni MGP Reagent, cobas omni Lysis Reagent, cobas omni Specimen Diluent, and cobas omni Wash Reagent for use on the **cobas®** 6800/8800 Systems.
2. Reliable results depend on proper sample collection, storage, and handling procedures.
3. This test has been validated only for use with EDTA plasma and serum. Testing of other sample types may result in inaccurate results.
4. Quantitation of HCV RNA is dependent on the number of virus particles present in the samples and may be affected by sample collection methods, patient factors (i.e., age, presence of symptoms), and/or stage of infection.
5. Though rare, mutations within the highly conserved regions of a viral genome covered by cobas® HCV may affect primer and/or probe binding resulting in the under-quantitation of virus or failure to detect the presence of virus.
6. Drug interference studies were performed in vitro and may not assess the potential interferences that might be seen after the drugs are metabolized in vivo.
7. cobas® HCV has not been approved for use as a screening test for the presence of HCV in blood or blood products.
8. Significant differences exist in the quantitative values generated by different laboratory HCV viral load assays. It is recommended that serial patient testing be performed using a consistent method. Quantitation of HCV RNA is dependent on the number of virus particles present in the specimen and may be affected by specimen collection methods, patient factors (e.g. age, presence of symptoms), and stage of infection. Though rare, mutations in the highly conserved regions of the viral genome covered by the primers and/or probe used in this assay method may result in the under-quantitation of or failure to detect the presence of the virus in this circumstance. All results must be interpreted within the context of all relevant and clinical laboratory findings.

References:

1. **cobas®** HCV Package Insert, Version 1.0, October 2015