



June 1, 2017

**cobas® 6800 HCV Viral Load Assay**  
**- New Platform -**

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**Ordering Information and Suggested Codes:**

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

**Effective Date:** Testing offered beginning on Monday, June 5<sup>th</sup>, 2017.

**Performed:** Monday through Friday, dayshift

**Reference Range:**

<b>Age Range</b>	
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<sub>10</sub> IU/mL), and patient samples with high-level viral loads were used for the high pool (target >5 log<sub>10</sub> 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<sub>10</sub> 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
<b>Low Pool</b>	10	4.62	0.054	1.16%	20	4.62	0.050	1.09%
<b>High Pool</b>	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<sub>10</sub> 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
HCV 7	7.575

X: Excluded from calculations

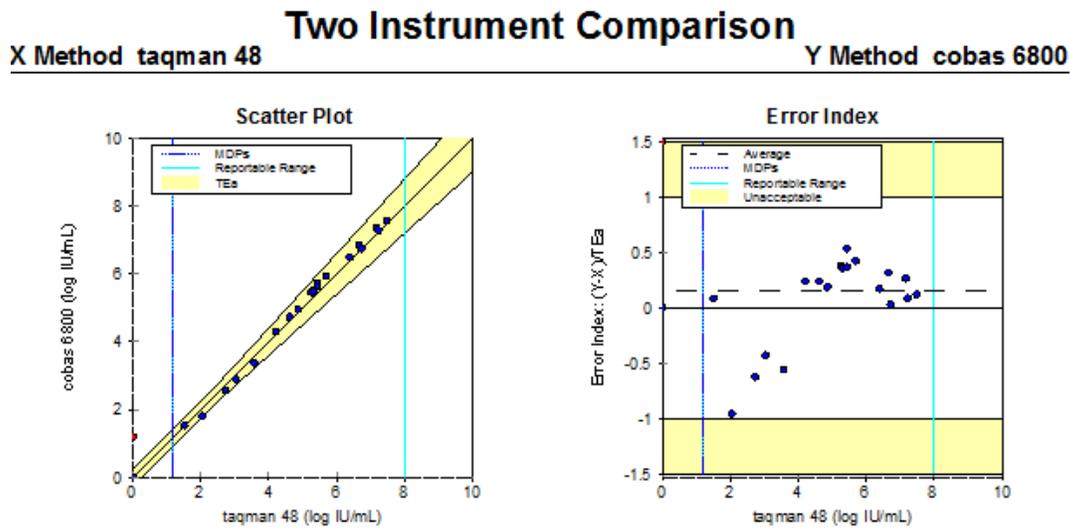
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Reportable Range	1.18 to 8.00 log IU/mL
RR-Low Range	0.000 to 2.360 log IU/mL
RR-High Range	6.800 to 9.200 log IU/mL

**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<sub>10</sub> 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 Allowable Total 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 occurred 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**

<b>Y = Slope * X + Intercept</b>	
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



**References:**

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