SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Real-time PCR test

Device Trade Name: **cobas**® HCV

Device Procode: MZP

Applicant's Name and Address: Roche Molecular Systems, Inc.
4300 Hacienda Drive
Pleasanton, CA 94588-2722

Date of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P150015

Date of Notice of Approval: October 14, 2015

II. INDICATIONS FOR USE

cobas® HCV is an in vitro nucleic acid amplification test for both the detection and quantitation of hepatitis C virus (HCV) RNA, in human EDTA plasma or serum, of HCV antibody positive or HCV-infected individuals. Specimens containing HCV genotypes 1 to 6 are validated for detection and quantitation in the assay.

cobas® HCV is intended for use as an aid in the diagnosis of HCV infection in the following populations: individuals with antibody evidence of HCV with evidence of liver disease, individuals suspected to be actively infected with HCV antibody evidence, and individuals at risk for HCV infection with antibodies to HCV. Detection of HCV RNA indicates that the virus is replicating and therefore is evidence of active infection.

cobas® HCV is intended for use as an aid in the management of HCV-infected patients undergoing anti-viral therapy. The assay can be used to measure HCV RNA levels at baseline, during treatment, at the end of treatment, and at the end of follow up of treatment to determine sustained or non-sustained viral response. The results must be interpreted within the context of all relevant clinical and laboratory findings.

cobas® HCV has not been approved for use as a screening test for the presence of HCV in blood or blood products.

Assay performance characteristics have been established for individuals treated with certain direct-acting antiviral agents (DAA) regimens. No information is available on the assay's predictive value when other DAA combination therapies are used.

III. CONTRAINDICATIONS

There are no known contraindications for use for this test.

IV. WARNINGS AND PRECAUTIONS

Warnings and precautions can be found in the labeling for the **cobas**® HCV.

V. DEVICE DESCRIPTION

cobas® HCV test Description

The **cobas**® HCV is a quantitative test performed on the **cobas**® 6800 System and **cobas**® 8800 System. The **cobas**® HCV enables both the detection and quantitation of HCV RNA in EDTA plasma or serum of HCV antibody positive or HCV infected patients. Dual probes are used to detect and quantify, but not discriminate between genotypes 1-6. The viral load is quantified against a non-HCV armored RNA quantitation standard (RNA-QS), which is introduced into each specimen during sample preparation. The RNA-QS also functions as an internal control for sample preparation and PCR amplification process. In addition, the test utilizes three external controls: a high titer positive, a low titer positive, and a negative control.

The **cobas**® HCV test system consists of:

- the **cobas**® 6800/8800 Systems
- the **cobas**® HCV Assay Specific Analysis Package (ASAP) software
- **cobas**® HCV in cassettes
- **cobas**® HBV/HCV/HIV-1 Control Kit (HPC and LPC) in cassettes
- **cobas**® NHP Negative Control Kit in cassettes
- Specimen preparation reagents (**cobas**® omni Reagents)

The **cobas**® HCV is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The **cobas**® 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the **cobas**® 6800/8800 Systems Software which assigns results to all specimens and controls tested. Results can be viewed directly on the system screen, exported, or printed as a report.

Principle of Procedure

1) Sample Preparation (Nucleic Acid Extraction and Purification):

Nucleic acid from patient samples, external controls and added armored RNA-QS molecules are simultaneously extracted. Viral nucleic acids are released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities (denatured proteins, cellular debris and potential PCR inhibitors) are removed with subsequent wash reagent steps. Purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature.

2) Nucleic Acid Amplification:
Selective amplification of target nucleic acid from the patient sample is achieved by the use of target virus-specific forward and reverse primers which are selected from highly conserved regions of the HCV genome. Selective amplification of RNA-QS is achieved by the use of sequence-specific forward and reverse primers which are selected to have no homology with the HCV genome. A thermostable DNA polymerase enzyme is used for both reverse-transcription and PCR amplification. The target and RNA-QS sequences are amplified simultaneously utilizing a universal PCR amplification profile with predefined temperature steps and number of cycles.

The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythymidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon). Any contaminating amplicons from previous PCR runs are eliminated by the AmpErase enzyme, which is included in the PCR mix, during the first thermal cycling step. However, newly formed amplicons are not eliminated since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

3) Nucleic Acid Detection:
The **cobas**® HCV master mix contains two detection probes specific for the HCV target sequences and one for the RNA-QS. The probes are labeled with target-specific fluorescent reporter dyes allowing simultaneous detection of HCV target and RNA-QS in two different target channels.

When not bound to the target sequence, the fluorescent signals of the intact probes are suppressed by a quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5'-to-3' nuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probe is generated and the cumulative signal of the reporter dye increases concomitantly. Real-time detection and discrimination of PCR products is accomplished by measuring the fluorescence of the released reporter dyes for the viral targets and RNA-QS. Since the two specific reporter dyes are measured at defined wavelengths, simultaneous detection and discrimination of the amplified HCV target and the RNA-QS is possible.

Instrumentation and Software
The **cobas**® 6800/8800 platform is configured in two instrument versions: the **cobas**® 6800 System and the **cobas**® 8800 System.

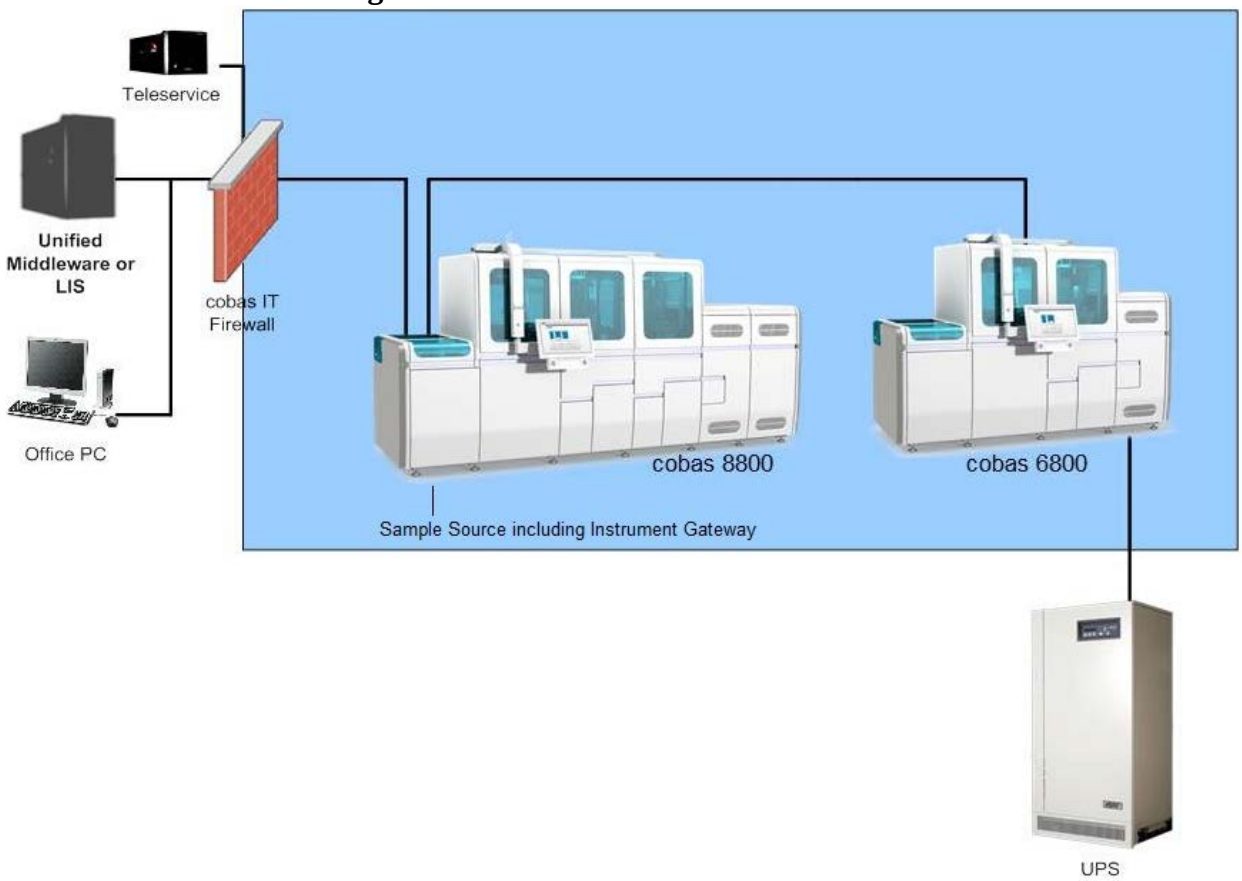
Each system is comprised of a **cobas**® 6800 or **cobas**® 8800 instrument, system software, Assay Specific Analysis Packages (ASAP), and a sample source unit, which can be connected to a conveyor system for automated transport of samples to and from the system. The test kits consist of assay-specific reagents and omni reagents (or common reagents) which can be used with any of the **cobas**® 6800/8800 assays, and on either the **cobas**® 6800 or the **cobas**® 8800 instrument.

In addition, the omni (common) reagents and consumables, such as such as the P-plates, racks, AD-plates, waste bags, pipette tips, and secondary tubes, can be used by any of the **cobas**® 6800/8800 System assays, and on either the **cobas**® 6800 or the **cobas**® 8800 instrument.

Either system can be interfaced to an uninterruptible power supply (UPS), a customer's Laboratory Information System (LIS), or middleware, and office PCs for some remote viewing and messaging functionalities.

The following figure depicts the **cobas**® 6800/8800 Platform:

Figure 1: The cobas® 6800/8800 Platform



Interpretation of Results

Results are determined automatically by the **cobas**® software and are shown in the following table:

Table 1: Individual Target Result Interpretation

Result Read-Out from cobas®	**Analytical Interpretation**	**Clinical Interpretation**
Target Not Detected	HCV RNA not detected. Report results as "HCV not detected."	No current HCV infection For HCV Diagnosis: No further testing indicated.* For Viral Load Assessment: Routine clinical follow-up according to national HCV guidelines.
< Titer Min	HCV RNA detected but not quantified. Calculated titer is below the Lower Limit of Quantitation (LLoQ) of the assay. Report results as "HCV detected, less than (Titer Min)" Titer min = 15 IU/mL	Low-level HCV viremia, may indicate previous spontaneous or treatment-related resolution of HCV infection. For HCV Diagnosis: Results must be interpreted within the context of all relevant clinical and laboratory findings* For Viral Load Assessment: Routine clinical follow-up according to national HCV guidelines.
15 IU/mL≤ Titer < 25 IU/mL	HCV RNA detected and quantified. Calculated titer is within the Linear Range of the assay – greater than or equal to 15 IU/mL and less than 25 IU/mL. Report results as "(Titer) of HCV detected".	Low-level HCV viremia, may indicate previous spontaneous or treatment-related resolution of HCV infection*. For HCV Diagnosis and Viral Load Assessment: Provide patient with appropriate counseling and link to care and treatment according to current national HCV treatment guidelines.
25 IU/mL≤ Titer ≤ Titer Max	HCV RNA detected and quantified. Calculated titer is within the Linear Range of the assay – greater than or equal to 25 IU/mL and less than or equal to Titer Max. Report results as "(Titer) of HCV detected".	Current HCV Infection. For HCV Diagnosis and Viral Load Assessment: Provide patient with appropriate counseling and link to care and treatment according to current national HCV treatment guidelines.
> Titer Max	Calculated titer is above the Upper Limit of Quantitation (ULoQ) of the assay. Report results as "HCV detected, greater than (Titer Max)." Titer max = 1.00E+08 IU/mL	Current HCV Infection. For HCV Diagnosis and Viral Load Assessment: Provide patient with appropriate counseling and link to care and treatment according to current national HCV treatment guidelines.

* Repeat HCV RNA testing if the person tested is suspected to have had HCV exposure within the past 6 months or has clinical evidence of HCV disease, or if there is concern regarding the handling or storage of the test specimen.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are currently several FDA approved in *vitro* diagnostic tests for the quantitation of HCV RNA. The patient's medical history and thorough clinical examination, in addition to serology, PCR or nucleic acid testing (NAT), determination of liver enzyme levels, and biopsy of the liver, will provide further information on the status of an HCV infection. Each alternative has its own advantages and disadvantages

VII. MARKETING HISTORY

The **cobas**® HCV is marketed in multiple countries. The device has not been withdrawn from marketing for any reasons related to its safety or effectiveness. The following table provides a list of countries where the product is distributed:

Table 2: Countries in which the cobas® HCV is Marketed

Austria	Greece	Norway
Belgium	Hungary	Poland
Bulgaria	Iceland	Portugal
Croatia	Ireland	Romania
Cyprus	Italy	Slovakia
Czech Republic	Latvia	Slovenia
Denmark	Liechtenstein	Spain
Estonia	Lithuania	Sweden
Finland	Luxembourg	Switzerland
France	Malta	Turkey
Germany	Netherlands	United Kingdom

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

When used according to the instructions in the package insert, there are no known potential direct adverse effects to health. Failure of the test to perform as indicated or human error during performance of the test may lead to improper diagnosis of patient HCV infection status or improper patient management.

The diagnosis of HCV infection requires the evaluation of the patient's blood for anti-HCV antibodies where a positive result is followed up with nucleic acid testing for HCV RNA.

A false target-not-detected (false negative) HCV RNA result may lead to a patient with HCV infection going unidentified and not receiving treatment. Under these circumstances, there is a safety concern for both the patient and the public, since they may be capable of transmitting HCV infection. However, if a patient is known to be at high risk of HCV infection, or is symptomatic, and the physician's suspicion of HCV infection is high, HCV RNA testing is

often repeated within a certain timeframe. If a patient has completed treatment, a false result of target-not-detected on follow-up would lead a doctor to conclude that the patient is "cured" when HCV RNA may still be present. This is not as big a concern given that with the new direct acting antiviral (DAAs) therapies the patient is on treatment for a finite time and the response during treatment is not used to guide decisions about the duration of treatment.

A false reactive (false positive) result using an HCV RNA assay is not considered a patient or public health concern because amount of HCV RNA will also be determined and if it is low, the patient should be retested within 6 months. Treatment of the patient with chronic HCV infection is initiated after additional clinical, laboratory, and behavioral assessment of the patient.

IX. SUMMARY OF PRE-CLINICAL STUDIES

Laboratory Studies

All non-clinical studies were performed at Roche Molecular Systems using both **cobas**® 6800 and 8800 systems. The HCV Secondary Standard used in these studies is traceable to the WHO International Standard.

Limit of Detection (LOD): WHO International Standard: The limit of detection (LoD) of **cobas**® HCV was determined for the WHO International Standard (genotype 1a) and for genotype 1b through 6. The overall LoD was 12.0 IU/mL for EDTA plasma and 13.7 IU/mL for serum.

The limit of detection of **cobas**® HCV for the WHO International Standard was determined by analysis of serial dilutions of the WHO International Standard for Hepatitis C Virus RNA for Nucleic Acid Amplification Technology Assays (4th WHO International Standard) genotype 1a obtained from NIBSC in HCV-negative human EDTA plasma and serum, using sample processing volumes of 500 µL. The minimum sample requirement for a sample to be processed by **cobas**® 6800/8800 Systems is 650 µL. Panels of six concentration levels plus a negative were tested with 500 µL sample processing volume over three lots of **cobas**® HCV test reagents, multiple runs, days, operators, and instruments.

The results for EDTA plasma and serum are shown in Table 3 and Table 4, respectively.

Table 3: HCV RNA WHO International Standard Limit of Detection in EDTA Plasma

Instrument	LoD by PROBIT at 95% hit rate (IU/mL)	95% confidence interval (IU/mL)
cobas® 6800 System	8.5	7.5-9.79
cobas® 8800 System	8.3	7.28-9.55

Table 4: HCV RNA WHO International Standard Limit of Detection in Serum

Instrument	LoD by PROBIT at 95% hit rate (IU/mL)	95% confidence interval (IU/mL)
cobas® 6800 System	9.6	8.70-10.95
cobas® 8800 System	12.4	10.93-14.69

LoD: Genotypes 1b through 6: The limit of detection of **cobas**® HCV for genotypes 1b through 6 was determined by analysis of serial dilutions from each genotype, in HCV-negative human EDTA plasma and serum. Panels of six concentration levels plus a negative were tested using four lots of **cobas**® HCV test reagents, over multiple runs, days, operators, and instruments. The results for EDTA plasma and serum are shown in Table 5 and Table 6, respectively.

Table 5: HCV RNA Genotype Limit of Detection in EDTA Plasma

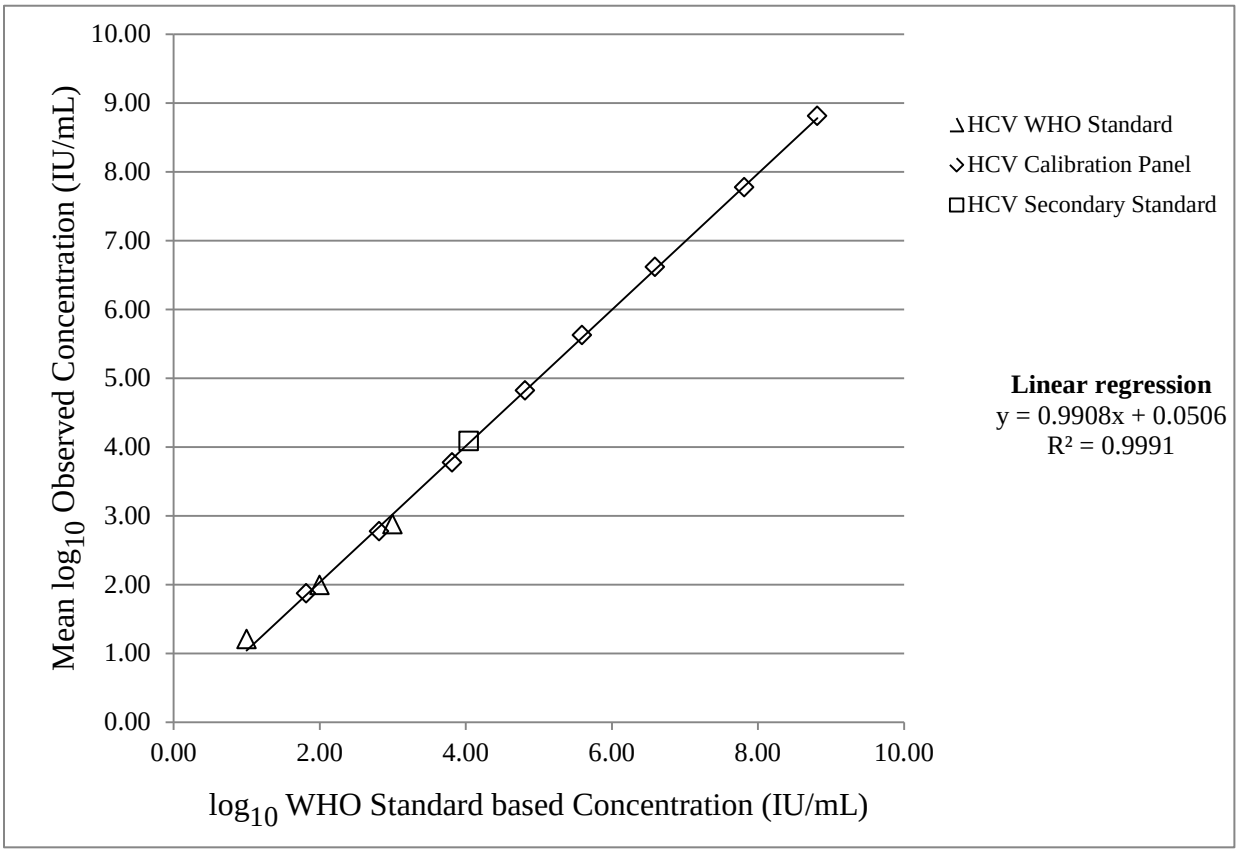
	cobas® 6800 System		**cobas® 8800 System**	
Genotype	**95% LoD by Probit (IU/mL)**	**95% Confidence Interval (IU/mL)**	**95% LoD by Probit (IU/mL)**	**95% Confidence Interval (IU/mL)**
GT 1b	11.4	9.82 – 14.24	10.2	8.68-13.04
GT 2	9.3	8.09 – 11.49	9.5	8.43-11.55
GT 3	8.5	7.30 – 10.73	9.2	7.65-11.98
GT 4	12.0	10.30 – 15.33	11.4	9.86-14.28
GT 5	10.5	8.93 – 13.65	8.3	7.32-10.19
GT 6	11.9	9.36 – 17.55	10.6	9.09-13.48

Table 6: HCV RNA Genotype Limit of Detection in Serum

	cobas® 6800 System		**cobas® 8800 System**	
Genotype	**95% LoD by Probit (IU/mL)**	**95% Confidence Interval (IU/mL)**	**95% LoD by Probit (IU/mL)**	**95% Confidence Interval (IU/mL)**
GT 1b	13.7	11.45-18.34	9.9	8.65-12.05
GT 2	11.5	9.67-15.22	11.8	10.02-15.17
GT 3	6.8	5.90-8.50	8.0	6.73-10.13
GT 4	11.3	9.90-13.90	13.7	11.26-18.95
GT 5	11.9	9.94-15.79	13.1	10.78-17.94
GT 6	10.5	9.16-12.92	10.4	8.89-13.26

Traceability to the WHO Standard: Several standards and controls have been used during development of this test to provide traceability to the WHO standard. The standards used during development of the test include the HCV WHO Standard, the RMS HCV Secondary Standard, and the RMS HCV Calibration Panel. The Standards and the Calibration Panel were tested. The concentration range tested for the HCV WHO Standard was from 1.00E+01 IU/mL to 1.00E+03 IU/mL (1.00 – 3.00 $\log_{10}$ IU/mL), the RMS HCV Secondary Standard was tested at 1.10E+04 IU/mL (4.04 $\log_{10}$ IU/mL), and the RMS HCV Calibration Panel was tested from 6.50E+01 to 6.50E+08 IU/mL (1.81 – 8.81 $\log_{10}$ IU/mL).
All materials behaved similarly and demonstrated co-linear dilution performance across the linear range of **cobas**® HCV (Figure 2). Based on these results, the calibration and standardization process of **cobas**® HCV provides quantitation values for the RMS HCV calibration panel, the RMS HCV Secondary Standard, and the HCV WHO Standard that are similar to the expected values with deviation of not more than 0.21 $\log_{10}$ IU/mL. The maximum deviation was obtained around the test LLoQ using combined regression analyses for the RMS HCV Calibration Panel, the RMS HCV Secondary Standard, and the HCV WHO Standard.

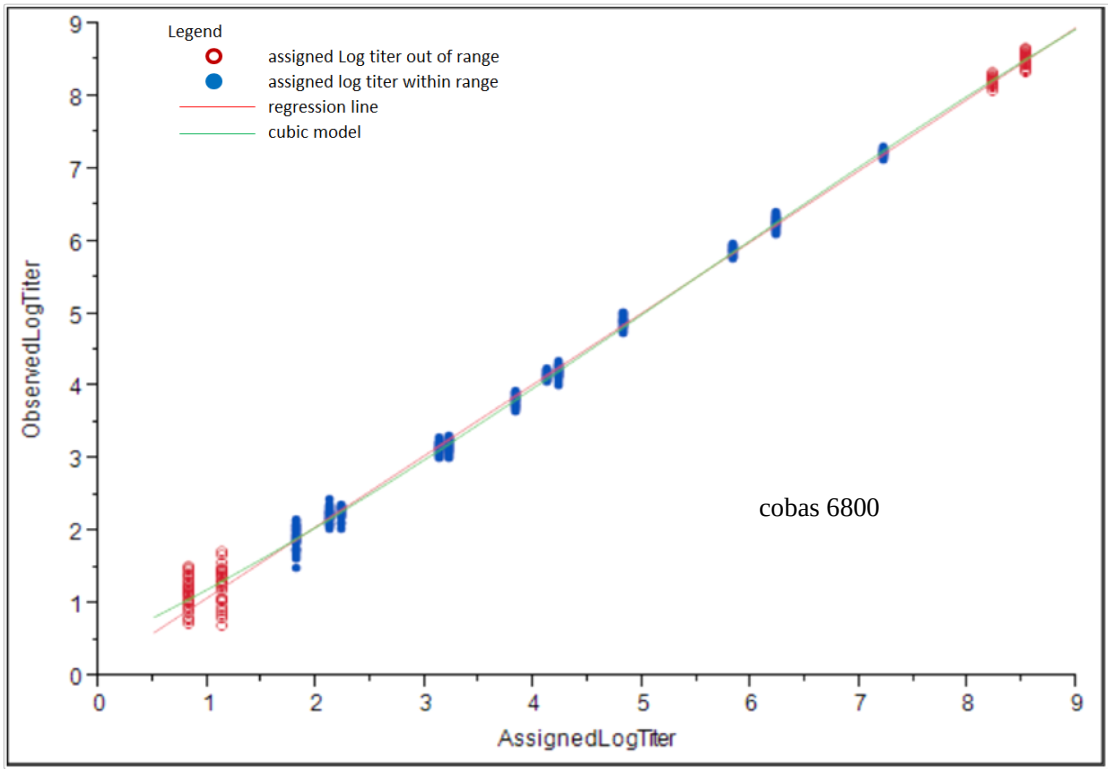
Figure 2: Traceability to WHO International Standard (Mean Observed $\log_{10}$ titer Versus $\log_{10}$ WHO Standard Based Titer) using cobas® HCV



Linear Range – Genotype 1: The linearity study was performed with a dilution series consisting of 16 panel members spanning the intended linear range for the predominant genotype (GT 1). High titer panel members were prepared from a high titer armored RNA (arRNA) stock whereas the lower titer panel members were prepared from clinical samples (CS). The linearity panel was designed to have an approximately 2 $\log_{10}$ titer overlap between the two material sources. The expected linear range of **cobas**® HCV is from LLoQ (15 IU/mL) to ULoQ (1.00E+08 IU/mL). The linearity panel was designed to range from one concentration below LLoQ (e.g., 7.5 IU/mL) to one concentration level above ULoQ (e.g., 2.0E+08 IU/mL) and to include medical decision points.

With 500 µL processing volume, **cobas**® HCV is linear for EDTA plasma and serum from 15 IU/mL to 1.00E+08 IU/mL and shows an absolute deviation from the better fitting non-linear regression of less than ± 0.24 $\log_{10}$. Across the linear range, the accuracy of the test was within ± 0.24 $\log_{10}$. See Figure 3 and Figure 4 below:

Figure 3: Linearity in EDTA Plasma



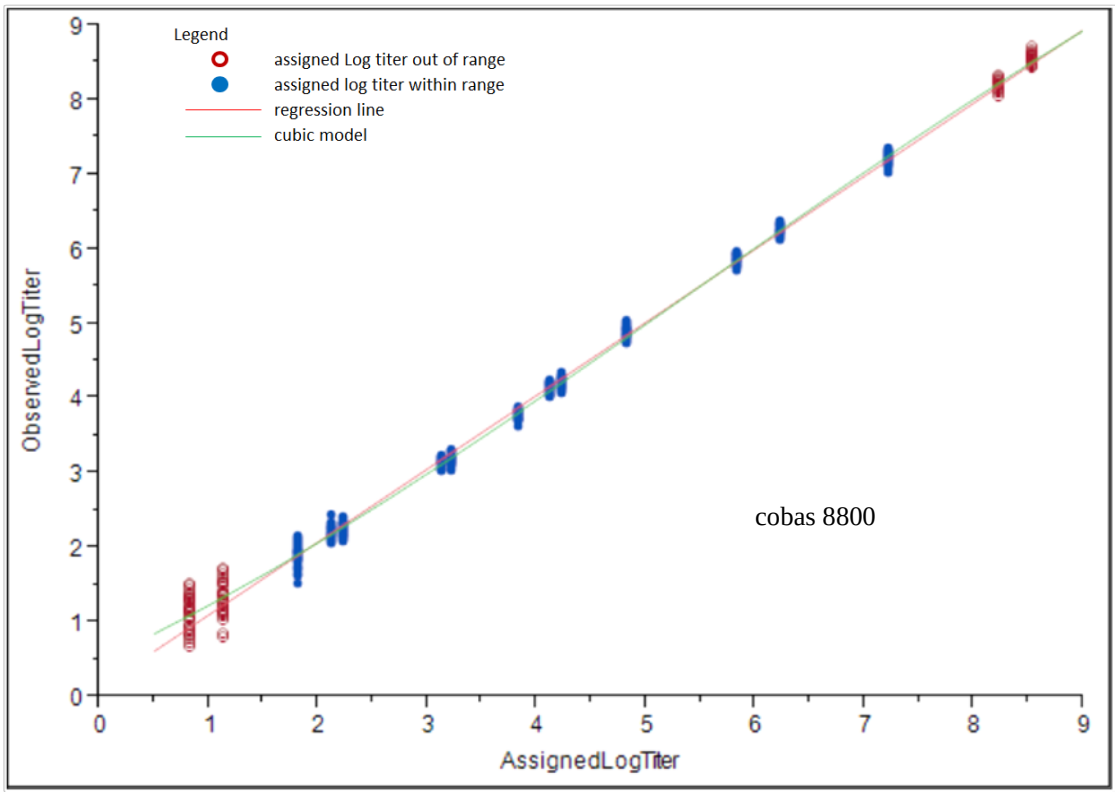
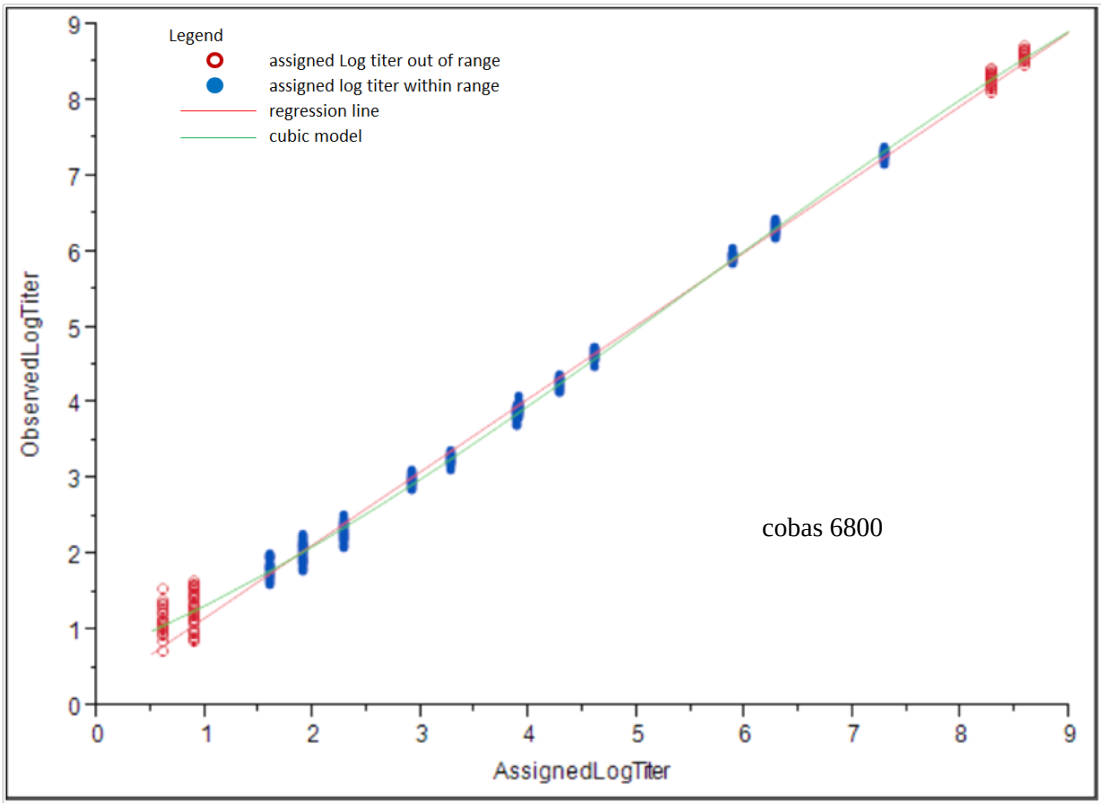
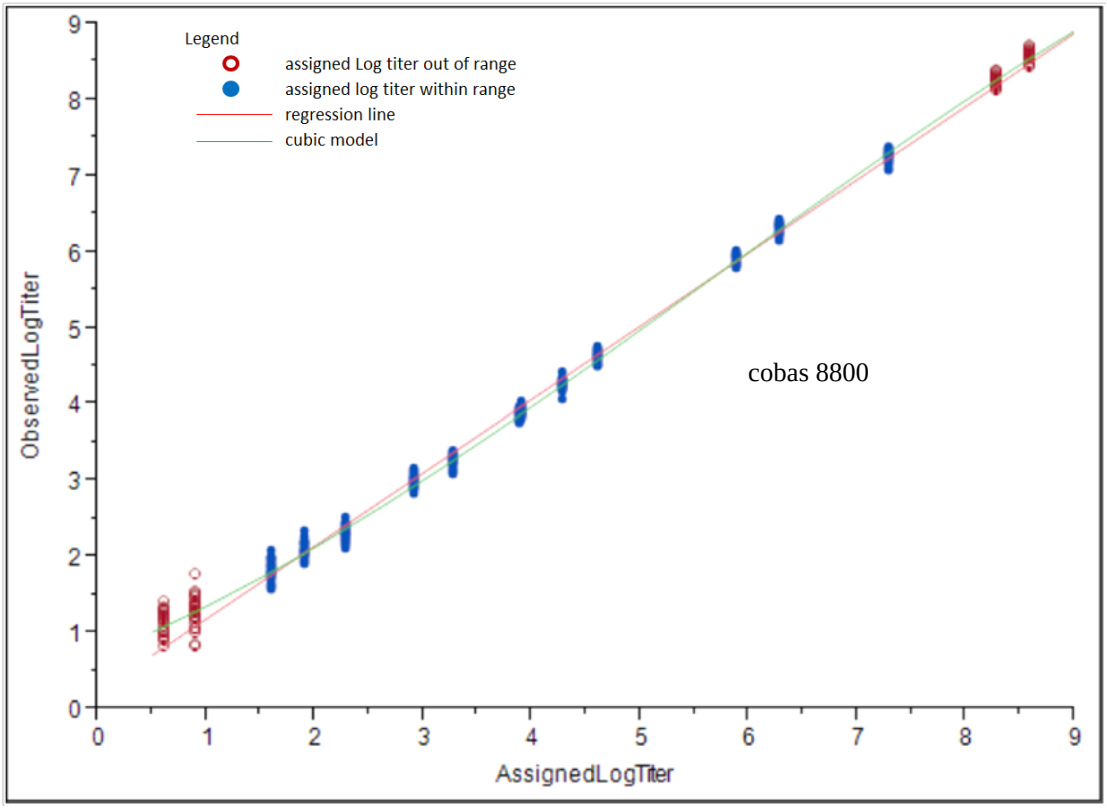


Figure 4: Linearity in Serum





Linearity Range (Genotypes 2 through 6): The dilution series used in the verification of linearity for genotypes 2-6 consisted of nine panel members for each genotype spanning the intended linear range. High titer panel members were prepared from a high titer arRNA stock whereas the lower titer panel members were made from a high titer clinical sample (CS). The linearity panel for each genotype was designed to have an approximately 2 $\log_{10}$ titer overlap between the two material sources. The linear range that was tested spanned from the LLoQ (15 IU/mL) to the ULoQ (1.00E+08 IU/mL). Testing was conducted with three lots of **cobas**® HCV reagent; 15 replicates per level were tested in EDTA plasma and serum on the **cobas**® 6800 and **cobas**® 8800 Systems.

The linearity within the claimed linear range of **cobas**® HCV was verified for all five genotypes (2, 3, 4, 5, and 6). The results are summarized in Table 7

Table 7: cobas® HCV Linearity Using HCV Genotypes 2 Through 6

	EDTA plasma		Serum	
GT	**Linear Equation HCV Genotype Linearity Study**	**Maximum Difference Between 1st Order Model and Higher Order Model ($\log_{10}$ IU/mL)**	**Linear Equation HCV Genotype Linearity Study**	**Maximum Difference Between 1st Order Model and Higher Order Model ($\log_{10}$ IU/mL)**
2	y= 0.9601x + 0.1827	0.15	y= 0.9758x + 0.2241	0.20
3	y= 0.9807x + 0.0920	0.09	y= 0.9432x + 0.2217	0.11
4	y= 0.9814x + 0.1570	0.18	y= 0.9834x + 0.0068	0.08
5	y= 0.9788x + 0.1595	0.12	y= 0.9410x + 0.2800	0.19
6	y= 0.9809x + 0.1990	0.14	y= 0.9498x + 0.3068	0.17

Precision – Within Laboratory: The precision of **cobas**® HCV was determined by analysis of serial dilutions of clinical HCV (Genotype 1) samples or of arRNA in HCV-negative EDTA plasma or in serum. Thirteen dilution levels were tested in plasma and 12 levels were tested in serum in two replicates for each level in two runs across 12 days for a total of 48 replicates per concentration. Each sample was carried through the entire **cobas**® HCV test procedure on fully automated **cobas**® 6800/8800 Systems. The study was performed with three lots of **cobas**® HCV test reagents. The results are shown in Tables 8 and 9.

The **cobas**® HCV showed the following precision for three lots of reagents tested across a concentration range of 1.00E+01 IU/mL to 1.0E+07 IU/mL for plasma and serum (Table 8 and Table 9):

Table 8: Within Laboratory Precision of cobas® HCV (EDTA Plasma Samples*)

			cobas® 6800				cobas® 8800			
Nominal Concentration (IU/mL)	Assigned Concentration (IU/mL)	Source Material	Lot 1	Lot 2	Lot 3	All Lots Pooled	Lot 1	Lot 2	Lot 3	All Lots Pooled
			SD	SD	SD	SD	SD	SD	SD	SD
1.00E+07	1.67E+07	arRNA	0.04	0.05	0.03	0.04	0.04	0.09	0.04	0.06
1.00E+06	1.67E+06	arRNA	0.05	0.05	0.06	0.05	0.04	0.05	0.04	0.04
4.00E+05	6.69E+05	arRNA	0.03	0.04	0.05	0.04	0.05	0.06	0.04	0.06
5.00E+04	6.69E+04	CS	0.08	0.06	0.06	0.06	0.04	0.08	0.06	0.06
1.00E+04	1.67E+04	arRNA	0.05	0.05	0.04	0.05	0.05	0.05	0.05	0.05
1.00E+04	1.34E+04	CS	0.03	0.06	0.05	0.05	0.06	0.07	0.06	0.06
4.00E+03	6.69E+03	arRNA	0.05	0.06	0.06	0.06	0.06	0.05	0.06	0.05
1.00E+03	1.34E+03	CS	0.05	0.06	0.05	0.05	0.05	0.06	0.04	0.05
1.00E+03	1.67E+03	arRNA	0.05	0.07	0.05	0.06	0.06	0.08	0.06	0.06
1.00E+02	1.34E+03	CS	0.06	0.09	0.05	0.07	0.06	0.08	0.08	0.07
1.00E+02	1.67E+02	arRNA	0.10	0.06	0.06	0.08	0.07	0.08	0.07	0.07
5.00E+01	6.69E+01	CS	0.09	0.17	0.10	0.13	0.17	0.15	0.08	0.14
1.00E+01	1.34E+01	CS	0.26	0.21	0.13	0.21	0.21	0.26	0.17	0.22

*Titer data are considered to be log-normally distributed and are analyzed following $\log_{10}$ transformation. Standard deviations (SD) columns present the total of the log-transformed titer for each of the three reagent lots.

Table 9: Within-Laboratory Precision of cobas® HCV (Serum Samples*)

			cobas® 6800				cobas® 8800			
Nominal Concentration (IU/mL)	Assigned Concentration (IU/mL)	Source Material	Lot 1	Lot 2	Lot 3	All Lots Pooled	Lot 1	Lot 2	Lot 3	All Lots Pooled
			SD	SD	SD	SD	SD	SD	SD	SD
1.00E+07	1.92E+07	arRNA	0.03	0.07	0.04	0.05	0.05	0.08	0.04	0.06
1.00E+06	1.92E+06	arRNA	0.05	0.06	0.04	0.05	0.06	0.06	0.04	0.05
4.00E+05	7.69E+05	arRNA	0.03	0.07	0.03	0.05	0.05	0.06	0.03	0.05
5.00E+04	4.05E+04	CS	0.07	0.06	0.04	0.06	0.05	0.06	0.06	0.06
1.00E+04	1.92E+04	arRNA	0.06	0.06	0.04	0.05	0.05	0.06	0.06	0.06
1.00E+04	8.11E+03	CS	0.05	0.06	0.04	0.05	0.04	0.06	0.04	0.05
4.00E+03	7.69E+03	arRNA	0.04	0.08	0.04	0.06	0.06	0.05	0.04	0.05
1.00E+03	8.11E+02	CS	0.05	0.06	0.06	0.05	0.05	0.09	0.07	0.07
1.00E+03	1.92E+03	arRNA	0.06	0.05	0.05	0.05	0.05	0.07	0.04	0.05
1.00E+02	8.11E+01	CS	0.10	0.18	0.10	0.13	0.07	0.11	0.09	0.09
1.00E+02	1.92E+02	arRNA	0.07	0.08	0.09	0.08	0.08	0.10	0.07	0.09
5.00E+01	4.05E+01	CS	0.09	0.14	0.18	0.14	0.09	0.12	0.09	0.10

*Titer data are considered to be log-normally distributed and are analyzed following $\log_{10}$ transformation. Standard deviations (SD) columns present the total of the log-transformed titer for each of the three reagent lots.

Performance with HCV Negative Specimens: The performance of the **cobas**® HCV was determined by analyzing HCV negative EDTA plasma and serum samples from individual donors. Three hundred individual EDTA plasma and 300 individual serum samples (600 total results) were tested with two lots of **cobas**® HCV reagents. All samples tested negative for

HCV RNA. In the test panel, the overall result of all specimens tested with the **cobas**® HCV was 100% "Target Not Detected" (two-sided 95% confidence limit: 99.4%-100%)

Analytical Specificity – Cross Reactivity: The analytical specificity of **cobas**® HCV was evaluated by diluting a panel of microorganisms with HCV RNA positive and HCV RNA negative EDTA plasma (Table 10). The microorganisms were added at $1x10^6$ pfu/mL or cfu/mL to normal, HCV virus-negative human EDTA plasma and tested with and without 50 IU/mL HCV RNA added. Negative results were obtained with **cobas**® HCV for all microorganism samples without HCV target and positive results were obtained on all of the microorganism samples with HCV target. Furthermore, the mean $\log_{10}$ titer of each of the positive HCV samples containing potentially cross-reacting organisms was within ± 0.3 $\log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

Table 10: Microorganisms Tested for Cross-Reactivity

Viruses		**Bacteria**	**Yeast**
Adenovirus type 5	West Nile Virus	Propionibacterium acnes	Candida albicans
Cytomegalovirus	St. Louis encephalitis Virus	Staphylococcus aureus	
Epstein-Barr Virus	Murray Valley encephalitis Virus		
Hepatitis A Virus	Dengue Virus types 1, 2, 3, and 4		
Hepatitis B Virus	FSME Virus (strain HYPR)		
Hepatitis D Virus	Yellow Fever Virus		
Human Immunodeficiency Virus-1	Human Herpes Virus Type-6		
Human T-Cell Lymphotropic Virus types 1 and 2	Herpes Simplex Virus Type-1 and 2		
Human Papillomavirus	Influenza A Virus		
Varizella-Zoster Virus	Zika Virus		

Analytical Specificity – Interfering Substances: Elevated levels of triglycerides (34.5g/L), conjugated bilirubin (0.25 g/L), unconjugated bilirubin (0.25 g/L), albumin (58.7 g/L), hemoglobin (2.9 g/L) and human DNA (2 mg/L) in EDTA plasma samples were tested in the presence and absence of 50 IU/mL HCV RNA. The tested endogenous interferences were shown not to interfere with the test performance of **cobas**® HCV.

Moreover, the presence of autoimmune diseases such as antinuclear antibody (ANA), systemic lupus erythematosus (SLE) and rheumatoid arthritis (RF) were tested. An initial set of specimens from patients diagnosed with autoimmune diseases (22 ANA, 6 SLE, 7 RF) showed interference (false negative results) in at least one of the 3 replicates tested from two SLE donors, one RF donor and four ANA donors with **cobas**® HCV when tested at 50 IU/mL of HCV RNA. Although a root-cause investigation into the observed interference did not

reveal the source of the interference, a second set of samples was tested (16 ANA, 15 SLE, 15 RF), and no interference in the presence of autoimmune disease states was observed. Negative results were obtained with **cobas**® HCV for all samples without HCV target and positive results were obtained on all of the samples with HCV target. Furthermore, the mean $\log_{10}$ titer of each of the positive HCV samples containing potentially interfering substances was within ± 0.3 $\log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

In addition, the drug compounds listed in Table 11 were tested at three times the Cmax. All drug compounds tested were shown not to interfere with the specificity and quantitation of HCV RNA by **cobas**® HCV.

Table 11: Drug Compounds Tested for Interference with the Quantitation of HCV RNA by cobas® HCV

Class of Drug	Generic Drug Name	
Immune Modulator	Peginterferon alfa-2a Peginterferon alfa-2b	Ribavirin
HIV entry inhibitor	Maraviroc	
HIV Integrase Inhibitor	Elvitegravir/Cobicistat	Raltegravir
Non-nucleoside HIV Reverse Transcriptase Inhibitor	Efavirenz Etravirine	Nevirapine Rilpivirine
HIV protease inhibitor	Atazanavir Tipranavir Darunavir Fosamprenavir	Lopinavir Nelfinavir Ritonavir Saquinavir
HCV Protease Inhibitor	Boceprevir Simeprevir	Telaprevir
Reverse transcriptase or DNA polymerase inhibitors	Abacavir Emtricitabine Entecavir Foscarnet Cidofovir Lamivudine Telbivudine	Tenofovir Adefovir dipivoxil Zidovudine Aciclovir Valganciclovir Ganciclovir Sofosbuvir
Compounds for Treatment of Opportunistic Infections	Azithromycin Clarithromycin Ethambutol Fluconazole Isoniazid	Pyrazinamide Rifabutin Rifampicin Sulfamethoxazole (SMX) Trimethoprim (TMP)

Matrix Equivalency – EDTA Plasma versus Serum: One hundred and ninety paired EDTA plasma and serum samples were analyzed for matrix equivalency. Of these samples, 73 paired samples were HCV positive samples. The HCV positive samples covered genotypes 1 to 5 in the measuring range.

The mean titer deviation measured for the matched EDTA plasma and serum samples was -0.13 $\log_{10}$ (95% Confidence Interval: -0.19; -0.07). The results show a correlation of $y = 0.99x-0.08$ with $R^2 = 0.96$.

Cross Contamination: The cross contamination rate for **cobas**® HCV was determined by testing 240 replicates of a normal, virus-negative (HIV, HCV and HBV) human EDTA-plasma sample and 225 replicates of a high titer HCV sample at 4.0E+07 IU/mL. In total, five runs were performed with positive and negative samples in a checkerboard configuration.

Two hundred and thirty-nine of 240 replicates of the negative samples were valid and detected negative, resulting in a cross contamination rate of 0.42%. The two-sided 95% exact confidence interval was 0.01% - 2.3%.

Reproducibility: Lot-to-Lot Variability (1 Site): Lot-to-lot variability testing was performed for genotypes 1 through 6 at one test site, using three reagent lots. Two operators at the site tested each lot for 6 days. Two runs were performed each day.

Table 12 below shows attributable percentages of total variance, total precision SDs, and lognormal CVs by genotype and expected $\log_{10}$ HCV RNA concentration for the **cobas**® 6800 System.

Table 12: Attributable Percentage of Total Variance, Total Precision Standard Deviation and lognormal CV(%) of HCV RNA Concentration ($\log_{10}$ IU/mL) by Genotype and Positive Panel Member on the cobas® 6800 System (Lot to Lot)

	HCV RNA Concentration ($\log_{10}$ IU/mL)			Percent Contribution to Total Variance (Lognormal CV(%))					Total Precision	
Geno-type	Expected	Mean[a]	No. of Tests[b]	Lot	Oper-ator	Day	Run	Within-Run	SD[c]	Log-normal CV(%)[d]
1	1.477	1.482	68	0% (0.00)	0% (0.00)	0% (0.00)	25% (22.14)	75% (39.26)	0.1899	45.91
	2.000	1.890	72	8% (10.98)	1% (3.68)	0% (0.00)	10% (12.12)	81% (35.75)	0.1672	39.97
	3.699	3.457	72	0% (0.00)	0% (0.00)	0% (0.00)	82% (32.85)	18% (14.84)	0.1531	36.38
	4.699	4.443	72	3% (7.26)	0% (0.00)	0% (0.00)	86% (37.29)	11% (12.88)	0.1693	40.51
	5.699	5.552	72	0% (0.00)	0% (0.00)	0% (0.00)	83% (33.86)	17% (14.96)	0.1570	37.36

	HCV RNA Concentration (log_{10} IU/mL)			Percent Contribution to Total Variance (Lognormal CV(%))					Total Precision	
Geno-type	Expected	Mean[a]	No. of Tests[b]	Lot	Oper-ator	Day	Run	Within-Run	SD[c]	Log-normal CV(%)[d]
	6.699	6.453	71	47% (17.58)	0% (0.00)	0% (0.00)	25% (12.71)	28% (13.35)	0.1100	25.74
	7.699	7.103	72	54% (28.85)	0% (0.00)	0% (0.00)	24% (19.14)	22% (18.00)	0.1670	39.92
2	1.477	1.611	72	5% (9.52)	0% (0.00)	8% (11.25)	0% (0.00)	87% (39.60)	0.1776	42.67
	2.000	2.125	72	0% (0.00)	0% (0.00)	0% (0.00)	25% (12.12)	75% (21.10)	0.1047	24.47
	3.699	3.714	72	9% (5.63)	0% (0.00)	0% (0.00)	47% (12.66)	44% (12.17)	0.0798	18.53
	4.699	4.743	72	0% (0.00)	0% (0.00)	0% (0.00)	54% (16.10)	46% (14.97)	0.0949	22.12
	5.699	5.806	72	7% (4.24)	0% (0.00)	0% (0.00)	22% (7.39)	71% (13.32)	0.0684	15.85
	6.699	6.187	72	41% (20.03)	0% (0.00)	0% (0.00)	17% (12.73)	42% (20.44)	0.1348	31.80
	7.699	7.080	72	40% (17.99)	1% (2.73)	0% (0.00)	0% (0.00)	59% (21.87)	0.1223	28.73
3	1.477	1.474	72	0% (0.00)	3% (8.35)	0% (0.00)	43% (32.35)	54% (36.31)	0.2084	50.89
	2.000	1.946	72	13% (13.11)	0% (0.00)	0% (0.00)	49% (25.49)	38% (22.49)	0.1562	37.16
	3.699	3.636	72	14% (6.76)	0% (0.00)	0% (0.00)	27% (9.30)	59% (13.76)	0.0776	18.01
	4.699	4.597	72	0% (1.38)	0% (0.00)	0% (0.00)	52% (14.95)	47% (14.24)	0.0894	20.80
	5.699	5.504	72	0% (0.00)	1% (1.62)	0% (0.00)	43% (13.51)	57% (15.54)	0.0893	20.77
	6.699	6.451	72	28% (14.47)	0% (0.00)	3% (5.08)	0% (0.00)	69% (23.03)	0.1189	27.91
	7.699	7.149	71	21% (18.47)	0% (0.00)	8% (11.62)	0% (0.00)	71% (34.88)	0.1747	41.90

	HCV RNA Concentration (log_{10} IU/mL)			Percent Contribution to Total Variance (Lognormal CV(%))					Total Precision	
Geno-type	Expected	Mean[a]	No. of Tests[b]	Lot	Oper-ator	Day	Run	Within-Run	SD[c]	Log-normal CV(%)[d]
4	1.477	1.358	69	7% (14.37)	0% (0.00)	1% (5.44)	0% (0.00)	91% (53.25)	0.2269	56.03
	2.000	1.827	72	10% (9.40)	0% (0.00)	1% (2.80)	8% (8.35)	81% (27.09)	0.1283	30.21
	3.699	3.416	72	20% (7.82)	0% (0.00)	0% (0.00)	42% (11.23)	38% (10.61)	0.0750	17.40
	4.699	4.405	72	22% (8.06)	0% (0.00)	0% (0.00)	13% (6.30)	65% (14.06)	0.0752	17.46
	5.699	5.069	71	5% (8.88)	0% (0.00)	24% (19.47)	13% (14.23)	57% (30.31)	0.1699	40.66
	6.699	6.070	72	27% (23.68)	0% (0.00)	12% (15.28)	34% (26.55)	27% (23.52)	0.1940	47.00
	7.699	6.930	72	37% (30.60)	0% (0.00)	22% (23.53)	11% (16.70)	30% (27.73)	0.2149	52.68
5	1.477	1.575	72	5% (8.30)	0% (0.00)	0% (0.00)	10% (11.53)	85% (35.32)	0.1611	38.42
	2.000	2.049	72	9% (7.51)	0% (0.00)	0% (0.00)	0% (0.00)	91% (24.38)	0.1093	25.57
	3.699	3.606	72	4% (3.63)	0% (0.00)	0% (0.00)	59% (14.11)	38% (11.28)	0.0797	18.51
	4.699	4.616	72	20% (8.86)	0% (0.00)	0% (0.00)	37% (12.19)	43% (13.21)	0.0867	20.17
	5.699	5.678	72	7% (4.63)	0% (0.00)	0% (0.00)	33% (10.36)	60% (13.93)	0.0777	18.04
	6.699	6.505	71	54% (19.49)	0% (0.00)	19% (11.53)	0% (0.00)	27% (13.77)	0.1143	26.79
	7.699	7.592	72	35% (11.59)	1% (2.25)	12% (6.72)	4% (3.94)	47% (13.37)	0.0842	19.58
6	1.477	1.494	70	0% (0.00)	0% (0.00)	0% (0.00)	3% (7.34)	97% (47.65)	0.1990	48.33
	2.000	1.940	72	9% (9.29)	0% (0.00)	0% (0.00)	2% (4.14)	90% (30.32)	0.1361	32.13
	3.699	3.417	72	0% (0.00)	0% (0.00)	0% (0.00)	81% (37.28)	19% (17.38)	0.1737	41.64
	4.699	4.541	72	0% (0.00)	0% (0.00)	0% (0.00)	70% (26.40)	30% (17.27)	0.1351	31.88
	5.699	5.611	72	0% (0.00)	0% (0.00)	0% (0.00)	74% (22.82)	26% (13.36)	0.1136	26.62
	6.699	6.414	72	49% (22.99)	0% (0.00)	9% (10.03)	16% (12.88)	26% (16.83)	0.1413	33.42

	HCV RNA Concentration (log_{10} IU/mL)			Percent Contribution to Total Variance (Lognormal CV(%))					Total Precision	
Geno-type	Expected	Mean[a]	No. of Tests[b]	Lot	Oper-ator	Day	Run	Within-Run	SD[c]	Log-normal CV(%)[d]
	7.699	7.529	71	48% (19.63)	1% (2.67)	2% (4.25)	22% (13.15)	28% (14.96)	0.1225	28.78

Note: The table only includes results with detectable viral load.
[a] Calculated using the SAS MIXED procedure.
[b] Number of valid tests with detectable viral load.
[c] Calculated using the total variability from the SAS MIXED procedure.
[d] Lognormal CV(%) = sqrt(10^[SD^2 * ln(10)] - 1) * 100
CV(%) = percent coefficient of variation; HCV = hepatitis C virus; No. = number; RNA = Ribonucleic acid; SD = standard deviation; sqrt = square root.

Reproducibility (3 Sites): Reproducibility testing was performed at three sites for genotypes 1 through 3, using one reagent lot. Two operators at each site tested for 6 days. Two runs were performed each day.

The following table shows attributable percentages of total variance, total precision SDs, and lognormal CV by genotype and expected log_{10} HCV RNA concentration on the **cobas**® 6800 System:

Table 13: Attributable Percentage of Total Variance, Total Precision Standard Deviation and Lognormal CV(%) of HCV RNA Concentration (log_{10} IU/mL) by Genotype and Positive Panel Member on the cobas® 6800 System (Reproducibility)

	HCV RNA Concentration (log_{10} IU/mL)			Percent Contribution to Total Variance (Lognormal CV(%))					Total Precision	
Geno-type	Expected	Mean[a]	No. of Tests[b]	Site	Oper-ator	Day	Run	Within-Run	SD[c]	Log-normal CV(%)[d]
1	1.477	1.373	68	1% (6.43)	0% (0.00)	0% (0.00)	20% (25.63)	78% (52.96)	0.2437	60.84
	2.000	1.866	72	4% (7.25)	0% (0.00)	0% (0.00)	17% (15.81)	79% (34.64)	0.1644	39.24
	3.699	3.466	72	0% (0.00)	0% (0.00)	0% (0.00)	83% (29.77)	17% (13.35)	0.1391	32.87
	4.699	4.444	72	7% (10.74)	0% (0.00)	0% (0.00)	83% (37.40)	9% (12.16)	0.1721	41.24
	5.699	5.579	72	4% (6.84)	0% (0.00)	0% (0.00)	74% (30.53)	22% (16.27)	0.1504	35.70
	6.699	6.439	72	52% (16.35)	9% (6.91)	0% (0.00)	9% (6.74)	30% (12.36)	0.0979	22.84
	7.699	7.091	72	76% (45.80)	0% (0.00)	0% (0.00)	7% (12.87)	17% (20.92)	0.2170	53.25

	HCV RNA Concentration (log_{10} IU/mL)			Percent Contribution to Total Variance (Lognormal CV(%))					Total Precision	
Geno-type	Expected	Mean[a]	No. of Tests[b]	Site	Oper-ator	Day	Run	Within-Run	SD[c]	Log-normal CV(%)[d]
2	1.477	1.631	72	10% (11.41)	0% (0.00)	0% (0.00)	0% (0.00)	90% (35.77)	0.1586	37.77
	2.000	2.096	72	2% (3.71)	0% (0.00)	0% (0.00)	35% (14.49)	63% (19.44)	0.1057	24.70
	3.699	3.699	72	4% (3.47)	0% (0.00)	0% (0.00)	49% (11.99)	47% (11.76)	0.0742	17.22
	4.699	4.745	72	0% (0.00)	0% (0.00)	0% (0.00)	59% (17.39)	41% (14.45)	0.0975	22.75
	5.699	5.824	72	19% (7.91)	0% (0.00)	0% (0.00)	24% (8.99)	57% (13.89)	0.0794	18.43
	6.699	6.177	72	51% (20.74)	0% (1.59)	0% (0.00)	9% (8.47)	40% (18.27)	0.1246	29.30
	7.699	7.069	72	17% (13.08)	0% (0.00)	0% (0.00)	0% (0.00)	83% (29.26)	0.1367	32.28
3	1.477	1.457	72	0% (0.00)	0% (0.00)	0% (0.00)	34% (24.33)	66% (34.06)	0.1776	42.67
	2.000	1.911	72	16% (13.76)	0% (0.00)	0% (0.00)	27% (18.01)	58% (26.79)	0.1504	35.70
	3.699	3.628	72	10% (6.12)	0% (0.00)	0% (0.00)	18% (8.09)	71% (16.06)	0.0821	19.07
	4.699	4.587	72	2% (2.23)	0% (0.00)	0% (0.00)	55% (13.21)	44% (11.85)	0.0774	17.96
	5.699	5.524	72	0% (0.00)	0% (0.00)	0% (0.00)	44% (12.53)	56% (14.30)	0.0822	19.10
	6.699	6.442	71	22% (11.89)	0% (0.00)	0% (0.00)	0% (0.00)	78% (22.66)	0.1100	25.73
	7.699	7.109	71	10% (13.36)	0% (0.00)	21% (19.65)	0% (0.00)	69% (35.94)	0.1827	44.01

Note: The table only includes results with detectable viral load.
[a] Calculated using the SAS MIXED procedure.
[b] Number of valid tests with detectable viral load.
[c] Calculated using the total variability from the SAS MIXED procedure.
[d] Lognormal CV(%) = sqrt(10^[SD^2 * ln(10)] - 1) * 100
CV(%) = percent coefficient of variation; HCV = hepatitis C virus; No. = number; ; RNA = Ribonucleic acid; SD = standard deviation; sqrt = square root.

Reproducibility (Comparison between cobas® 6800 and cobas® 8800 Systems): An identical sample set was tested for lot-to-lot variability and reproducibility of **cobas®** HCV on the **cobas®** 8800 system. The performance of the two systems is comparable. The following table lists the precision performance achieved in the reproducibility portion of the study for both the **cobas®** 6800 and **cobas®** 8800 System across the linear range of **cobas®** HCV:

Table 14: Comparison of Precision Standard Deviation of HCV RNA Concentration (log_{10}IU/mL) for Genotypes 1 - 3 on cobas® 6800 and 8800 Systems (Reproducibility)

	Precision Standard Deviation[a] (No. of Tests[b])					
	cobas® 6800			cobas® 8800		
Concentration Level (IU/mL)	Genotype 1	Genotype 2	Genotype 3	Genotype 1	Genotype 2	Genotype 3
1.0 E+01 ≤ X < 1.0 E+02	0.24 (68) 0.16 (72)	0.16 (72)	0.18 (72) 0.15 (72)	0.23 (47) 0.15 (47)	0.14 (48)	0.17 (47) 0.17 (48)
1.0 E+02 ≤ X < 1.0 E+03	-	0.11 (72)	-	-	0.12 (48)	-
1.0 E+03 ≤ X < 1.0 E+04	0.14 (72)	0.07 (72)	0.08 (72)	0.13 (48)	0.07 (48)	0.08 (48)
1.0 E+04 ≤ X < 1.0 E+05	0.17 (72)	0.10 (72)	0.08 (72)	0.11 (48)	0.06 (48)	0.08 (48)
1.0 E+05 ≤ X < 1.0 E+06	0.15 (72)	0.08 (72)	0.08 (72)	0.11 (48)	0.07 (47)	0.10 (48)
1.0 E+06 ≤ X < 1.0 E+07	0.10 (72)	0.12 (72)	0.11 (71)	0.09 (48)	0.13 (48)	0.11 (48)
1.0 E+07 ≤ X < 1.0 E+08	0.22 (72)	0.14 (72)	0.18 (71)	0.16 (48)	0.10 (48)	0.19 (48)

Note: Grouping of observed precisions to concentration levels are based on the median test results on the untransformed scale (IU/mL). The table only includes results with detectable viral load. SD = standard deviation.
'-' Indicates no applicable results for this level.
[a] Precision Standard Deviation in log_{10} units
[b] Number of valid tests with detectable viral load.

Specimen Stability: Specimen stability studies demonstrated that for the **cobas®** HCV specimens should be stored as follows:

Whole blood collected in EDTA plasma or serum preparation tubes stored for up to 24 hours at 2°C to 25°C before further processing and matrix separation

- Resultant plasma and serum samples are stable for:
  - Up to 6 days at 2°C to 8°C or up to 6 months at -15°C to -80°C
  - 6 days at 2°C to 8°C followed by 6 months at -75°C ± 15°C after matrix separation
- Plasma and serum samples are stable for up to 4 freeze/thaw cycles when frozen at -18°C or -80°C

Real-Time Reagent (including Controls) Stability: Expiration dating for the **cobas®** HCV reagents has been established and approved at 16 months when stored at 2-8°C.

Antimicrobial Effectiveness (AET): The AET was performed on one lot of new component used in the **cobas®** HCV test and the **cobas®** HCV Control Kit in combination with the new universal reagents

(**cobas**® omni Specimen Diluent, **cobas**® omni Reagent, and **cobas**® omni Wash Reagent). Results of the study are compared to the requirements of USP 51. All reagents met the USP requirements for antimicrobial effectiveness testing.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

Clinical studies included evaluation of

- the ability of the assay to predict clinical outcome in patients undergoing treatment (Section A, below) and
- the ability of the assay to correctly diagnose anti-HCV positive subjects with active HCV infection (Section B below).

A. Clinical Performance (prediction of clinical outcome)

Study design and demographics

The purpose of this study was to evaluate the clinical performance of the **cobas**® HCV on the **cobas**® 6800 system with specimens from patients undergoing treatment. The study was designed to evaluate the ability of the assay to predict clinical outcome.

Treatment Plan 1 included four treatment regimens, containing a combination of direct acting antiviral compounds with or without pegylated interferon and ribavirin (pegIFN/RBV). Subjects were infected with HCV genotype 1 and were partial or null responders during a previous course of pegIFN/RBV combination therapy.

Treatment Plan 2 included subjects infected with genotype 2 or 3, who were treatment naïve and received a course of pegIFN/RBV combination therapy.

Testing with the **cobas**® HCV was performed at four sites. Each site was equipped with one **cobas**® 6800 System. One site had both the **cobas**® 6800 and 8800 Systems. Three kit lots of reagents were used in the study; each sample was tested with one kit lot.

Table 15 below shows the demographic and baseline characteristics of subjects whose samples were tested on the **cobas**® 6800 System.

The demographic distribution of the subjects in this study was consistent with that of chronic HCV patients in the US, the majority being male, over 40 years of age, and infected with HCV genotype 1. Subjects with HCV genotypes 1, 2, and 3 were enrolled. HCV infection with genotypes 4, 5, and 6 is rare in the US.

Table 15: Demographics and Baseline Characteristics for cobas® 6800 and 8800 Systems

	cobas® 6800		**cobas® 8800**	
Characteristics	**Statistics**	**Subjects**	**Statistics**	**Subjects**
Total	N	401	N	353
Treatment Plan				
1	n (%)	307 (76.6%)	n (%)	287 (81.3%)
2	n (%)	94 (23.4%)	n (%)	66 (18.7%)
Age Category (years)				
< 40	n (%)	90 (22.4%)	n (%)	81 (22.9%)
≥ 40	n (%)	311 (77.6%)	n (%)	272 (77.1%)
Age (years)				
	Mean ± SD	49 ± 11.1	Mean ± SD	49 ± 11.2
	Median	52	Median	52
	Range	20 - 76	Range	20 – 71
Gender				
MALE	n (%)	276 (68.8%)	n (%)	245 (69.4%)
FEMALE	n (%)	125 (31.2%)	n (%)	108 (30.6%)
Race / Ethnicity				
Asian	n (%)	3 (0.7%)	n (%)	2 (0.6%)
African American	n (%)	13 (3.2%)	n (%)	12 (3.4%)
White/Caucasian	n (%)	357 (89.0%)	n (%)	318 (90.1%)
Other	n (%)	28 (7.0%)	n (%)	21 (5.9%)
Genotype				
1A	n (%)	174 (43.4%)	n (%)	159 (45.0%)
1B	n (%)	133 (33.2%)	n (%)	128 (36.3%)
Overall 1	**n (%)**	307 (76.6%)	n (%)	287 (81.3%)
2	n (%)	31 (7.7%)	n (%)	22 (6.2%)
3	n (%)	63 (15.7%)	n (%)	44 (12.5%)
Overall Non-1	**n (%)**	94 (23.4%)	n (%)	66 (18.7%)
Baseline HCV RNA (log_{10} IU/mL)				
	Mean ± SD	6.32 ± 0.58	Mean ± SD	6.33 ± 0.56
	Median	6.41	Median	6.41
	Range	2.57 - 7.52	Range	2.77 - 7.52
HCV RNA Category at Baseline				
< 400,000 IU/mL	n (%)	36 (9.0%)	n (%)	32 (9.1%)
≥ 400,000 IU/mL	n (%)	363 (90.5%)	n (%)	304 (86.1%)
Missing	n (%)	2 (0.5%)	n (%)	17 (4.8%)

HCV = hepatitis C virus; RNA = Ribonucleic acid; SD = standard deviation

Prediction of Response to Antiviral Therapy

Definitions**:**

- Week 2 viral load (VL) = HCV RNA < LLoQ = LoD = 15 IU/mL at Week 2 of antiviral therapy
- Week 4 VL: HCV RNA < LLoQ at Week 4 of antiviral therapy
- Week 8 VL: HCV RNA < LLoQ at Week 8 of antiviral therapy
- Week 12 VL: Either at least a 2 $\log_{10}$ drop in HCV RNA level compared to baseline or HCV RNA < LLoQ at Week 12 of antiviral therapy
- Week 24 VL [End of Treatment (EOT)]: HCV RNA < LLoQ at Week 24 of antiviral therapy
- Sustained Virologic Response (SVR)12: HCV RNA < LLoQ at Week 12 after completion of antiviral therapy measured with an independent HCV RNA test.

Predictive Value of VR to Success of Antiviral Therapy:
In this study, the Positive Predictive Value (PPV) for Week 4 to predict SVR12 was 78.1% (95% CI: 72.7 to 82.8%) in genotype 1 subjects and 84.7% (95% CI: 73.5 to 91.8%) in subjects with non-1 genotypes (Table 17). Therefore, VL at Week 4 measured by **cobas**® HCV was a useful predictor of SVR12.

For Treatment Plan 1, as a representative of a highly efficacious DAA containing regimen, VL measured on **cobas**® HCV at both Week 12 and 24 strongly predict SVR12 in genotype 1 subjects, with PPVs of 77.0% and 78.6%, respectively. The absence of VL at Week 12 or 24 predicts non-response, with Negative Predictive Values (NPVs) of 87.5% and 100%, respectively (Table 17). Additional analysis of Week 2 VL to predict SVR12 shows a PPV of 79.4% but a low NPV of 29.9%. The most recent AASLD Guidelines from 2014 have not included any earlier virologic decline assessments than Week 4.

In Treatment Plan 2, Week 12 VL using **cobas**® HCV in genotype 2 and 3 was highly predictive of SVR12, with a PPV of 75.3%. Due to the rarity of non-response, absence of VL at Week 12 is not a useful measure of outcome in this population. The NPV was 50% and the number of non-responders was small in this study (Table 16).

Overall, this study demonstrated the clinical performance of **cobas**® HCV and continued value of the assessment of Week 4, Week 12, and Week 24 HCV RNA responses in patients undergoing treatment for chronic HCV infection.

Table 16: Probability of Achieving Sustained Virological Response (SVR12) Given Virologic Response (< 15 IU/mL) at a Specific On-Treatment Visit for the cobas® 6800 System

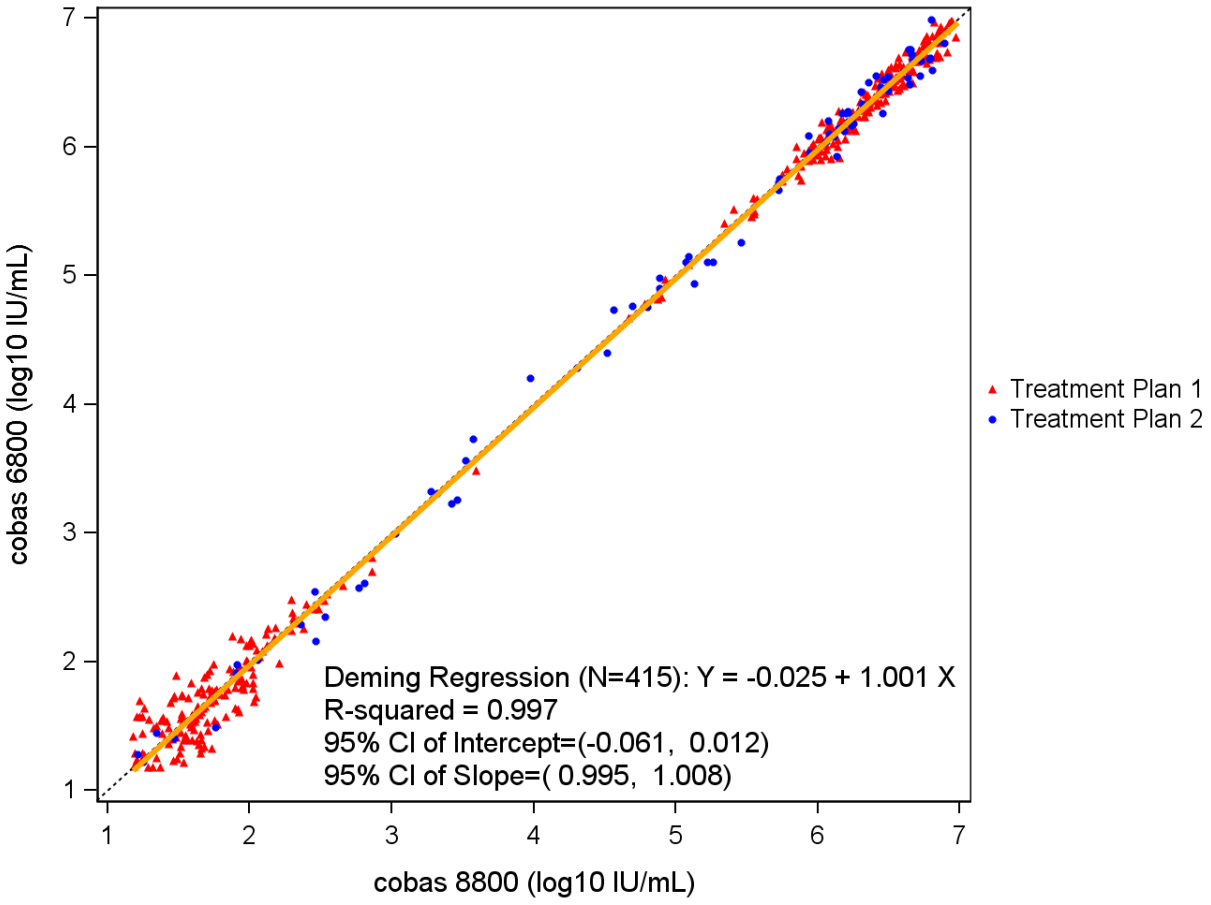
				PPV (%)		NPV (%)		OR
Treat-ment Plan	**Geno-type**	**On-Treatment Visit**	**Eligible Subjects**	**Estimate (95% CI)**	**n / N**	**Estimate (95% CI)**	**n / N**	**Estimate (95% CI)**
1	1	Week 2	290	79.4 (71.5, 85.5)	100 / 126	29.9 (23.4, 37.3)	49/164	1.64 (0.95, 2.83)
		Week 4	290	78.1 (72.7, 82.8)	200 / 256	50.0 (34.1, 65.9)	17 / 34	3.57 (1.71, 7.45)
		Week 8	285	76.8 (71.5, 81.4)	212 / 276	66.7 (35.4, 87.9)	6 / 9	6.63 (1.61, 27.24)
		Week 12	286	77.0 (71.7, 81.5)	214 / 278	87.5 (52.9, 97.8)	7 / 8	23.41 (2.83,193.80)
		Week 24	282	78.6 (73.4, 83.0)	217 / 276	100.0 (61.0, 100.0)	6 / 6	47.52 (2.64,855.66)
2	Non-1	Week 4	82	84.7 (73.5, 91.8)	50 / 59	47.8 (29.2, 67.0)	11 / 23	5.09 (1.72, 15.04)
		Week 12	83	75.3 (64.9, 83.4)	61 / 81	50.0 (9.5, 90.5)	1 / 2	3.05 (0.18, 51.04)

Positive Predictive Value (PPV) = TP / (TP + FP) or the probability of being an SVR12 given the subject was a viral responder at a specific visit. SVR12 is achieved if the subject has HCV RNA < 15 IU/mL at 12 weeks after the last dose.
Negative Predictive Value (NPV) = TN / (FN + TN) or the probability of not being an SVR12 given the subject was not a viral responder at a specific visit.
Odds Ratio (OR) = (TP • TN) / (FP • FN)
CI = confidence interval; FN = false negative; FP = false positive; HCV = hepatitis C virus; SVR12 = sustained virological response 12 weeks after the last dose; TN = true negative; TP = true positive.

Comparison between cobas® 6800 and cobas® 8800 Systems

A subset of samples was tested for clinical performance of **cobas®** HCV on the **cobas®** 8800 system. The systems demonstrate highly correlated performance that was not significantly different. Figure 5 below shows a Deming regression plot of VLs (log_{10} IU/mL) greater than 15 IU/mL at all applicable time points on treatment:

Figure 5: Deming Linear Regression Plot of Viral Loads (log10 IU/mL) from Baseline, Week 2, and Week 4 (cobas® 6800 and 8800 Systems.



CI = confidence interval.

B. Diagnostic Utility

The study was designed to evaluate the ability of the assay to correctly diagnose anti-HCV positive subjects with active HCV infection. The following table shows the demographic and clinical characteristics of subjects whose samples were tested on the **cobas**® 6800 and 8800 Systems:

Table 17: Demographic and Clinical Characteristics by System (HCV Antibody Positive) Subjects

Characteristics	cobas® 6800	cobas® 8800
Total, N	235	230
Clinical Condition		
HCV Antibody Positive, n(%)		
HCV RNA Positive	154 (65.5%)	150 (65.2%)
HCV RNA Negative	81 (34.5%)	80 (34.8%)
Age (years)		
Mean ± SD	48 ± 11.9	49 ± 11.9
Median	50	50
Range	20 - 88	20 - 88
Gender, n(%)		
Male	132 (56.2%)	127 (55.2%)
Female	103 (43.8%)	103 (44.8%)
Race, n(%)		
Black / African-American	49 (20.9%)	48 (20.9%)
White / Caucasian	183 (77.9%)	179 (77.8%)
Other	3 (1.3%)	3 (1.3%)
Risk Factor, n(%)		
Baby Boomers (Born: 1945-1965) only	114 (48.5%)	112 (48.7%)
IVD Users only	22 (9.4%)	22 (9.6%)
Baby Boomers and IVD Users	23 (9.8%)	22 (9.6%)
Undisclosed*, HCV antibody positive	76 (32.3%)	74 (32.2%)

[a] VERSANT HCV Test result was used to determine HCV RNA status. For subjects whose VERSANT HCV Test result was not available, the APTIMA HCV Test result was used. If both Versant and Aptima results were not available then **COBAS**® AMPLICOR HCV Test, v2.0 result was used.

* Undisclosed includes those subjects for whom both the risk factors are either missing or 'No', or those for whom one risk factor is missing and the other has a value of 'No'.

APTIMA = Aptima HCV RNA Qualitative Assay; HCV = hepatitis C Virus; IVD = Intravenous Drug Use.

SD = standard deviation; VERSANT= VERSANT HCV RNA Qualitative Assay.

The sensitivity of the **cobas**® HCV was evaluated in subjects, who had previous exposure to HCV and tested positive for HCV antibodies, on both the **cobas**® 6800 and 8800 Systems (Table 18. According to current AASLD Guidelines, an FDA-approved quantitative or qualitative NAT with a detection level of 25 IU/mL or lower should be used to detect HCV RNA. The agreement of the **cobas**® HCV with the patient infection status (PIS) was determined using a cutoff of < 25 IU/mL to define the absence of active HCV infection (Table 18).

Table 18: Agreement of the cobas® HCV on the cobas® 6800 and 8800 Systems with the PIS Using a Cutoff of 25 IU/mL

	Patient Infected Status (PIS)					
cobas® HCV	**cobas® 6800 System**			**cobas® 8800 System**		
	HCV Positive	**HCV Negative**	**Total**	**HCV Positive**	**HCV Negative**	**Total**
HCV RNA Detected Above 25 IU/mL	152	0	152	149	1	150
HCV RNA not Detected or detected below 25 IU/mL	0	81	81	0	79	79
Total	152	81	233	149	80	229
Positive Percent Agreement (95% score CI)	100.0 % (97.5, 100.0)	NA	NA	100.0 % (97.5, 100.0)	NA	NA
Negative Percent Agreement (95% score CI)	NA	100.0 % (95.5, 100.0)	NA	NA	98.8 % (93.3, 99.8)	NA

Note: Only valid results from **cobas**® HCV among the HCV Antibody Positive specimens are included in this table.
CI = confidence interval; **cobas**® HCV = **cobas**® HCV for use on the **cobas**® 6800/8800 systems; HCV = hepatitis C virus; NA = not applicable.

This study demonstrates the ability of the **cobas**® HCV to correctly diagnose subjects with ongoing active HCV RNA infection and to distinguish them from subjects with inactive infections in a population with prior exposure to HCV (HCV antibody-positive serology).

Diagnostic Utility: Comparison between cobas® 6800 and cobas® 8800 Systems
A subset of samples was tested for the confirmation of active HCV infection on both the **cobas**® 6800 and 8800 systems. The specificity of the **cobas**® HCV, in a variety of liver diseases for which active HCV infection was not the underlying cause, was also 100%. The agreement of the **cobas**® HCV on the **cobas**® 8800 System with the PIS, using a cutoff of <25 IU/mL to define the absence of active HCV infection was 99.6%. These results indicate that the **cobas**® 6800 and 8800 Systems are comparable for the diagnosis of active HCV infection using the **cobas**® HCV.

Cross-reactivity in Subjects with Non-HCV Related Liver Disease
The cross-reactivity of the **cobas**® HCV was evaluated with specimens that represented a variety of liver diseases for which active HCV infection was not the underlying cause (Tables 19-21).

Table 19: Demographic and Clinical Characteristics by System (Subjects with Non HCV Related Liver Disease)

Characteristics	cobas® 6800	cobas® 8800
Total, N	247	181
Clinical Condition		
HCV RNA Negative, n(%)		
Alcoholic Liver Disease	33 (13.4%)	20 (11.0%)
Autoimmune Hepatitis	37 (15.0%)	32 (17.7%)
Chronic HBV	30 (12.1%)	30 (16.6%)
Fatty Liver Disease	66 (26.7%)	38 (21.0%)
Non-Alcoholic Steatohepatitis (NASH)	41 (16.6%)	30 (16.6%)
Nonspecific Cirrhosis	6 (2.4%)	3 (1.7%)
Primary Billiary Cirrhosis	33 (13.4%)	28 (15.5%)
Unknown [a]	1 (0.4%)	
Age (years)		
Mean ± SD	54 ± 13.1	54 ± 13.5
Median	56	56
Range	20 - 81	20 - 81
Gender, n(%)		
Male	71 (28.7%)	44 (24.3%)
Female	104 (42.1%)	74 (40.9%)
Unknown	72 (29.1%)	63 (34.8%)
Race, n(%)		
Asian	11 (4.5%)	1 (0.6%)
Black / African-American	13 (5.3%)	11 (6.1%)
White / Caucasian	70 (28.3%)	48 (26.5%)
Other	7 (2.8%)	1 (0.6%)
Unknown	146 (59.1%)	120 (66.3%)
Baby Boomers (Born: 1945-1965), n(%)		
Yes	80 (32.4%)	63 (34.8%)
No	64 (25.9%)	53 (29.3%)
Undisclosed	103 (41.7%)	65 (35.9%)

[a] Hepatic Steatosis disease is presented as 'Unknown.'

HBV = hepatitis B virus; HCV = hepatitis C virus; SD = standard deviation.

The specificity of **cobas**® HCV was evaluated with specimens that represented a variety of liver diseases for which active HCV infection was not the underlying cause (Table 20).

Table 20: Number of HCV RNA Negative Samples on the cobas® 6800 System with Non HCV-related Liver Diseases with Test Result Categories by Clinical Condition

		Number of Valid Tests					
Clinical Condition	**Target Not Detected**	**< 1.50E+01 IU/mL**	**1.50E+01 IU/mL ≤ X ≤ 2.5E+01 IU/mL**	**2.50E+01 IU/mL ≤ X ≤ 1.00E+08 IU/mL**	**> 1.00E+08 IU/mL**	**Total**	**Specificity[a] % (95% CI)[b]**
Alcoholic Liver Disease	33	0	0	0	0	33	100.0 (89.4, 100.0)
Autoimmune Hepatitis	37	0	0	0	0	37	100.0 (90.5, 100.0)
Chronic HBV	30	0	0	0	0	30	100.0 (88.4, 100.0)
Fatty Liver Disease	66	0	0	0	0	66	100.0 (94.6, 100.0)
NASH	40	1*	0	0	0	41	97.6 (87.1, 99.9)
Nonspecific Cirrhosis	6	0	0	0	0	6	100.0 (54.1, 100.0)
Primary Billiary Cirrhosis	33	0	0	0	0	33	100.0 (89.4, 100.0)
Total	245	1*	0	0	0	246	99.6 (97.8, 100.0)

Note: Only valid results from **cobas**® HCV among the HCV Antibody negative specimens (non-HCV-related liver disease) are included in this table.
The single subject with Hepatic Steatosis liver disease was excluded.
[a] Clinical Specificity: percentage of number of RNA negative result to the total number of HCV Antibod y negatie specimens among valid test results.
[b] 95% CI: 95% exact confidence interval.
* Sample reported <LLOQ, HCV RNA detected at ~ 1.5 IU/mL.
CI = confidence interval; HBV = hepatitis B virus; HCV = hepatitis C virus; NASH = non-alcoholic steatohepatitis.

Table 21: Number of HCV RNA Negative Samples on the cobas® 8800 System with Non HCV-related Liver Diseases with Test Result Categories by Clinical Condition

		Number of Valid Tests					
Clinical Condition	**Target Not Detected**	**< 1.50E+01 IU/mL**	**1.50E+01 IU/mL ≤ X ≤ 2.5E+01 IU/mL**	**2.50E+01 IU/mL ≤ X ≤ 1.00E+08 IU/mL**	**> 1.00E+08 IU/mL**	**Total**	**Specificity[a] % (95% CI)[b]**
Alcoholic Liver Disease	20	0	0	0	0	20	100.0 (83.2 100.0)
Autoimmune Hepatitis	32	0	0	0	0	32	100.0 (89.1, 100.0)
Chronic HBV	30	0	0	0	0	30	100.0 (88.4, 100.0)
Fatty Liver Disease	38	0	0	0	0	38	100.0 (90.7, 100.0)
NASH	30	0	0	0	0	30	100.0 (88.4, 100.0)
Nonspecific Cirrhosis	3	0	0	0	0	3	100.0 (29.2, 100.0)
Primary Billiary Cirrhosis	28	0	0	0	0	28	100.0 (87.7, 100.0)
Total	181	0	0	0	0	181	100.0 (98.0, 100.0)

Note: Only valid results from **cobas®** HCV among the HCV Antibody negative specimens (non-HCV-related liver disease) are included in this table.
The single subject with Hepatic Steatosis liver disease was excluded.
[a] Clinical Specificity: percentage of number of RNA negative result to the total number of HCV Antibody negative specimens among valid test results.
[b] 95% CI: 95% exact confidence interval.
CI = confidence interval; HBV = hepatitis B virus; HCV = hepatitis C virus; NASH = non-alcoholic steatohepatitis.

The **cobas®** HCV also demonstrated the ability to determine absence of active HCV infection in subjects with a range of liver diseases due to causes other than HCV. When real-time PCR assays such as the **cobas®** HCV are used to aid in the diagnosis of HCV infection, a cut-off of 25 IU/mL should be applied to distinguish between non-active and active HCV infection. The HCV RNA concentration, together with other markers of active liver disease, need to be evaluated if antiviral treatment is being considered.

C. Safety and Effectiveness Results

1. Safety Results
   There were no adverse effects of the device reported while the study was conducted.

2. Effectiveness Results
   The effectiveness of the **cobas**® HCV was assessed for two purposes: 1) ability to detect RNA in anti-HCV positive individuals (diagnostic utility) and 2) ability of the test to measure RNA levels at baseline, during, and after treatment (clinical performance). See results in Section X. A and B, above.

   A subset of patient samples were tested on both the **cobas**® 6800 and 8800 Systems to assess equivalent performance between the two systems for both purposes indicated above.

   Overall, the clinical studies demonstrate the effectiveness of the **cobas**® HCV in accurately detecting the presence of HCV RNA in samples from anti-HCV positive individuals and in accurately measuring HCV RNA levels in patients undergoing treatment.

3. Subgroup Analysis
   Not Applicable.

D. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included 7 investigators. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2.(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XI. PANEL RECOMMENDATIONS

In accordance with the provisions of section 515(c)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the FDA Microbiology Devices Advisory Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

- The effectiveness of the **cobas**® HCV has been demonstrated when used for the quantitation of HCV RNA in human EDTA plasma or serum for diagnosis in patients who are anti-HCV positive and for the management of patients undergoing treatment.
- There are no issues with endogenous interferents at physiological levels or with commonly administered medications.
- Whole blood collected in EDTA plasma or serum preparation tubes may be stored for up to 24 hours at 2°C to 25°C before further processing and matrix separation.
- EDTA plasma and serum samples are stable for up to 6 days at 2°C to 8°C or up to 6 months at -15°C to -80°C
- EDTA plasma and serum samples are stable for up to 4 freeze/thaw cycles when frozen at -18°C or -80°C.
- The preservatives that the **cobas**® HCV reagents and controls contain have been shown to meet USP Chapter 51 criteria.
- The **cobas**® HCV reagents can be stored at 2-8°C for up to 16 months.
- Reproducibility of the **cobas**® HCV was acceptable from run to run, day to day, reagent lot to reagent lot, and site to site. The between run and within run components contributed the majority of the variability for all of the genotypes tested.
- The clinical performance was evaluated in an ethnically diverse population representative of the intended use population: anti-HCV positive individuals (see diagnostic utility studies, above) and patients undergoing HCV therapy (see clinical performance studies, above).
- The following studies were conducted on both the **cobas**® 6800 and 8800 Systems: analytical studies (LoD, Linearity, Precision, and Reproducibility). A subset of the samples tested in the, diagnostic utility and clinical performance were tested on both the **cobas**® 6800 and 8800 systems. The results from both instruments were similar and the instruments were found to be equivalent.

B. Safety Conclusions

Based on the results of the analytical and clinical laboratory studies, the **cobas**® HCV, when used according to the provided directions and in conjunction with other laboratory results and clinical information, should be safe and pose minimal risk to the patient due to false test results.

C. Benefit-Risk Conclusions

The benefits outweigh the risks at the level of performance observed in the pivotal clinical study. Complimentary analytical studies strengthen this conclusion. Accurate detection and quantitation of HCV RNA is an essential component of the diagnosis of active HCV infection and the treatment of HCV. In an era of highly effective treatment for active hepatitis C virus infection, the identification of patients with active infection as candidates for treatment and ascertainment of sustained virological response has substantial individual benefit (i.e., reduction of the risk of progressive liver disease and the incidence of hepatocellular carcinoma) and public health benefit (i.e., interruption of transmission).

Risks include false positive/false negative test results and inaccurate quantification. These are substantially mitigated by device design (i.e., use of controls), and the likelihood of additional testing for false positive or false negative results. Errors of quantification in the current era of highly active antiviral therapy are unlikely to be significant outside of unique circumstances.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. The rate of false positive and false negative results is within acceptable limits compared with previously approved tests. Therefore, this device should benefit the physician in the diagnosis and management of HCV infected patients when used according to the directions for use in the labeling.

XIII. CDRH DECISION

CDRH issued an approval order on October 14, 2015. The final conditions of approval cited in the approval order are described below.

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.
Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.
Post-approval Requirements and Restrictions: See approval order.