COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0

FOR *IN VITRO* DIAGNOSTIC USE.

COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0	**HC2**	72 Tests	P/N: 05480442 190
COBAS® AmpliPrep/COBAS® TaqMan® Wash Reagent	**PG WR**	5.1 Liters	P/N: 03587797 190

INTENDED USE

The COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 is an *in vitro* nucleic acid amplification test for both the detection and quantitation of hepatitis C (HCV) RNA genotypes 1 to 6 in human EDTA plasma or serum of HCV-infected individuals using the COBAS® AmpliPrep Instrument for automated specimen processing and the COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 48 Analyzer for automated amplification and detection.

The test is intended for use as an aid in the diagnosis of HCV infection in the following populations: individuals with antibody evidence of HCV infection 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.

The test is intended for use as an aid in the management of HCV-infected patients undergoing anti-viral therapy in conjunction with clinical and laboratory markers of infection. 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.

Assay performance characteristics have been established for individuals treated with peginterferon alfa-2a plus ribavirin. No information is available on the assay's predictive value when other therapies are used.

The COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 is not intended for use as a screening test for the presence of HCV in blood or blood products.

SUMMARY AND EXPLANATION OF THE TEST

Hepatitis C virus is considered to be the principal etiologic agent responsible for 90% to 95% of the cases of post-transfusion hepatitis[1-4]. HCV is a single-stranded, positive sense RNA virus with a genome of approximately 9,500 nucleotides coding for 3,000 amino acids. As a blood-borne virus, HCV is transmitted by blood and blood products. Widespread adoption of HCV blood screening measures has markedly lowered the risk of transfusion-associated hepatitis. The incidence of HCV infection is highest in association with intravenous drug abuse and to a lesser extent with other percutaneous exposures[4]. The global prevalence of HCV infection is estimated to be 3%[5] and the prevalence in the USA was 1.6% between 1999 and 2003. Following exposure, 75% to 85% of HCV-infected individuals develop chronic hepatitis, with up to 20% of these chronic cases progressing to cirrhosis. In cirrhotic patients, hepatocellular carcinoma is observed in 1% to4% of the population every year[6, 7].

Quantitation of HCV RNA for measuring baseline viral loads and for on-treatment monitoring has been well established in demonstrating the efficacy of antiviral response to pegylated interferon plus ribavirin combination therapy[8–12]. Current guidelines for the management and treatment of HCV recommend quantitative testing for HCV RNA before the start of antiviral therapy, during therapy (response guided therapy), and generally 12 to 24 weeks following the end of treatment. Absence of detectable HCV RNA by a sensitive test, 24 weeks after the end of treatment, is the goal of treatment and indicates that a sustained virologic response (SVR) has been achieved[13]. During antiviral therapy an early virologic response (EVR), defined as a two-log or greater decrease in HCV RNA or undetectable HCV RNA after 12 weeks of therapy, is commonly observed. Failure to achieve EVR has a high negative predictive value for achieving a SVR and has been incorporated in futility (stopping) rules for pegylated interferon plus ribavirin therapies[7-13]. A rapid viral response (RVR), undetectable levels of HCV RNA after 4 weeks of therapy, has a high positive predictive value for SVR[14]. 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 24 weeks, following the end of treatment[15,16].

HCV RNA can be detected in either plasma or serum using nucleic acid extraction and amplification technologies. The COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 uses real-time PCR technology[17] combined with a novel dual probe approach for the accurate quantitation of HCV RNA. Novel primers allow for the detection and quantitation of genotypes 1, 2, 3, 4, 5 and 6. The assay is standardized against the WHO International Standard for Hepatitis C Virus RNA for Nucleic Acid Amplification Technology Assays and titer results are reported in International Units per milliliter (IU/mL).

PRINCIPLES OF THE PROCEDURE

The COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 is a nucleic acid amplification test for the quantitation of hepatitis C virus (HCV) RNA in human serum or EDTA plasma. Specimen preparation is automated using the COBAS® AmpliPrep Instrument with

amplification and detection automated using the COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 48 Analyzer.

The COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 is based on three major processes: (1) specimen preparation to isolate HCV RNA; (2) reverse transcription of the target RNA to generate complementary DNA (cDNA), and (3) simultaneous PCR amplification of target cDNA and detection of cleaved dual-labeled oligonucleotide detection probe specific to the target.

Specimen Preparation

The COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 uses automated specimen preparation on the COBAS® AmpliPrep Instrument by a generic silica-based capture technique. The sample input volume is 650 µL, whereas the procedure processes 500 µL of EDTA plasma or serum. The HCV virus particles are lysed by incubation at elevated temperature with a protease and chaotropic lysis/binding buffer to release nucleic acids and protect the released HCV RNA from RNases in serum or EDTA plasma. Protease and a known number of HCV Quantitation Standard (QS) RNA molecules are introduced into each specimen along with the lysis reagent and magnetic glass particles. Subsequently, the mixture is incubated and the HCV RNA and HCV QS RNA are bound to the surface of the magnetic glass particles. Unbound substances, such as salts, proteins and other cellular impurities, are removed by washing the magnetic glass particles. After separating the magnetic glass particles and completing the washing steps, the adsorbed nucleic acids are eluted at elevated temperature with an aqueous solution. The processed specimen, containing the released HCV RNA and HCV QS RNA, is added to the amplification mixture and transferred to the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer.

Reverse Transcription and PCR Amplification

The COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 uses reverse transcription of HCV RNA to complementary DNA (cDNA) and PCR amplification of cDNA using primers that define a sequence within the highly conserved region of the 5'-untranslated region of the HCV genome[18]. The nucleotide sequence of the primers has been optimized to yield comparable amplification of HCV genotypes 1 through 6. The reverse transcription and PCR amplification reaction is performed with an optimized blend of thermostable recombinant enzymes: ZO5 and ZO5D DNA polymerase. In the presence of manganese (Mn^{2+}) and under the appropriate buffer conditions, ZO5 and ZO5D have both reverse transcriptase and DNA polymerase activity. This allows both reverse transcription and PCR amplification to occur together with real-time detection of the amplicon.

Processed specimens are added to the amplification mixture in amplification tubes (K-tubes) where both reverse transcription and PCR amplification occur. The reaction mixture is heated to allow a downstream primer to anneal specifically to the HCV target RNA and to the HCV QS RNA. In the presence of Mn^{2+} and excess deoxynucleotide triphosphates (dNTPs), including deoxyadenosine, deoxyguanosine, deoxycytidine and deoxyuridine triphosphates, Z05 and Z05D polymerases extend the annealed primers forming a DNA strand complementary to the RNA target.

Target Amplification

Following reverse transcription of the HCV target RNA and the HCV QS RNA, the Thermal Cycler in the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer heats the reaction mixture to denature the RNA:cDNA hybrid and to expose the specific primer target sequences. As the mixture cools, the primers anneal to the target DNA. The thermostable DNA Polymerases (Z05 and Z05D) in the presence of Mn^{2+} and excess deoxynucleotide triphosphates (dNTPs), extends the annealed primers along the target template to produce a double-stranded DNA molecule termed an amplicon. The COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer automatically repeats this process for a designated number of cycles, with each cycle intended to double the amount of amplicon DNA. The required number of cycles is preprogrammed into the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer. Amplification occurs only in the region of the HCV genome between the primers; the entire HCV genome is not amplified.

Selective Amplification

Selective amplification of target nucleic acid from the specimen is achieved in the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 by the use of AmpErase (uracil-N-glycosylase) enzyme and deoxyuridine triphosphate (dUTP). The AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine[19], but not DNA containing deoxythymidine. Deoxyuridine is not present in naturally occurring DNA, but is always present in amplicon due to the use of deoxyuridine triphosphate as one of the dNTPs in the Master Mix reagent; therefore, only amplicon contains deoxyuridine. Deoxyuridine renders contaminating amplicon susceptible to destruction by the AmpErase enzyme prior to amplification of the target DNA. Also, any nonspecific product formed after initial activation of the Master Mix by manganese is destroyed by the AmpErase enzyme. The AmpErase enzyme, which is included in the Master Mix reagent, catalyzes the cleavage of deoxyuridine-containing DNA at the deoxyuridine residues by opening the deoxyribose chain at the C1-position. When heated in the first thermal cycling step, the amplicon DNA chain breaks at the position of the deoxyuridine, thereby rendering the DNA non-amplifiable. The AmpErase enzyme remains inactive for a prolonged period of time once exposed to temperatures above 55ºC, i.e., throughout the thermal cycling steps, and therefore does not destroy target amplicon formed throughout the duration of the PCR reaction.

Detection of Cleaved Dual-Labeled probes and HCV RNA Quantitation

The COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 utilizes real-time[20] PCR technology. The use of dual-labeled fluorescent probes allows for real-time detection of PCR product accumulation by monitoring the emission intensity of fluorescent reporter dyes released during the amplification process. The probes consist of HCV and HCV QS-specific oligonucleotide probes with a reporter dye and a quencher dye. In the COBAS® AmpliPrep/ COBAS® TaqMan® HCV Test, v2.0 the HCV and HCV QS probes are labeled with different fluorescent reporter dyes. When these probes are intact, the fluorescence of the reporter dye is suppressed by the proximity of the quencher dye due to Förster-type energy transfer effects. During PCR, the probe hybridizes to a target sequence and is cleaved by the $5' \rightarrow 3'$ nuclease activity of the thermostable Z05 and Z05D DNA polymerases. Once the reporter and quencher dyes are released and separated, quenching no longer occurs, and the fluorescent activity of the reporter dye is increased. The amplification of HCV RNA and HCV QS RNA are measured independently at different wavelengths. This process is repeated for a designated number of cycles, each cycle effectively increasing the emission intensity of the individual reporter dyes, permitting independent identification of HCV RNA

and HCV QS RNA. The PCR cycle where a growth curve starts exponential growth is related to the amount of starting material at the beginning of the PCR.

The quantitation of HCV viral RNA is performed using the HCV Quantitation Standard. The standard compensates for effects of inhibition and controls the preparation and amplification processes, allowing a more accurate quantitation of HCV RNA in each specimen. The HCV QS is a non-infectious armored RNA (aRNA) construct that contains fragments of HCV sequences with identical primer binding sites as the HCV target RNA and a unique probe binding region that allows HCV QS amplicon to be distinguished from the HCV target amplicon.

The HCV QS is added to each specimen at a known copy number and is carried through the specimen preparation, reverse transcription, PCR amplification and detection of cleaved dual-labeled oligonucleotide detection probes. The COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer calculates the HCV RNA concentration in the test specimens by comparing the HCV signal to the HCV QS signal for each specimen and control.

During the extension phase of the PCR on the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer, the specimens are illuminated and excited by filtered light and filtered emission fluorescence data are collected for each specimen. The readings from each specimen are then corrected for instrumental fluctuations. These fluorescence readings are sent by the instrument to the AMPLILINK software and stored in a database. Pre-Checks are used to determine if the HCV RNA and HCV QS RNA data represent sets that are valid. Flags are generated as notification to the user when the data lie outside the preset limits. After all Pre-Checks are completed and passed, the fluorescence readings are processed to generate cycle threshold (Ct) values for the HCV RNA and the HCV QS RNA. The lot-specific calibration constants provided with the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 are generated using calibration material and are used to calculate the titer value for the specimens and controls based upon the HCV RNA and HCV QS RNA Ct values.

REAGENTS

Refer to the **REAGENTS** section and **WARNING AND PRECAUTIONS** section for the hazard information for the product.

COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 (HC2) **72 Tests** (P/N 05480442 190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning[a]**
HCV v2.0 CS1 **(HCV Magnetic Glass Particles Reagent Cassette)**	Magnetic glass particles Tris buffer 0.09% Sodium azide 0.1 % Methylparaben	**1 x 72 Tests** (1 x 7.0 mL)	Not applicable
HCV v2.0 CS2 **(HCV Lysis Reagent Cassette)**	Sodium citrate dihydrate 42.5% Guanidine thiocyanate[b] < 6% Polydocanol[b] 0.9% Dithiothreitol[b]	**1 x 72 Tests** (1 x 78.0 mL)	 **DANGER** H302 + H332: Harmful if swallowed or if inhaled. H318: Causes serious eye damage. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P273: Avoid release to the environment. P280: Wear protective gloves/eye protection/face protection. P304 + P340 + P312: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER or doctor/ physician if you feel unwell. P305+P351+P338 + P310: IF IN EYES Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor/ physician.

COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 (HC2) **72 Tests** (P/N 05480442 190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning[a]**
HCV v2.0 CS3 (HCV Multi Reagent Cassette)1	HCV Multi Reagent Cassette containing: **Pase** (Proteinase Solution) Tris buffer < 0.05% EDTA Calcium chloride Calcium acetate ≤7.8% Proteinase[b] Glycerol	**1 x 72 Tests** 1 x 3.8 mL	 **DANGER** H315: Causes skin irritation. H318: Causes serious eye damage. H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P280: Wear eye protection/ face protection. P284: Wear respiratory protection. P304 + P340: IF INHALED: Remove person to fresh air and keep comfortable for breathing. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor/ physician. P342 + P311: If experiencing respiratory symptoms: Call a POISON CENTER or doctor/ physician.
	EB (Elution Buffer) Tris-base buffer 0.09% Sodium azide	1 x 8.1 mL	Not applicable

COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 (HC2) **72 Tests** (P/N 05480442 190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning[a]**
HCV v2.0 CS4 (HCV Test-Specific Reagent Cassette)	HCV Test-Specific Reagent Cassette containing:	**1 x 72 Tests**	Not applicable
	HCV QS (HCV Quantitation Standard) Tris buffer EDTA < 0.002% Poly rA RNA (synthetic) < 0.001% Armored HCV RNA construct containing HCV primer binding sequences and a unique probe binding region (non-infectious RNA in MS2 bacteriophage) 0.05% Sodium azide	1 x 3.6 mL	
	HCV MMX (HCV Master Mix) Tricine buffer Potassium acetate Potassium hydroxide < 20% Dimethyl sulfoxide Glycerol < 0.004% dATP, dCTP, dGTP, dUTP < 0.002% Upstream and downstream HCV primers to the 5' UTR region of HCV < 0.001% Fluorescent-labeled oligonucleotide probes specific for HCV and the HCV Quantitation Standard < 0.001% Oligonucleotide aptamer < 0.05% Z05 and Z05D DNA Polymerase (microbial) < 0.1% AmpErase (uracil-N-glycosylase) enzyme (microbial) 0.09% Sodium azide	1 x 3.5 mL	
	Mn^{2+} (COBAS® AmpliPrep/ COBAS® TaqMan® Manganese Solution) < 0.5% Manganese acetate[b] Glacial acetic acid 0.09% Sodium azide	1 x 19.8 mL	

COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 (HC2) **72 Tests** (P/N 05480442 190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning[a]**
HCV H(+)C, v2.0 **(HCV High Positive Control)**	< 0.001% Armored HCV RNA construct containing HCV sequences (non-infectious RNA in MS2 bacteriophage) Negative Human Plasma, non-reactive by US FDA licensed tests for antibody to HCV, antibody to HIV-1/2, HIV p24 antigen and HBsAg; HIV-1 RNA, HCV RNA and HBV DNA not detectable by PCR methods 0.1% ProClin® 300 preservative[b]	6 x 0.85 mL	 **WARNING** H317: May cause an allergic skin reaction. P261: Avoid breathing mist/ vapours. P272: Contaminated work clothing should not be allowed P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/container to an approved waste disposal plant.
HCV L(+)C, v2.0 **(HCV Low Positive Control)**	< 0.001% Armored HCV RNA construct containing HCV sequences (non-infectious RNA in MS2 bacteriophage) Negative Human Plasma, non-reactive by US FDA licensed tests for antibody to HCV, antibody to HIV-1/2, HIV p24 antigen and HBsAg; HIV-1 RNA, HCV RNA and HBV DNA not detectable by PCR methods 0.1% ProClin® 300 preservative[b]	6 x 0.85 mL	 **WARNING** H317: May cause an allergic skin reaction. P261: Avoid breathing mist/ vapours. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/container to an approved waste disposal plant.
CTM (–) C [COBAS® TaqMan® Negative Control (Human Plasma)]	Negative Human Plasma, non-reactive by US FDA licensed tests for antibody to HCV, antibody to HIV-1/2, HIV p24 antigen and HBsAg; HIV-1 RNA, HCV RNA and HBV DNA not detectable by PCR methods 0.1% ProClin® 300 preservative[b]	6 x 1.0 mL	 **WARNING** H317: May cause an allergic skin reaction. P261: Avoid breathing mist/ vapours. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/container to an approved waste disposal plant.

COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 (HC2) 72 Tests (P/N 05480442 190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning[a]**
Nonreagent Kit Components			
HCV H(+)C, v2.0 Clip **(HCV High Positive Control Barcode Clip)**	Not applicable	1 x 6 Clips	Not applicable
HCV L(+)C, v2.0 Clip **(HCV Low Positive Control Barcode Clip)**	Not applicable	1 x 6 Clips	Not applicable
HCV (–) C, v2.0 Clip **(HCV Negative Control Barcode Clip)**	Not applicable	1 x 6 Clips	Not applicable

[a] Product safety labeling primarily follows EU GHS guidance

[b] Hazardous substance

Kit	Reagent ingredients	Quantity per kit	Safety symbol and warning
PG WR **(COBAS® AmpliPrep/ COBAS® TaqMan® Wash Reagent)** (P/N 03587797 190)	Sodium citrate dehydrate < 0.1% N-Methylisothiazolone-HCl	1 x 5.1 L	Not applicable

WARNINGS AND PRECAUTIONS

As with any test procedure, good laboratory technique is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

A. **FOR *IN VITRO* DIAGNOSTIC USE**.

B. This test is for use with human serum or plasma collected in the anticoagulant EDTA.

C. Do not pipette by mouth.

D. Do not eat, drink or smoke in laboratory work areas. Wear protective disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

E. Avoid microbial and ribonuclease contamination of reagents when removing aliquots from control vials.

F. The use of sterile disposable pipettes and RNase-free pipette tips is recommended.

G. Do not pool controls from different lots or from different vials of the same lot.

H. Do not mix reagent cassettes or controls from different kits.

I. Do not open COBAS® AmpliPrep cassettes and exchange, mix, remove or add bottles.

J. Dispose of unused reagents, waste and specimens in accordance with country, federal, state and local regulations.

K. Do not use a kit after its expiration date.

L. Safety Data Sheets (SDS) are available on request from your local Roche office.

M. Specimens and controls should be handled as if infectious using safe laboratory procedures such as those outlined in the US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*[21] and in the Clinical and Laboratory Standards Institute (CLSI) document M29-A4[22]. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.

Note: Commercial liquid household bleach typically contains sodium hypochlorite at a concentration of 5.25%. A 1:10 dilution of household bleach will produce a 0.5% sodium hypochlorite solution.

N. **CAUTION**: **CTM (–) C, HCV L(+)C, v2.0** and **HCV H(+)C**, **v2.0** contain Human Plasma derived from human blood. The source material has been tested and found non-reactive for the presence of Hepatitis B Surface Antigen (HBsAg), antibodies to HIV-1/2 and HCV, and HIV p24 Antigen. Testing of Negative Human Plasma by PCR methods showed no detectable HIV-1 RNA, HCV RNA or HBV DNA. No known test methods can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all human sourced material, including **CTM (–) C, HCV L(+)C, v2.0** and **HCV H(+)C, v2.0** should be handled as if infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories*[19] and in the CLSI Document M29-A4[22]. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.

O. **MGP, EB, QS, Mn^{2+},** and **HCV MMX** contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. While disposing of sodium azide-containing solutions down laboratory sinks, flush the drains with a large volume of water to prevent azide buildup.

P. Wear eye protection, laboratory coats, and disposable gloves when handling any reagent. Avoid contact of these materials with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated. If spills of these reagents occur, dilute with water before wiping dry.

Q. When disposing of used COBAS® AmpliPrep Sample Processing Units (SPUs), which contain guanidine thiocyanate, avoid any contact with sodium hypochlorite (bleach) solution or acids. These mixtures can produce a highly toxic gas.

STORAGE AND HANDLING REQUIREMENTS

A. ***Do not freeze reagents or controls***.

B. Store **HCV v2.0 CS1, HCV v2.0 CS2, HCV v2.0 CS3,** and **HCV v2.0 CS4** at 2ºC to 8ºC. Unused, these reagents are stable until the expiration date indicated. Once opened, these reagents are stable for 70 days at 2°C to 8°C or until the expiration date, whichever comes first. **HCV v2.0 CS1, HCV v2.0 CS2, HCV v2.0 CS3** and **HCV v2.0 CS4** can be used for a maximum of 96 hours cumulative on-board the COBAS® AmpliPrep Instrument. Reagents must be stored at 2°C to 8°C between instrument cycles.

C. Store **HCV H(+)C, v2.0 , HCV L(+)C, v2.0** and **CTM (–) C** at 2°C to 8°C. The controls are stable until the expiration date indicated. Once opened, any unused portion must be discarded.

D. Store Barcode clips [**HCV H(+)C, v2.0 Clip, HCV L(+)C, v2.0 Clip** and **HCV (–) C Clip**] at 2°C to 30°C.

E. Store **PG WR** at 2°C to 30°C. Unopened **PG WR** is stable until the expiration date indicated. Once opened, this reagent is stable for 28 days at 2°C to 30°C or until the expiration date, whichever comes first.

MATERIALS PROVIDED

A. COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 **HC2**

HCV v2.0 CS1
(HCV Magnetic Glass Particles Reagent Cassette)

HCV v2.0 CS2
(HCV Lysis Reagent Cassette)

HCV v2.0 CS3
(HCV Multi-Reagent Cassette)

HCV v2.0 CS4
(HCV Test-Specific Reagent Cassette)

HCV H(+)C, v2.0
(HCV High Positive Control)

HCV L(+)C, v2.0
(HCV Low Positive Control)

CTM (–) C
[COBAS® TaqMan® Negative Control (Human Plasma)]

HCV H(+)C, v2.0 Clip
(HCV High Positive Control Barcode Clip)

HCV L(+)C, v2.0 Clip
(HCV Low Positive Control Barcode Clip)

HCV (–) C, v2.0 Clip
(HCV Negative Control Barcode Clip)

B. COBAS® AmpliPrep/COBAS® TaqMan® Wash Reagent **PG WR**

PG WR
(COBAS® AmpliPrep/COBAS® TaqMan® Wash Reagent)

MATERIALS REQUIRED BUT NOT PROVIDED

Instrumentation and Software

- COBAS® AmpliPrep Instrument
- COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer
- Optional: Docking Station
- Optional: **cobas p** 630 Instrument
- AMPLILINK Software, Version 3.3 or Version 3.4 Series
- Control Unit for the AMPLILINK software, with printer
- Instrument and Software Manuals:
  - COBAS® AmpliPrep Instrument Manual for use with the AMPLILINK Software Version 3.3 and 3.4 Series
  - COBAS® TaqMan® Analyzer Instrument Manual for use with the AMPLILINK Software Version 3.3 and 3.4 Series
  - COBAS® TaqMan® 48 Analyzer Instrument Manual for use with the AMPLILINK Software Version 3.3 and 3.4 Series
  - AMPLILINK Software Version 3.3 Series Application Manual for use with the COBAS® AmpliPrep Instrument, COBAS® TaqMan® Analyzer, COBAS® TaqMan® 48 Analyzer, COBAS® AMPLICOR® Analyzer and **cobas p** 630 Instrument

    or
  - AMPLILINK Software Version 3.4 Series Application Manual
  - Optional: **cobas p** 630 Instrument Operator's Manual Software Version 2.2
- Test definition File (TDF). See Product Information Card, provided with the kit, for name and current version of the TDF.

OTHER MATERIALS

- Sample Rack (SK 24 rack)
- Reagent Rack
- SPU rack
- K-carrier
- K-carrier Transporter
- K-carrier Rack
- Pipettors with aerosol barrier or positive displacement RNase-free tips (capacity 1,000 µL)*
- Disposable gloves, powderless
- Vortex mixer

**Pipettors should be accurate within 3% of stated volume. Aerosol barrier or positive displacement RNase-free tips must be used where specified to prevent specimen and amplicon cross-contamination.*

Disposables

- Sample processing units: SPUs
- Sample input tubes (S-tubes) with barcode clips
- Racks of K-tips
- K-tube Box of 12 x 96

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

Note: Handle all specimens and controls as if they are capable of transmitting infectious agents.

Specimen Collection and Storage

The COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 is for use with serum or EDTA plasma specimens. Blood should be collected in Serum Separation Tubes, BD Vacutainer® PPT™ Plasma Preparation Tubes for Molecular Diagnostic Test Methods or in sterile tubes using EDTA (lavender top) as the anticoagulant.

Follow the manufacturer's instructions for collection tube handling. Freshly drawn specimens (whole blood) may be stored at 2°C to 25°C for up to 24 hours prior to centrifugation. After centrifugation, transfer serum or EDTA plasma to sterile polypropylene tubes. It is recommended that specimens be stored in approximately 1,000 µL aliquots in sterile, 2.0 mL polypropylene screw-cap tubes (such as 2 mL screw cap micro tube from Sarstedt). Serum or EDTA plasma specimens may be stored:

At 2-8°C for up to 72 hours

At -20°C to -80°C for up to 6 weeks

Serum and EDTA plasma specimens may be frozen and thawed up to 5 times without loss of HCV RNA.

Specimen Transport

Transportation of whole blood, serum or EDTA plasma must comply with country, federal, state and local regulations for the transport of etiologic agents[23]. Whole blood must be transported at 2°C to 25°C and centrifuged within 24 hours of collection. EDTA plasma or serum may be transported at 2°C to 8°C or frozen at -20°C to -80°C or colder, within the specimen storage period described above.

INSTRUCTIONS FOR USE

For detailed operating instructions, descriptions of the possible configurations, printing results and interpreting flags, comments and error messages, can be found within the AMPLILINK Software 3.3 or Version 3.4 Series manual in the section Instrumentation and Software.

Batch Size and Workflow

Each kit contains reagents sufficient for 72 tests, which may be performed in batches of 12 to 24 tests. At least one of each control **CTM (–) C, HCV L(+)C, v2.0,** and **HCV H(+)C, v2.0** must be included in each batch (see "Quality Control" section). The COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer run must be started within 120 minutes following completion of specimen and control preparation. DO NOT FREEZE or STORE processed specimens and controls at 2°C to 8°C.

Specimen and Control Preparation

If using frozen specimens, place the specimens at room temperature until completely thawed and vortex for 3 to 5 seconds before use. Controls should be removed from 2°C to 8°C storage and equilibrated to room temperature and vortexed for 3 to 5 seconds before use.

COBAS® AmpliPrep Instrument Set-up

Part A. Maintenance and Priming

A1. The COBAS® AmpliPrep Instrument is ready for operation in stand-by mode.

A2. Turn the Control Unit for the AMPLILINK software ON. Prepare the Control Unit as follows:

1. Log onto Microsoft Windows® Operating System.
2. Double click the AMPLILINK software icon.
3. Log onto AMPLILINK software by entering the assigned User ID and Password.

A3. Check the supply of **PG WR** using the **Status** Screen and replace if necessary.

A4. Perform all Maintenance that is listed in the Due Tab. The COBAS® AmpliPrep Instrument will automatically prime the system.

Part B. Loading of Reagent Cassettes

Note: All reagent cassettes should be removed from 2°C to 8°C storage, immediately loaded onto the COBAS® AmpliPrep Instrument and allowed to equilibrate to ambient temperature on the instrument for at least 30 minutes before the first specimen is to be processed. Do not let reagent cassettes come to ambient temperature outside the instrument as condensation may form on the barcode labels. Do not wipe off condensation if it appears on the barcode labels.

B1. Place **HCV v2.0 CS1** onto a reagent rack. Place **HCV v2.0 CS2, HCV v2.0 CS3,** and **HCV v2.0 CS4** onto a separate reagent rack.

B2. Load the reagent rack containing **HCV v2.0 CS1** onto rack position **A** of the COBAS® AmpliPrep Instrument.

B3. Load the reagent rack containing **HCV v2.0 CS2, HCV v2.0 CS3** and **HCV v2.0 CS4** onto rack position **B, C, D** or **E** of the COBAS® AmpliPrep Instrument (refer to the appropriate Instrument Manuals for additional and detailed information).

Part C. Loading of Disposables

Note: Determine the number of COBAS® AmpliPrep reagent cassettes, Sample Processing Units (SPUs), Input Sample tubes (S-tubes), K-tips and K-tubes needed. One SPU, one Input S-tube, one K-tip and one K-tube are needed for each specimen or control.

Multiple configurations for use of the COBAS® AmpliPrep Instrument with the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer are possible.. Depending on the configuration used, load the appropriate number of reagent cassette racks, sample racks with Input S-tubes, SPU racks, K-tip racks, K-tube racks and K-carriers on K-carrier racks onto the respective rack positions of the COBAS® AmpliPrep Instrument.

C1. Place the SPUs in the SPU rack(s) and load the rack(s) onto rack position **J, K,** or **L** of the COBAS® AmpliPrep Instrument.

C2. Depending on the configuration used, load full K-tube rack(s) onto rack position **M, N, O,** or **P** of the COBAS® AmpliPrep Instrument.

C3. Load full K-tip rack(s) onto rack position **M, N, O,** or **P** of the COBAS® AmpliPrep Instrument.

C4. Depending on the configuration used, load K-carriers on K-carrier rack(s) onto rack position **M, N, O,** or **P** of the COBAS® AmpliPrep Instrument.

Part D. Ordering and Loading of Specimens

D1. Prepare sample racks as follows: Attach a barcode label clip to each sample rack position where a specimen (S-tube) is to be placed. Attach one of the specific barcode label clips for the controls [**CTM (–) C, HCV L(+)C, v2.0,** and **HCV H(+)C, v2.0**] to each sample rack position where the controls (S-tube) are to be placed. The barcode label clips for controls should have the same control lot number as the lot number on the control vials in the kit. Take care in assigning the right control to the position with the appropriate control barcode clip. Place one Input S-tube into each position containing a barcode label clip.

D2. Using the AMPLILINK software, create specimen orders for each specimen and control in the **Orders** window **Sample** folder. Select the appropriate test file and complete by saving.

D3. Assign specimen and control orders to sample rack positions in the **Orders** window **Sample Rack** folder. The sample rack number must be for the rack prepared in Step D1.

D4. Print the **Sample Rack Order** report to use as a worksheet.

D5. Prepare specimen and control racks in the designated area for specimen and control addition as follows: Vortex each specimen and control [**CTM (–) C, HCV L(+)C, v2.0,** and **HCV H(+)C, v2.0**] for 3 to 5 seconds. Avoid contaminating gloves when manipulating the specimens and controls.

D6. Transfer 650 µL of each specimen and control [**CTM (–) C, HCV L(+)C, v2.0,** and **HCV H(+)C, v2.0**] to the appropriate barcode labeled Input S-tube using a micropipettor with an aerosol barrier or positive displacement RNase-free tip. ***Avoid transferring particulates and/or fibrin clots from the original specimen to the Input S-tube.*** Specimens and controls should be transferred to tube positions as assigned and recorded on the worksheet (see step D4). The barcode label clips for controls should have the same control lot number as the lot number on the control vials in the kit. Assign the correct control to the position with the appropriate control barcode clip. ***Avoid contaminating the upper part of the S-tubes with specimens or controls.***

D7. If using the **cobas p 630** instrument for preparation of specimens, refer to the **cobas p 630** instrument Operators Manual.

D8. Depending on the configuration used, load the sample rack(s) filled with Input S-tubes onto rack positions F, G or H of the COBAS® AmpliPrep Instrument.

D9. Depending on the configuration used, load sample rack(s) with Input S-tubes and K-tubes (one for each Input S-tube, loaded in the right position adjacent to Input S-tubes) onto rack position F, G or H of the COBAS® AmpliPrep Instrument.

Part E. Start of COBAS® AmpliPrep Instrument Run

E1. Start the COBAS® AmpliPrep Instrument using the AMPLILINK software.

Part F. End of COBAS® AmpliPrep Instrument Run and Transfer to COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer (Only for manual transfer)

F1. Check for flags or error messages using the AMPLILINK software.

F2. Remove processed specimens and controls from the COBAS® AmpliPrep Instrument from sample racks (for COBAS® TaqMan® Analyzer without Docking Station) or K-carrier racks (for COBAS® TaqMan® 48 Analyzer), depending on the configuration.

F3. Remove waste from the COBAS® AmpliPrep Instrument.

Note: Processed specimens and controls should not be exposed to light after completion of specimen and control preparation.

Amplification and Detection

COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer Set-up

The COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer run must be started within 120 minutes following completion of specimen and control preparation.

Note: Do not freeze or store processed specimens and controls at 2°C to 8°C.

Part G. Loading Processed Specimens

G1. Depending on the instrument configuration, perform the appropriate steps to transfer the K-tubes to the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer.

Part H. Start of COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer Run

H1. Start the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer depending on the configuration used.

Part I. End of COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer Run

I1. At the completion of the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer run, print the Results Report. Check for flags or error messages in the Result section of the software. Specimens with flags and comments are interpreted as described in the Results section. After acceptance, store data in the archive.

I2. Remove and discard used K-tubes from the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer.

QUALITY CONTROL

One COBAS® TaqMan® Negative Control, one HCV Low Positive Control and one HCV High Positive Control must be included in each test batch. The batch is valid if no flags appear for any of the controls [**HCV L(+)C**, **v2.0**, **HCV H(+)C**, **v2.0** and **CTM (–) C**].

There are no requirements regarding the position of the controls on the sample rack.

Check the batch printout for flags and comments to ensure that the batch is valid.

Negative Control

The **CTM (–) C** must yield a "Target Not Detected" result. If the **CTM (–) C** is flagged as invalid, then the entire batch is invalid. Repeat the entire process (specimen and control preparation, amplification and detection). If **CTM (–) C** is consistently invalid in multiple batches, contact your local Roche office for technical assistance.

Positive Controls

The assigned range for **HCV L(+)C**, **v2.0** and **HCV H(+)C**, **v2.0** is specific for each reagent, and is provided on Test reagent cassette barcodes.

The HCV RNA IU/mL for **HCV L(+)C**, **v2.0** and **HCV H(+)C**, **v2.0** should fall within their assigned ranges. If one or both of the positive controls are flagged as invalid, then the entire batch is invalid. Repeat the entire process (specimen and control preparation, amplification and detection). If the HCV RNA titer of one or both of the positive controls is consistently outside the assigned ranges in multiple batches, contact your local Roche office for technical assistance.

RESULTS

The COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 48 Analyzer automatically determines the HCV RNA concentration for the specimens and controls. The HCV RNA concentration is expressed in International Units per milliliter (IU/mL).

AMPLILINK Software:

- Determines the cycle threshold (Ct) value for the HCV RNA and the HCV QS RNA.
- Determines the HCV RNA concentration based upon the Ct values for the HCV RNA and HCV QS RNA and the lot-specific calibration coefficients provided on the cassette barcodes.
- Determines that the calculated IU/mL for **HCV L(+)C**, **v2.0** and **HCV H(+)C**, **v2.0** fall within their assigned ranges.

Batch Validation

Check AMPLILINK software results window or printout for flags and comments to ensure that the batch is valid.

For control orders, a check is made to determine if the IU/mL value for the control is within its specified range. If the IU/mL value for the control lies outside of its range, a flag is generated to show the control has failed.

The batch is valid if no flags appear for any of the controls [**HCV L(+)C, v2.0, HCV H(+)C, v2.0,** or **CTM (–) C**].

The batch is valid if no flags appear for any of the HCV Controls [**HCV L(+)C, v2.0, HCV H(+)C, v2.0,** or **CTM (–) C**].

The batch is not valid if any of the following flags appear for the HCV Controls:

Negative Control

Flag	Result	Interpretation
NC_INVALID	Invalid	An invalid result or the calculated titer result for the negative control is not negative, i.e., the result Target Not Detected is not generated.

HCV Low Positive Control

Flag	Result	Interpretation
LPCINVALID	Invalid	An invalid result or the calculated titer result for the low positive control is not within the assigned range.

HCV High Positive Control

Flag	Result	Interpretation
HPCINVALID	Invalid	An invalid result or the calculated titer result for the high positive control is not within the assigned range.

If the batch is invalid, repeat the entire batch including specimen and control preparation, amplification and detection.

Interpretation of Results

For a valid batch, check each individual specimen for flags or comments on the result printout. Interpret the results as follows:

- A valid batch may include both valid and invalid specimen results depending on whether flags and/or comments are obtained for the individual specimens.

Specimen results are interpreted as follows:

Result Read-Out from cobas®	**Analytical Interpretation**	**Clinical Intepretation**
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.

PROCEDURAL LIMITATIONS

1. This test has been validated only for use with only human serum or plasma collected in EDTA anticoagulant. Testing of other specimen types may result in inaccurate results.
2. Though rare, mutations within the highly conserved regions of the viral genome covered by the test's primers and/or probes may result in the under-quantitation of or failure to detect the presence of the virus.
3. 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.

4. Reliable results are dependent on adequate specimen collection, transport, storage and processing procedures.
5. The presence of AmpErase enzyme in the COBAS® AmpliPrep/COBAS® TaqMan® HCV Master Mix reduces the risk of amplicon contamination. However, contamination from HCV positive controls and clinical specimens can be avoided only by good laboratory practices and careful adherence to the procedures specified in this Package Insert.
6. Use of this product should be limited to personnel trained to operate the **cobas p 630** instrument (optional), the COBAS® AmpliPrep Instrument and the COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 48 Analyzer. The operator should have a thorough knowledge of the applications run on the instruments and should follow good laboratory practices.
7. This product can only be used with the **cobas p 630** instrument (optional), COBAS® AmpliPrep Instrument and the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer.
8. Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next; users perform method correlation studies in their laboratory to quantify technology differences.

Interfering Substances

Elevated levels of triglycerides (3,300 mg/dL), conjugated bilirubin (25 mg/dL), unconjugated bilirubin (20 mg/dL), albumin (6,000 mg/dL), hemoglobin (200 mg/dL) and human DNA (40 mg/dL) in specimens as well as the presence of autoimmune diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and antinuclear antibody (ANA) have been shown not to interfere with the quantitation of HCV RNA by the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0.

The following drug compounds tested at the Peak Plasma Level (Cmax) and at 3 times the Cmax have been shown not to interfere with the quantitation of HCV RNA by the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0:

<table>
<tr>
<td rowspan="2">Nucleotide Reverse Transcriptase and DNA Polymerase Inhibitors
Adefovir dipivoxil
Tenofovir</td>
<td>Nucleoside Reverse Transcriptase Inhibitors
Lamivudine
Zidovudine
Stavudine
Abacavir
Didanosine
Emtricitabine
Entecavir
Telbivudine</td>
</tr>
<tr>
<td>Non-nucleoside HIV Reverse Transcriptase Inhibitors
Nevirapine
Efavirenz</td>
</tr>
<tr>
<td rowspan="3">HIV Protease Inhibitors
Atazanavir
Saquinavir
Ritonavir
Nelfinavir
Amprenavir
Lopinavir/Ritonavir
Darunavir
Tipranavir
Fosamprenavir</td>
<td>HIV Fusion Inhibitor
Enfurvitide</td>
</tr>
<tr>
<td>HIV Entry Inhibitors
Maraviroc</td>
</tr>
<tr>
<td rowspan="2">Immune Modulator
Peginterferon alfa-2b
Ribavirin
Peginterferon alfa-2a</td>
<td>HIV Integrase Inhibitor
Raltegravir</td>
</tr>
<tr>
<td>Compounds for Treatment of Herpes Viruses
Ganciclovir
Valganciclovir
Acyclovir</td>
</tr>
</table>

NON-CLINICAL PERFORMANCE EVALUATION

A. Limit of Detection Using the WHO International Standard

The limit of detection of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 was determined by analysis of serial dilutions of the WHO International Standard for Hepatitis C Virus RNA for Nucleic Acid Amplification Technology Assays (NIBSC code 06/100[24]), for Hepatitis C Virus RNA for Nucleic Acid Amplification Technology Assays, genotype 1a, obtained from NIBSC, in HCV negative human EDTA plasma or serum. Three independent dilution series were analyzed for each matrix. A total of up to 252 replicates per concentration level were tested for each matrix type. The study was performed over three days with three lots of COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 reagents.

The results for EDTA plasma and serum are shown in Table 1 and Table 2 and demonstrate that the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 detected HCV RNA at concentrations of 15 IU/mL or greater with a hit rate of ≥ 95%. The difference between EDTA plasma and serum was not statistically significant.

Table 1
Limit of Detection in EDTA Plasma determined with the WHO International Standard for Hepatitis C Virus RNA for Nucleic Acid Amplification Technology Assays

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	180	71
2.5	251	121	48
0	250	0	0
LOD by Hit Rate	**15 IU/mL**		
LOD by PROBIT at 95 % Hit Rate	**11 IU/mL 95% confidence range: 10 – 13 IU/mL**		

Table 2
Limit of Detection in Serum determined with the WHO International Standard for Hepatitis C Virus RNA for Nucleic Acid Amplification Technology Assays

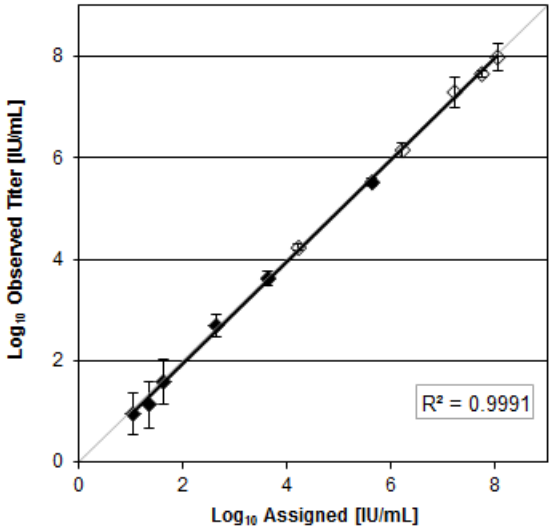
Input Titer (HCV RNA IU/mL)	Number of Valid Replicates	Number of Positives	Hit Rate in %
50	188	188	100
25	189	188	99
15	189	185	98
10	189	172	91
5	189	140	74
2.5	189	92	49
0	189	0	0
LOD by Hit Rate	**15 IU/mL**		
LOD by PROBIT at 95 % Hit Rate	**12 IU/mL 95% confidence range: 10 – 14 IU/mL**		

B. Linear Range

Two linearity panels were used to evaluate the linear range of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0. These panels consisted of dilutions of a HCV RNA-positive clinical specimen for the lower and middle part of the dynamic range (up to 3.0E+05 IU/mL) and armored HCV RNA (aRNA) for the high end of the dynamic range (up to 2.0E+08 IU/mL in either EDTA plasma or serum. The study was performed with two lots of COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 reagents in accordance with methods defined in CLSI document –EP6A[25]. All 11 panel members for EDTA plasma and all 14 panel members for serum were tested in up to 16 replicates per concentration level, matrix and reagent lot.

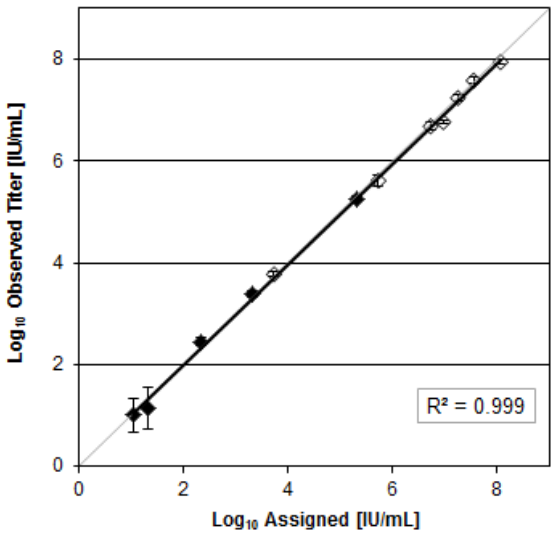
The COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 is linear from 15 IU/mL to at least 1.00E+08 IU/mL using an acceptable absolute deviation from +/- 0.2 $\log_{10}$ (see Figure 1 and Figure 2 for representative results). Across the linear range, the accuracy of the test was within +/- 0.2 $\log_{10}$.

Figure 1
Linear Range Determination for the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 in EDTA Plasma Specimens



The regression plot in Figure 1 shows the mean observed sample results for clinical samples (filled diamond) and aRNA samples (open diamond) plotted against the assigned $\log_{10}$ titer. The regression line (bold; from 11 to 1.1E+08 IU/mL) is shown together with the line of unity (grey) in order to visualize the linear behavior of the test. Standard deviation of $\log_{10}$ titer is shown as error bars, the R^2 is presented.

Figure 2
Linear Range Determination for the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 in Serum Specimens



The regression plot in Figure 2 shows the mean observed sample results for clinical samples (filled diamond) and aRNA samples (open diamond) plotted against the assigned $\log_{10}$ titer. The regression line (bold; from 11 to 1.2E+08 IU/mL) is shown together with the line of unity (grey) in order to visualize the linear behavior of the test. Standard deviation of $\log_{10}$ titers is shown as error bars, the R^2 is presented.

C. Precision – Within Laboratory

Precision of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 was determined by analysis of serial dilutions of clinical HCV specimens (Genotype 1a) or of armored HCV RNA (aRNA) in HCV negative human EDTA plasma or in serum.

Six dilution levels were tested in 3 replicates per level in 12 runs on 4 days. Each sample was carried through the entire COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 testing procedure, including specimen preparation, amplification and detection. Therefore, the precision reported here represents all aspects of the test procedure. The study was performed with 3 lots of COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 reagents, and the results are shown in Table 3.

The COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 was precise for 3 lots of reagents tested across a concentration range of 3.0E+02 IU/mL to 1.0E+08 IU/mL.

Table 3
Within-Laboratory Precision of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 (EDTA Plasma and Serum Samples)*

Nominal Concentration [IU/mL]	Source Material	EDTA Plasma						Serum					
		Lot 1		Lot 2		Lot 3		Lot 1		Lot 2		Lot 3	
		SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
3.0E+02	CS	0.22	54%	0.07	16%	0.09	21%	0.07	16%	0.05	12%	0.09	21%
3.0E+03	CS	0.15	36%	0.07	16%	0.07	16%	0.06	14%	0.06	14%	0.06	14%
3.0E+04	aRNA	0.06	14%	0.05	12%	0.07	16%	0.05	12%	0.07	16%	0.08	19%
3.0E+05	CS	0.08	19%	0.07	16%	0.08	19%	0.05	12%	0.05	12%	0.04	9%
3.0E+06	aRNA	0.14	33%	0.04	9%	0.07	16%	0.11	26%	0.06	14%	0.07	16%
1.0E+08	aRNA	0.07	16%	0.05	12%	0.11	26%	0.07	16%	0.06	14%	0.08	19%

* 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 3 reagent lots.

D. Inclusivity

The performance of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 on HCV genotypes was evaluated by (i) verifying the Limit of Detection for genotypes 1 through 6 and (ii) verifying of the Linear Range for genotypes 1 through 6.

Verification of Limit of Detection for genotypes 1 through 6

HCV RNA clinical specimens for 8 different genotypes/subtypes (1a, 1b, 2a, 2b, 3, 4, 5 and 6) were diluted to 3 different concentration levels in EDTA plasma or serum and a hit rate determination was performed for each level with up to 70 replicates. Testing was conducted with one lot of COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 reagents.

The results for EDTA plasma and serum are shown in Table 4 and Table 5 and verify that the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 detected HCV RNA for 8 different genotypes/subtypes at concentrations of 15 IU/mL or greater with a hit rate of ≥ 95%. The difference between serum and EDTA plasma was not statistically significant.

Table 4
HCV RNA Genotype Verification of Limit of Detection in EDTA Plasma

Genotype	5 IU/mL			15 IU/mL			45 IU/mL		
	Number of Valid Replicates	Number of Positives	Hit Rate in %	Number of Valid Replicates	Number of Positives	Hit Rate in %	Number of Valid Replicates	Number of Positives	Hit Rate in %
1a	63	44	70	63	63	100	63	63	100
1b	63	47	75	63	62	98	63	63	100
2a	63	43	68	63	61	97	62	61	98
2b	62	57	92	62	62	100	62	62	100
3	62	58	94	63	63	100	62	62	100
4	63	43	68	63	62	98	63	63	100
5	63	47	75	62	62	100	62	62	100
6	63	55	87	63	62	98	63	63	100

Table 5
HCV RNA Genotype Verification of Limit of Detection in Serum

Genotype	5 IU/mL			15 IU/mL			45 IU/mL		
	Number of Valid Replicates	Number of Positives	Hit Rate in %	Number of Valid Replicates	Number of Positives	Hit Rate in %	Number of Valid Replicates	Number of Positives	Hit Rate in %
1a	63	45	71	62	62	100	63	63	100
1b	62	48	77	63	63	100	63	63	100
2a	63	47	75	61	60	98	63	63	100
2b	63	42	67	63	61	97	63	63	100
3	63	58	92	63	63	100	63	63	100
4	63	41	65	63	62	98	63	63	100
5	62	46	74	61	60	98	62	62	100
6	70	58	83	70	69	99	69	69	100

Verification of Linear Range for Genotypes 1 through 6

HCV clinical specimens for 8 different genotypes/subtypes (1a, 1b, 2a, 2b, 3, 4, 5 and 6) were tested in up to 22 concentration levels. Test panels consisted of dilutions of HCV RNA-positive clinical genotype specimens for the lower and middle part of the dynamic range and genotype specific armored RNA (aRNA) for the high end of the dynamic range (genotypes 1a, 1b, 3 and 4 up to the upper limit of quantitation) or across the complete dynamic range (genotypes 2a, 2b, 5 and 6) in EDTA plasma. Testing was performed with one lot of COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 reagents. All 22 panel members were tested in up to 15 replicates.

The Linear Range of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 was verified with HCV genotypes 1 through 6 from 13 HCV RNA IU/mL to at least 1.4E+08 HCV RNA IU/mL using an acceptable absolute deviation from Linearity of +/- 0.2 $\log_{10}$.

E. Specificity

The specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 was determined by analyzing HCV RNA-and sero-negative EDTA plasma or serum samples from individual donors. Individual EDTA plasma and serum specimens (600 total results) were tested with 2 lots of COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 reagents. All specimens tested negative for HCV RNA. In the test panel the specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 was 100% (one-sided lower 95% confidence limit: ≥99.5%).

F. Analytical Specificity

The analytical specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 was evaluated by diluting different pathogens (see Table 6) with HCV RNA positive and HCV RNA negative human EDTA plasma specimens. None of the non-HCV pathogens interfered with test performance, or showed false positive results in the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0.

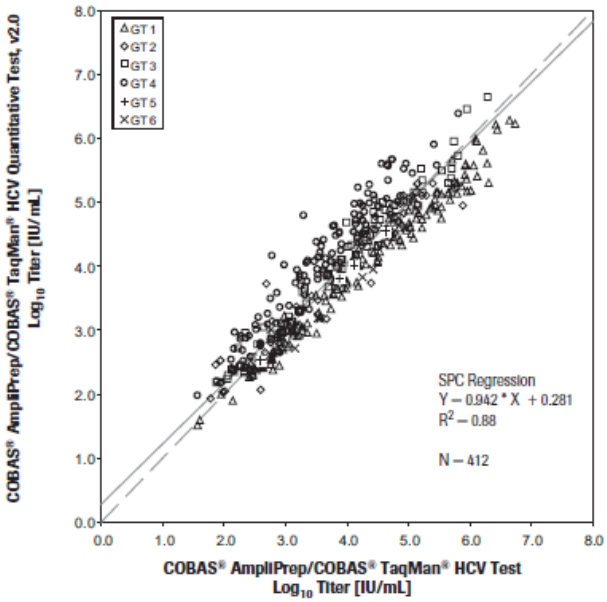
Table 6
Cross Reactivity Specimens

Viruses	Non-HCV Flavivirus
Adenovirus type 5	West Nile virus
Cytomegalovirus	St. Louis encephalitis virus
Epstein-Barr virus	Murray Valley encephalitis virus
Hepatitis A virus	Dengue virus type 1, 2, 3, and 4
Hepatitis B virus	Yellow fever virus
HIV-1	
Human T-Cell Lymphotropic virus types 1 and 2	Zika virus
Human herpes virus type 6	FSME virus (strain HYPR)
Herpes simplex virus types 1 and 2	
Influenza A	
Human papillomavirus	
Varicella zoster virus	
Bacteria	**Yeast**
Propionibacterium acnes	*Candida albicans*
Staphylococcus aureus	

G. Performance Compared to COBAS® AmpliPrep / COBAS® TaqMan® HCV Test

The performance of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 and the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test were compared by analysis of serum and EDTA plasma specimens from HCV infected patients. A total of 412 EDTA plasma and serum specimens across all HCV genotypes, analyzed in duplicate, were valid and within the quantitation range of both tests. Deming regression and Bland-Altman analysis were performed. The Deming regression results are shown in Figure 3.

Figure 3
Correlation of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 and the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test (GT= Genotype)

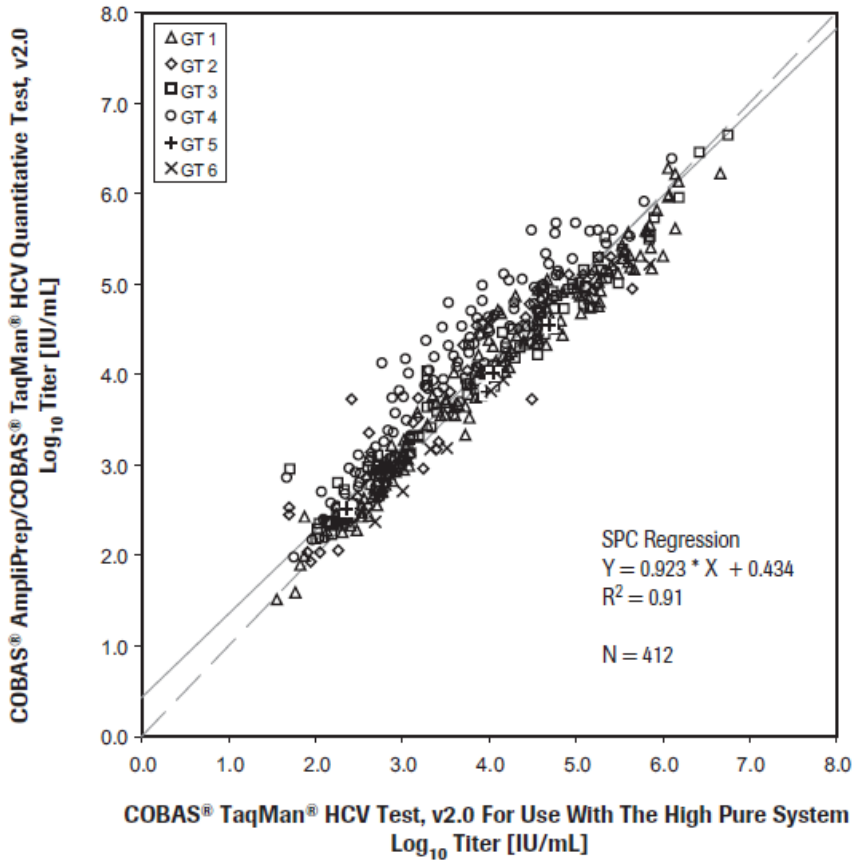


The Deming Regression analysis was performed and the R-squared value was 0.88 for all samples. After Bland-Altman analysis, the correlation showed a mean $\log_{10}$ titer difference of 0.1.

H. Performance Compared to COBAS® TaqMan® HCV Test, v2.0 for Use With The High Pure System

The performance of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 and the COBAS® TaqMan® HCV Test v2.0 for Use With The High Pure System were compared by analysis of serum and EDTA plasma specimens from HCV-infected patients. A total of 412 EDTA plasma and serum specimens across all HCV genotypes, analyzed in duplicate, were valid and within the quantitation range of both tests. Deming regression and Bland Altman analysis was performed. The Deming regression results are shown in Figure 4.

Figure 4
Correlation of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 and the COBAS® TaqMan® HCV Test, v2.0 For Use With The High Pure System



The Deming regression analysis was performed and the R-squared value was 0.91. After the Bland-Altman analysis, the correlation showed a mean $\log_{10}$ titer difference of 0.1.

I. Matrix Equivalency — Serum versus EDTA Plasma

Serum and plasma matrices from 25 negative (HCV RNA negative) and 25 HCV RNA positive matched samples were tested in the matrix equivalency evaluation. To achieve a distribution of HCV concentration levels across the linear range of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0, 10 samples with an HCV concentration level of approximately 100,000 IU/mL were diluted 1:10, 1:100, 1:1,000 and 1:10,000 with the appropriate matrix (HCV-negative serum or HCV-negative EDTA plasma).

The 25 serum and EDTA plasma samples were all tested negative with the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0. For the 25 HCV-positive specimens, an R-squared value of 0.99 was achieved (Deming regression analysis). When comparing the mean titer value of the plasma specimens to the titer obtained for the matched serum samples, an absolute mean $\log_{10}$ viral load titer of -0.1 $\log_{10}$ was obtained by Bland-Altman analysis.

CLINICAL PERFORMANCE STUDIES

A. Reproducibility

The reproducibility of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 was evaluated for HCV genotypes 1, 2, 3, 4, 5, and 6. Testing was performed by 2 operators at 4 clinical sites. Each operator performed 3 days of testing on each of 3 lots of reagents with assigned genotype panels. Each run consisted of a single genotype panel with each panel member tested in triplicate.

The results of the reproducibility study are summarized in Table 7.

Table 7
Attributable Percentage of Total Variance, Total Precision of HCV RNA Concentration (log_{10} IU/mL) by Genotype and Expected HCV RNA Concentration From Tests With Detectable Viral Load

	HCV RNA Concentration (log_{10} IU/mL)		HCV RNA Concentration (IU/mL)			Percent Contribution to Total Variance (Standard Deviation)						Total Precision	
Geno-type	Expected	Mean Observed	(Expected)	Lognormal Mean (Observed)	No. of Tests[a]	Lot	Site/ Instru-ment	Oper-ator	Day	Run	Within Run	SD	Log-normal CV(%)[b]
1	1.176**	1.074	15	18	314	5% (0.09)	1% (0.03)	4% (0.08)	6% (0.10)	0% (0.00)	85% (0.39)	0.42	124%
	1.398***	1.369	25	32	321	21% (0.16)	5% (0.08)	0% (0.00)	0% (0.02)	1% (0.03)	73% (0.30)	0.35	95%
	3.398	3.262	2,500	1,908	322	47% (0.09)	2% (0.02)	0% (0.00)	0% (0.00)	34% (0.07)	17% (0.05)	0.12	29%
	4.398	4.163	25,000	15,010	321	33% (0.06)	21% (0.05)	0% (0.00)	2% (0.01)	29% (0.06)	15% (0.04)	0.11	26%
	5.398	5.071	250,000	122,032	321	14% (0.05)	39% (0.08)	0% (0.00)	4% (0.02)	28% (0.06)	15% (0.05)	0.12	28%
	6.398	5.813	2,500,000	710,582	319	16% (0.07)	57% (0.14)	0% (0.00)	0% (0.00)	20% (0.08)	8% (0.05)	0.19	45%
	7.602	7.336	40,000,000	23,954,894	320	5% (0.05)	55% (0.14)	0% (0.00)	0% (0.00)	28% (0.10)	12% (0.07)	0.20	47%
2	1.176**	1.212	15	25	321	22% (0.18)	12% (0.14)	0% (0.00)	0% (0.00)	9% (0.12)	58% (0.30)	0.40	114%
	1.398***	1.501	25	45	323	13% (0.13)	21% (0.17)	0% (0.00)	2% (0.05)	0% (0.00)	64% (0.29)	0.37	102%
	3.398	3.534	2,500	3,573	324	43% (0.09)	14% (0.05)	0% (0.00)	0% (0.00)	28% (0.07)	14% (0.05)	0.13	31%
	4.398	4.429	25,000	27,479	324	31% (0.05)	2% (0.01)	0% (0.00)	0% (0.00)	37% (0.06)	30% (0.05)	0.10	22%
	5.398	5.340	250,000	224,857	324	14% (0.04)	15% (0.04)	2% (0.01)	0% (0.00)	34% (0.06)	34% (0.06)	0.10	24%
	6.398	5.923	2,500,000	877,267	323	31% (0.07)	22% (0.06)	1% (0.01)	4% (0.03)	24% (0.07)	18% (0.06)	0.13	31%
	7.602	6.980	40,000,000	10,120,461	324	12% (0.05)	20% (0.07)	0% (0.00)	0% (0.00)	47% (0.10)	21% (0.07)	0.15	35%
3	1.176**	1.685	15	56	318	17% (0.10)	6% (0.06)	0% (0.00)	0% (0.00)	12% (0.08)	64% (0.19)	0.23	58%
	1.398***	1.888	25	87	318	20% (0.09)	10% (0.07)	0% (0.00)	0% (0.00)	17% (0.09)	53% (0.15)	0.21	51%
	3.398	3.667	2,500	4,810	319	30% (0.07)	27% (0.06)	0% (0.00)	0% (0.00)	32% (0.07)	11% (0.04)	0.12	28%
	4.398	4.549	25,000	36,965	319	9% (0.04)	51% (0.09)	0% (0.00)	0% (0.00)	29% (0.07)	11% (0.04)	0.13	31%
	5.398	4.871	250,000	77,133	319	33% (0.07)	21% (0.06)	0% (0.00)	1% (0.01)	30% (0.07)	14% (0.05)	0.12	29%
	6.398	5.850	2,500,000	738,669	317	28% (0.07)	19% (0.06)	0% (0.00)	1% (0.01)	30% (0.07)	22% (0.06)	0.13	31%
	7.602	7.079	40,000,000	12,538,244	316	10% (0.04)	9% (0.04)	0% (0.00)	5% (0.03)	42% (0.08)	34% (0.08)	0.13	30%
4	1.176**	0.982	15	16	276	2% (0.06)	5% (0.10)	0% (0.00)	2% (0.07)	0% (0.00)	90% (0.41)	0.43	128%
	1.398***	1.236	25	28	311	9% (0.13)	6% (0.10)	3% (0.08)	2% (0.06)	0% (0.00)	80% (0.38)	0.42	126%
	3.398	3.337	2,500	2,257	320	49% (0.09)	13% (0.05)	0% (0.00)	0% (0.00)	18% (0.05)	20% (0.06)	0.12	29%
	4.398	4.653	25,000	46,109	320	35% (0.06)	7% (0.03)	0% (0.00)	0% (0.00)	41% (0.06)	16% (0.04)	0.10	23%
	5.398	5.567	250,000	379,490	319	20% (0.05)	4% (0.02)	0% (0.00)	0% (0.00)	58% (0.08)	18% (0.05)	0.11	25%
	6.398	6.616	2,500,000	4,209,953	319	19% (0.04)	1% (0.01)	0% (0.01)	0% (0.00)	57% (0.07)	22% (0.04)	0.09	21%
	7.602	7.511	40,000,000	33,209,396	319	0% (0.00)	0% (0.00)	0% (0.00)	0% (0.00)	72% (0.08)	28% (0.05)	0.09	22%

	HCV RNA Concentration (log_{10} IU/mL)		HCV RNA Concentration (IU/mL)			Percent Contribution to Total Variance (Standard Deviation)						Total Precision	
Geno-type	Expected	Mean Observed	(Expected)	Lognormal Mean (Observed)	No. of Tests[a]	Lot	Site/ Instru-ment	Oper-ator	Day	Run	Within Run	SD	Log-normal CV(%)[b]
5	1.176**	1.515	15	43	314	8% (0.09)	10% (0.10)	2% (0.05)	0% (0.00)	8% (0.09)	72% (0.27)	0.32	86%
	1.398***	1.791	25	71	314	12% (0.08)	11% (0.08)	2% (0.03)	3% (0.04)	14% (0.09)	58% (0.18)	0.23	58%
	3.398	3.650	2,500	4,589	317	37% (0.06)	8% (0.03)	2% (0.02)	4% (0.02)	29% (0.05)	19% (0.04)	0.10	23%
	4.398	4.398	25,000	25,594	315	45% (0.06)	4% (0.02)	0% (0.00)	4% (0.02)	30% (0.05)	17% (0.04)	0.09	21%
	5.398	5.191	250,000	161,079	312	30% (0.07)	26% (0.06)	0% (0.00)	4% (0.02)	29% (0.07)	11% (0.04)	0.12	29%
	6.398	6.183	2,500,000	1,573,763	315	18% (0.05)	16% (0.05)	1% (0.01)	8% (0.03)	34% (0.07)	24% (0.06)	0.11	27%
	7.602	7.408	40,000,000	26,333,634	314	6% (0.03)	18% (0.05)	0% (0.00)	14% (0.04)	44% (0.07)	17% (0.05)	0.11	26%
6	1.176**	0.909	15	14	300	1% (0.05)	14% (0.17)	0% (0.00)	4% (0.09)	3% (0.08)	77% (0.40)	0.45	141%
	1.398***	1.167	25	23	319	10% (0.12)	6% (0.10)	0% (0.00)	0% (0.00)	18% (0.17)	66% (0.32)	0.40	115%
	3.398	3.272	2,500	1,940	321	40% (0.07)	10% (0.04)	0% (0.00)	0% (0.00)	31% (0.06)	19% (0.05)	0.11	26%
	4.398	4.143	25,000	14,245	322	33% (0.05)	4% (0.02)	0% (0.00)	0% (0.00)	46% (0.06)	18% (0.04)	0.10	22%
	5.398	5.349	250,000	231,569	321	19% (0.05)	22% (0.06)	0% (0.00)	10% (0.04)	33% (0.07)	17% (0.05)	0.12	28%
	6.398	6.387	2,500,000	2,525,720	321	1% (0.01)	25% (0.06)	0% (0.00)	0% (0.00)	43% (0.08)	31% (0.07)	0.12	28%
	7.602	7.689	40,000,000	50,951,696	322	0% (0.00)	31% (0.07)	0% (0.00)	0% (0.00)	33% (0.07)	35% (0.08)	0.13	30%

Note: Within assay range results are from ≥ 1.50 E+1 IU/mL to ≤1.00E+8 IU/mL (1.18 log10 IU/mL to 8.00 log10 IU/mL). Results >1.00E+8 IU/mL were imputed using the upper limit of quantitation (ULOQ), 1.00E+8 IU/mL (8.00 log10 IU/mL).
SD = standard deviation.

[a] Number of valid tests with detectable viral load.

[b] Lognormal CV (%) $= 100 * \sqrt{10^{\sigma^2 \ln(10)} - 1}$, σ^2 are the variance estimates.

** Over all genotypes, 758 of 1843 test results were <1.50E+1 IU/mL.

*** Over all genotypes, 379 of 1906 test results were <1.50E+1 IU/mL.

B. Clinical Utility

Clinical Performance Previously Established with the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test

The following study was conducted with the original COBAS® AmpliPrep/COBAS® TaqMan® HCV Test assay reagent formulation.

The primary objective of this study was to evaluate the clinical utility of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, for the clinical management of patients infected with chronic hepatitis C (CHC) by estimating the NPV and PPV for achieving an SVR at established clinically relevant time points during antiviral treatment (Week 4/RVR, Week 12/EVR, and Week 24).

Study Population

Retrospectively collected specimens from patients enrolled in a Phase III, randomized, multi-center study comparing 48 weeks with 24 weeks of treatment with peginterferon alfa-2a given in combination with either a standard dose or a low dose of ribavirin were studied.[12]

The patient population included subjects with serologically proven CHC who had not been previously treated with an interferon or ribavirin. A total of 1311 patients were enrolled in the original study, 1284 of whom received treatment. Specimens from a total of 1281 subjects were available for testing, for at least one time point, which was performed at 5 US test sites.

Determination of HCV RNA viral levels at Screening/Baseline, Week 4, Week 12, and Week 24 were performed using the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test. End of Treatment (EOT) and End of Follow-up (EOF) results were determined using the FDA-approved the COBAS® AMPLICOR HCV Test, v2.0.

Three predictability analysis subsets were established from the cohort based on the availability of serum samples at the key established clinically relevant time points as follows: Week 4/RVR Analysis was performed for the subset of patients with viral load results available for Screening/Baseline, Week 4 and EOF time points. This subset contained 984 patients. Week 12/EVR Analysis was performed for the subset of patients with viral load results available for Screening/Baseline, Week 12 and EOF time points. This subset contained 991 patients. Week 24 Analysis was performed for the subset of patients with viral load results available for Screening/Baseline, Week 24 and EOF time points. This subset contained 982 patients.

Baseline demographics of the study population are presented in Table 8.

Table 8
Description of the Study Population at Baseline

Characteristic	Category	Summary Statistics	Combined Over All Four Treatment Arms
Total Number of Subjects		N	1281
Age	< 40	N (%)	503 (39.3)
	≥40	N (%)	778 (60.7)
Gender	Male	N (%)	837 (65.3)
	Female	N (%)	444 (34.7)
Genotype	1	N (%)	739 (57.7)
	2	N (%)	202 (15.8)
	3	N (%)	288 (22.5)
	4	N (%)	36 (2.8)
	5	N (%)	7 (0.5)
	6	N (%)	9 (0.7)
Week 0 HCV RNA	≤ 7.40E+5 IU/mL[1]	N (%)	306 (23.9)
	> 7.40E+5 IU/mL	N (%)	910 (71.0)
	Missing	N (%)	65 (5.1)
Baseline Biopsy Result	Cirrhotic	N (%)	91 (7.1)
	Non-Cirrhotic	N (%)	959 (74.9)
	Transition to Cirrhotic	N (%)	231 (18.0)
Baseline SGPT	≤ 3 * ULN[2]	N (%)	880 (68.7)
	> 3 * ULN	N (%)	401 (31.3)
Baseline Serum Creatinine (mg/dL)		Mean	0.9
		SD	0.2
Baseline Creatinine Clearance (mL/min)		Mean	97.5
		SD	25.3

[1] 2,000,000 copies/mL = 7.40E+5 IU/mL = 5.87 log10 IU/mL.

[2] ULN = Upper Limit of Normal Range.

SGPT, serum glutamic pyruvic transaminase

Predictability Analysis

Association between Baseline Covariates and Sustained Virologic Response

Established host-, viral-, and treatment-related baseline covariates predictive of SVR with peginterferon/ribavirin therapy were analyzed using the unadjusted odds ratios (univariate) shown in Table 9. The data subset used for this analysis comprises 1017 patients who have baseline and End of Follow-up responses. Distribution of subjects for various characteristics in this subset is similar to that shown in Table 8. These results demonstrate that genotype non-1 and low baseline viral load for genotype 1 (defined as <7.40E+5) are the two most significant positive predictors of SVR.

Table 9
Predictors of Sustained Virological Response at Baseline

Characteristic[1]	Category	N (%)	Percent with SVR	Odds Ratio (95% CI) Using Univariate Analysis
Age	≥ 40	612(60.2)	60.3	
	< 40	405(39.8)	71.9	1.7 (1.3, 2.2)
Gender	Male	663(65.2)	63.2	
	Female	354(34.8)	68.1	1.2 (0.9, 1.7)
Treatment[1]	A: 24-W LD RBV[2,3]	177(17.4)	54.8	
	B: 24-W HD RBV	244(24.0)	68.9	1.8 (1.2, 2.8)
	C: 48-W LD RBV	261(25.7)	59.0	1.2 (0.8, 1.8)
	D: 48-W HD RBV	335(32.9)	71.9	2.1 (1.4, 3.1)
Genotype	1	575(56.5)	49.0	
	Non-1	442(43.5)	85.5	6.1 (4.5, 8.5)
Week 0 HCV RNA for Genotype 1	> 7.40E+5 IU/mL[4]	434(42.7)	43.3	
	≤ 7.40E+5 IU/mL	141(13.9)	66.7	2.6 (1.7, 4.0)
Week 0 HCV RNA for Genotype non-1	> 7.40E+5 IU/mL	335(32.9)	84.5	
	≤7.40E+5 IU/mL	107(10.5)	88.8	1.5 (0.7, 3.1)
Baseline Biopsy Result	Cirrhotic/ Transition to Cirrhotic	250(24.6)	58.4	
	Non-Cirrhotic	767(75.4)	67.0	1.4 (1.1, 2.0)
Baseline SGPT	≤ 3*ULN	706(69.4)	60.5	
	> 3*ULN	311(30.6)	74.9	2.0 (1.4, 2.7)

[1] Treatment is 180 mcg/wk PEG-IFN + RBV.

[2] 24-W = 24-week therapy ; 48-W = 48-week therapy.

[3] LD = low dose of RBV, 800 mg/day; HD = high dose of RBV, 1,000 or 1,200 mg/day.

[4] 2,000,000 copies/mL = 7.40E+5 IU/mL = 5.87 $\log_{10}$ IU/mL, based on the AASLD Practice Guideline[13].

SGPT, serum glutamic pyruvic transaminase; ULN, upper limit of normal range.

Definitions of Prediction Rules, NPV, PPV, and Odds Ratios

- Rapid Virologic Response Analysis = HCV-RNA < LOD at Week 4 of antiviral therapy
- Early Virologic Response = achievement of either a 2–$\log_{10}$ drop or absence of HCV RNA at Week 12 of antiviral therapy
- Week 24 Virologic Response = HCV-RNA < LOD at Week 24 of antiviral therapy
- Positive Predictive Value = the probability of SVR given an on-treatment virologic response at Week 4, Week 12, or Week 24
- Negative Predictive Value = the probability of NO SVR given no on-treatment virologic response at Week 4, Week 12, or Week 24

Odds ratio (OR) describes the measure of association between virologic response and SVR and is equal to:

$$OR = \frac{NPV * PPV}{(1-NPV) * (1-PPV)}$$

The relationship between SVR and RVR, EVR, or Week 24 results was studied after adjusting for baseline covariates and treatment arm. Factors such as HCV genotype, baseline viral load, cirrhosis, age, ethnicity, and body weight are cited in the literature as predictors for SVR.

Each of the 3 study subsets were initially analyzed for both PPV and NPV as pooled data for all treatment arms and further stratified by individual treatment arms, genotype and predictive rule cut-off (where appropriate).

Predictive Values at Week 4 of Antiviral Therapy (RVR Analysis)

Table 10 presents the performance statistics by treatment arm for RVR evaluation. This table shows that the positive predictive value for all patients at Week 4 generally remains high when the analysis is done by individual treatment arm (A through D) compared to the pooled results of all groups, regardless of genotype. The NPV for not achieving an SVR also remains low, particularly in the non-1 genotype patients due to the high response rate in this population.

Table 10
NPV and PPV at Week 4 (RVR) and Corresponding Odds Ratios:
Treatment Arms A through D

			Negative Predictive Value (NPV)		Positive Predictive Value (PPV)		Odds Ratio (95% CI)	
Treatment Arm[1]	**Genotype**	**Prediction Rule**	**Estimate (95% CI)**	**N**	**Estimate (95% CI)**	**N**	**Unadjusted**	**Adjusted[2]**
A	1	<18 IU/mL[3]	0.88 (0.78, 0.95)	61/69	0.87 (0.60, 0.98)	13/15	49.6 (8.3, 487.5)	47.5 (8.0, 282.4)
	Non-1	<18 IU/mL[3]	0.47 (0.23, 0.72)	8/17	0.90 (0.80, 0.96)	63/70	8.0 (1.9, 32.5)	6.8 (1.7, 26.7)
B	1	<18 IU/mL[3]	0.81 (0.69, 0.89)	54/67	0.94 (0.80, 0.99)	31/33	64.4 (12.9, 587.4)	89.9 (14.9, 542.5)
	Non-1	<18 IU/mL[3]	0.43 (0.24, 0.63)	12/28	0.95 (0.89, 0.98)	100/105	15.0 (4.1, 60.1)	13.2 (3.9, 44.3)
C	1	<18 IU/mL[3]	0.61 (0.52, 0.69)	86/142	0.85 (0.68, 0.95)	28/33	8.6 (3.0, 29.9)	7.9 (2.6, 23.7)
	Non-1	<18 IU/mL[3]	0.29 (0.13, 0.51)	7/24	0.86 (0.74, 0.94)	49/57	2.5 (0.7, 9.3)	2.4 (0.7, 8.6)
D	1	<18 IU/mL[3]	0.46 (0.38, 0.54)	68/148	0.84 (0.71, 0.93)	42/50	4.5 (1.9, 11.7)	3.2 (1.3, 7.7)
	Non-1	<18 IU/mL[3]	0.07 (0.01, 0.24)	2/27	0.87 (0.79, 0.93)	86/99	0.5 (0.1, 2.6)	0.4 (<0.1, 2.0)

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.

[1] Treatment Arm A = 24-week PEG-IFN + low-dose RBV; Treatment Arm B = 24-week PEG-IFN + high-dose RBV Treatment Arm C = 48-week PEG-IFN + low-dose RBV; Treatment Arm D = 48-week PEG-IFN + high-dose RBV

[2] Based on the logistic regression model including covariates for treatment arm, genotype (non-1 vs 1), baseline viral load (≤ 7.40 E+5 IU/mL vs > 7.40 E+5 IU/mL), liver disease (non-cirrhotic vs cirrhotic), baseline SGPT (> 3*ULN vs ≤ 3*ULN) and age (<40 vs ≥40). Genotype and/or treatment arm covariates were excluded if the analysis was by genotype and/or treatment arm.

[3] Limit of detection for COBAS® AmpliPrep/COBAS® TaqMan® HCV Test is 18 IU/mL.

Table 11 presents the NPV and PPV for all 4 treatment arms at 12 weeks stratified by genotype. Note that the sample sizes for non-1 patients are too small due to high response rate in this subgroup and insufficient to make meaningful conclusions.

Table 11
NPV and PPV at Week 12 (EVR) and Corresponding Odds Ratios:
Treatment Arms A through D

			Negative Predictive Value (NPV)		Positive Predictive Value (PPV)		Odds Ratio (95% CI)	
Treatment Arm[1]	**Genotype**	**Prediction Rule**	**Estimate (95% CI)**	**N**	**Estimate (95% CI)**	**N**	**Unadjusted**	**Adjusted[2]**
A	1	2 Log Drop or No HCV	1.00 (0.81, 1.00)	18/18	0.34 (0.23, 0.46)	23/68	>8.7 (1.2, 378.6)[3]	>13.7 (1.4, 132.0)[3]
	Non-1	2 Log Drop or No HCV	0.50 (0.01, 0.99)	1/2	0.85 (0.75, 0.92)	72/85	NA	NA
B	1	2 Log Drop or No HCV	1.00 (0.75, 1.00)	13/13	0.51 (0.40, 0.61)	47/93	>12.3 (1.7, 535.0)[3]	>10.2 (1.1, 97.8)[3]
	Non-1	2 Log Drop or No HCV	1.00 (0.29, 1.00)	3/3	0.90 (0.84, 0.95)	117/130	NA	NA
C	1	2 Log Drop or No HCV	0.94 (0.71, 1.00)	16/17	0.54 (0.45, 0.62)	83/155	18.4 (2.7, 782.6)	17.0 (2.2, 134.1)
	Non-1	2 Log Drop or No HCV	1.00 (0.03, 1.00)	1/1	0.82 (0.72, 0.90)	65/79	NA	NA
D	1	2 Log Drop or No HCV	0.76 (0.50, 0.93)	13/17	0.65 (0.58, 0.72)	119/183	6.0 (1.8, 26.3)	6.5 (1.9, 21.8)
	Non-1	2 Log Drop or No HCV	1.00 (0.03, 1.00)	1/1	0.90 (0.83, 0.94)	113/126	NA	NA

NPV: The denominator is the number of patients with no EVR at 12 weeks; the numerator is the number of patients who did not achieve SVR among patients with no EVR at 12 weeks.

PPV: The denominator is the number of patients with EVR at 12 weeks; the numerator is the number of patients who achieved SVR among patients with EVR.

[1] Treatment Arm A = 24-week PEG-IFN + low-dose RBV; Treatment Arm B = 24-week PEG-IFN + high-dose RBV Treatment Arm C = 48-week PEG-IFN + low-dose RBV; Treatment Arm D = 48-week PEG-IFN + high-dose RBV

[2] Based on the logistic regression model including covariates for treatment arm, genotype (non-1 vs 1), baseline viral load (≤ 7.40 E+5 IU/mL vs > 7.40 E+5 IU/mL), liver disease (non-cirrhotic vs cirrhotic), baseline SGPT (> 3*ULN vs ≤ 3*ULN) and age (<40 vs ≥40). Genotype and/or treatment arm covariates were excluded if the analysis was by genotype and/or treatment arm.

[3] Since NPV = 1.0 odds ratio estimate is not available. Conservative estimates of unadjusted and adjusted odds ratio are obtained by artificially subtracting one (1) from the numerator.

NA: with ≤ 3 in the denominator, the performance of the device for EVR in Non-1 cannot be determined.

Predictive Values at Week 24 of Antiviral Therapy

Table 12 presents performance characteristics at Week 24 classified by treatment arm. This table shows that the NPV for 24 weeks for all subgroups are at least 0.96, independent of treatment duration and genotype. The numbers of patients in non-1 genotype subsets are too small due to high response rate in this subgroup and are insufficient to draw conclusions. Once again, the PPV is less predictive of SVR and varies by genotype.

Table 12
NPV and PPV at Week 24 and Corresponding Odds Ratios:
Treatment Arms A through D

			Negative Predictive Value (NPV)		Positive Predictive Value (PPV)		Odds Ratio (95% CI)	
Treatment Arm[1]	**Genotype**	**Prediction Rule**	**Estimate (95% CI)**	**N**	**Estimate (95% CI)**	**N**	**Unadjusted**	**Adjusted[2]**
A	1	<18 IU/mL[3]	1.00 (0.85, 1.00)	22/22	0.35 (0.24, 0.49)	22/62	>11.6 (1.6, 499.0)[4]	>18.7 (2.0, 176.4)[4]
	Non-1	<18 IU/mL[3]	1.00 (0.16, 1.00)	2/2	0.84 (0.74, 0.91)	69/82	NA	NA
B	1	<18 IU/mL[3]	1.00 (0.81, 1.00)	18/18	0.53 (0.42, 0.64)	45/85	>19.1 (2.7, 816.6)[4]	>13.5 (1.6, 111.2)[4]
	Non-1	<18 IU/mL[3]	1.00 (0.29, 1.00)	3/3	0.90 (0.84, 0.95)	120/133	NA	NA
C	1	<18 IU/mL[3]	0.97 (0.84, 1.00)	31/32	0.60 (0.51, 0.68)	83/139	45.9 (7.1, 1894.5)	36.0 (4.7, 274.7)
	Non-1	<18 IU/mL[3]	1.00 (0.16, 1.00)	2/2	0.82 (0.72, 0.90)	64/78	NA	NA
D	1	<18 IU/mL[3]	0.96 (0.80, 1.00)	25/26	0.70 (0.63, 0.77)	121/172	59.3 (9.0, 2454.6)	44.7 (5.8, 345.2)
	Non-1	<18 IU/mL[3]	1.00 (0.16, 1.00)	2/2	0.89 (0.82, 0.94)	110/124	NA	NA

NPV: The denominator is the number of patients with no response at 24 weeks; the numerator is the number of patients who did not achieve SVR among patients with no response at 24 weeks.

PPV: The denominator is the number of patients with response at 24 weeks; the numerator is the number of patients who did not achieve SVR among patients with a response at 24 weeks.

[1] Treatment Arm A = 24-week PEG-IFN + low-dose RBV; Treatment Arm B = 24-week PEG-IFN + high-dose RBV Treatment Arm C = 48-week PEG-IFN + low-dose RBV; Treatment Arm D = 48-week PEG-IFN + high-dose RBV

[2] Based on the logistic regression model including covariates for treatment arm, genotype (non-1 vs 1), baseline viral load (≤ 7.40 E+5 IU/mL vs > 7.40 E+5 IU/mL), liver disease (non-cirrhotic vs cirrhotic), baseline SGPT (> 3*ULN vs ≤ 3*ULN) and age (<40 vs ≥40). Genotype and/or treatment arm covariates were excluded if the analysis was by genotype and/or treatment arm.

[3] Limit of detection for COBAS® AmpliPrep/COBAS® TaqMan® HCV Test

[4] Since NPV = 1.0 odds ratio estimate is not available. Conservative estimates of unadjusted and adjusted odds ratio are obtained by artificially subtracting one (1) from the numerator.

NA: with ≤ 3 in the denominator, the performance of the device for Week 24 Virological Response in Non-1 cannot be calculated.

Within-Subject Variability in Absence of Treatment

The objective of this analysis is to estimate the change in viral load (in log units) between two successive measurements of patients not receiving anti-viral therapy. Baseline and screening serum sample results were available from 196 subjects enrolled in the clinical study to evaluate the effect of pegylated-interferon 2b treatment duration and Ribavirin dose. The screening samples were obtained 2 to 56 days before the collection of the baseline samples with an average of 38 days between collections of the two samples. Out of 196 subjects, 139 were genotype 1 patients and 57 were non-1 genotype. These two results were used to estimate within subject variability, which includes biological variability as well as total assay variability. The within subject variability from these results was estimated to be 0.62 $\log_{10}$ IU/mL for genotype 1 patients and 0.59 $\log_{10}$ IU/mL for non-1 genotype patients. To obtain an estimate biological variability, total assay variability is subtracted from within subject variability. Biological variability for genotype 1 patients is 0.60 $\log_{10}$ IU/mL and 0.54 $\log_{10}$ IU/mL for non-1 genotype patients. The mean change of viral load within a subject was estimated to be 0.67 $\log_{10}$ IU/mL for genotype 1 patients and 0.39 $\log_{10}$ IU/mL for genotype non-1 patients. Viral load between two visits varied as noted in Table 13.

Table 13
Summary of Viral Load Changes Between Two Visits

Genotype	Mean Difference (log_{10}IU/mL)	Middle 95% of all difference (log_{10}IU/mL)
1	0.67	-0.51 log_{10} IU/mL to 1.80 log_{10}IU/mL
Non-1	0.39	-1.39 log_{10} IU/mL to 1.80 log_{10}IU/mL

C. Confirmation of Clinical Utility with the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0

The objective of this study was to confirm the clinical utility of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 for the clinical management of patients with chronic HCV infection by estimating the odds ratio (OR), negative predictive value (NPV), and predictive positive value (PPV) for achieving a sustained virologic response (SVR) at the following key medical decision points during their antiviral therapy: Week 4 (rapid virologic response, RVR), Week 12 (early virologic response, EVR), and Week 24. A set of retrospectively collected samples from 328 patients undergoing treatment for HCV infection with pegylated interferon alfa-2a/2b plus ribavirin were included in this study. HCV RNA viral load testing at Week 4, Week 12, and Week 24 was performed using the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0. Screening/Baseline, end of treatment (EOT) and confirmation of SVR were determined using the FDA-approved COBAS® AmpliPrep/COBAS® TaqMan® HCV Test or medical records.

Three predictability analysis subsets were established from the study population based on the availability of serum or plasma samples at the key established clinically relevant time points as follows: Week 4 (RVR) analysis was performed for the subset of subjects (n=258) with viral load results available for baseline, Week 4 and SVR time points. Week 12 (EVR) analysis was performed for the subset of subjects (n=281) with viral load results available for baseline, Week 12 and SVR time points. Week 24 analysis was performed for the subset of patients (n=242) with viral load results available for baseline, Week 24 and SVR time points. Table 14 describes the demographic characteristics of the entire subject population at Week 0 (Baseline).

Baseline demographics of the study population are presented in Table 14.

Table 14
Description of Analyzed Subject Population at Baseline

Characteristic	Category	Combined for 24-Week and 48-Week Treatment N (%)
Total Number of Subjects		**328**
Age	< 40	100 (30.5)
	≥ 40	228 (69.5)
Gender	Female	126 (38.4)
	Male	202 (61.6)
Treatment	24-Week PEG/RBV	56 (17.1)
	48-Week PEG/RBV	272 (82.9)
Genotype	1	263 (80.2)
	2	23 (7.0)
	3	38 (11.6)
	4	3 (0.9)
	5	0 (0.0)
	6	1 (0.3)
Week 0 HCV RNA	≤ 400,000 IU/mL	44 (13.4)
	> 400,000 IU/mL	284 (86.6)

Predictability Analysis

Association between Baseline Covariates and Sustained Virologic Response

Established host-, viral-, and treatment-related baseline covariates predictive of SVR with peginterferon/ribavirin plus ribavirin were analyzed using odds ratios as shown in Table 15. The data subset used for this analysis comprised 328 subjects who have baseline and SVR responses. There were 3 significant predictors of SVR (confidence interval of the odds ratio does not include 1): younger age, genotype non-1, and lower baseline viral load (defined as < 400,000 IU/mL).

Table 15
Predictors of Sustained Viral Response at Baseline

Characteristic	Category	N	Percent with SVR	Odds Ratio (95% CI)
Age	< 40	100	72.0	2.0 (1.2, 3.5)
	≥ 40	228	55.7	
Gender	Female	126	62.7	1.1 (0.7, 1.9)
	Male	202	59.4	
Genotype	Non-1	65	80.0	3.2 (1.6, 6.6)
	1	263	55.9	
Week 0 HCV RNA	≤ 400,000 IU/mL	44	77.3	2.5 (1.1, 5.8)
	> 400,000 IU/mL	284	58.1	

Definitions of Prediction Rules, NPV, PPV, and Odds Ratios

- Rapid Virologic Response Analysis (RVR): HCV RNA < LLOQ at Week 4 of antiviral therapy.
- Early Virologic Response (EVR): 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 virologic response: HCV RNA < LLOQ at Week 24 of antiviral therapy.
- Odds ratio (OR): The odds of achieving a SVR if the subject had a RVR at Week 4 or an EVR at Week 12 (or <LLOQ at week 24).
- Positive Predictive Value (PPV): The probability of sustained virologic response (SVR) given an on-treatment virologic response at Week 4, Week 12, or Week 24 depending on the time point (week)
- Negative Predictive Value (NPV): The probability of non-SVR given the absence of on-treatment virologic response at Week 4, Week 12, or Week 24 depending on the time point (week) of interest.

Each of the 3 subsets were initially analyzed for both PPV and NPV as pooled data for all genotypes and further stratified by genotype 1 or non-1.

Predictive Value at Week 4 of Antiviral Therapy (RVR Analysis)

The definition of RVR requires a value for HCV RNA < LLOQ (the lower limit of quantitation of the assay, 15 IU/ml) at week 4 of antiviral therapy.

Table 16 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 absence of SVR, especially in the non-1 genotype population.

Table 16
NPV and PPV at Week 4 (RVR) and Corresponding Odds Ratio

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)

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)).

Predictive Value at Week 12 of Antiviral Therapy (EVR Analysis)

Patients which achieved either a ≥ 2-log drop from Baseline or < LLOQ at Week 12 of antiviral therapy were considered to have an EVR.

Table 17 presents the overall NPV, PPV, and OR for all genotypes combined and stratified by genotype 1 and non-genotype 1 at Week 12. The NPV is highest for genotype 1 patients (0.73). Additionally, the PPV was less predictive and different for genotype 1 and non-1 patient groups.

As in the week 4 analysis, the NPV for non-1 genotype was less predictive for non-SVR.

Table 17
NPV and PPV at Week 12 (EVR) and Corresponding Odds Ratio

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	≥ 2 log Drop or <LLOQ	0.69 (0.49, 0.85)	20 / 29	0.63 (0.57, 0.69)	160 /252	3.9 (1.6, 10.0)
1	≥ 2 log Drop or <LLOQ	0.73 (0.52, 0.88)	19 / 26	0.60 (0.53, 0.67)	123 /205	4.1 (1.5, 11.9)
Non-1	≥ 2 log Drop or <LLOQ	0.33 (0.01, 0.91)	1 / 3	0.79 (0.64, 0.89)	37 / 47	1.9 (0.0, 38.5)

NPV: The denominator is the number of patients with no EVR at 12 weeks; the numerator is the number of patients who did not achieve SVR among patients with no EVR at 12 weeks.

PPV: The denominator is the number of patients with EVR at 12 weeks; the numerator is the number of patients who achieved SVR among patients with EVR.

OR: The measure of association between virologic response and SVR that is equal to (NPV*PPV)/((1-NPV)*(1-PPV)).

Predictive Value at Week 24 of Antiviral Therapy

Subjects with HCV RNA <LLOQ at Week 24 were defined as responders at Week 24. Table 18 shows that the NPV at Week 24 for all patients was high (> 0.9). However, the PPV at Week 24 was 0.71 for all patients and less predictive of SVR and varies by genotype. The number of non-responders among subjects with non-1 HCV genotypes was too small to be able to reliably estimate NPV in this sub-group.

Table 18
NPV and PPV at Week 24 and Corresponding Odds Ratio

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	0.93 (0.76, 0.99)	26 / 28	0.71 (0.64, 0.77)	152 /214	31.9 (7.5, 281.3)
1	<LLOQ	0.93 (0.76, 0.99)	25 / 27	0.68 (0.60, 0.75)	107 /158	26.2 (6.1, 233.2)
Non-1	<LLOQ	1.00 (0.03, 1.00)	1 / 1	0.80 (0.68, 0.90)	45 / 56	Infinity (0.2, Infinity)

NPV: The denominator is the number of patients with no response at 24 weeks; the numerator is the number of patients who did not achieve SVR among patients with no response at 24 weeks.

PPV: The denominator is the number of patients with response at 24 weeks; the numerator is the number of patients who achieved SVR among patients with a response at 24 weeks.

OR: The measure of association between virologic response and SVR that is equal to (NPV*PPV)/((1-NPV)*(1-PPV)).

Diagnostic utility

The study was designed to evaluate the ability of the assay to correctly diagnose anti-HCV positive subjects with active HCV infection.

Table 19 below shows the demographic and clinical characteristics for HCV antibody-positive subjects whose samples were tested with the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0.

Table 19
Demographic and Clinical Utility Characteristics (HCV Antibody Positive Subjects)

Characteristics	Statistic
Total, N	217
Clinical Condition	
HCV Antibody Positive[a], n(%)	
HCV RNA Positive	137 (63.1%)
HCV RNA Negative	80 (36.9%)
Age (years)	
Mean ± SD	49 ± 12.1
Median	50
Range	20 - 88
Gender, n(%)	
Male	121 (55.8%)
Female	96 (44.2%)
Race, n(%)	
Black / African-American	46 (21.2%)
White / Caucasian	168 (77.4%)
Other	3 (1.4%)
Risk Factor, n(%)	
Baby Boomers (Born: 1945--1965) only	96 (44.2%)
IVD Users only	22 (10.1%)
Baby Boomers and IVD Users	29 (13.4%)
Unknown, HCV antibody positive*	70 (32.3%)

[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, version 2.0 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; MSM = men who have sex with men; SD = standard deviation; VERSANT = VERSANT HCV RNA Qualitative Assay.

The agreement of COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 with patient infection status, using a cutoff of < 25 IU/mL to define absence of active HCV infection, was analyzed to determine the HCV infection status (Table 20).

Table 20
Agreement of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 with the Patient Infection Status Using 25 IU/mL as the Cut-off

	Patient Infection Status (PIS)		
COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0	**HCV Positive**	**HCV Negative**	**Total**
HCV RNA Detected Above 25 IU/mL	136	0	136
HCV RNA not Detected or Detected Below 25 IU/mL	0	80	80
Total	136	80	216
Positive Percent Agreement (95% score CI)	100.0 % (97.3, 100.0)	NA	NA
Negative Percent Agreement (95% score CI)	NA	100.0 % (95.4, 100.0)	NA

Note: Only valid tests from the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 among the HCV Antibody Positive specimens are included in this table.
CI = confidence interval; HCV = hepatitis C virus; PIS = patient infection status; NA = not applicable.

This study demonstrates the clinical utility of COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 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).

When highly sensitive real-time quantitative PCR assays such as COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 are used to aid in the diagnosis of HCV infection, a cut-off of 25 IU/mL on an HCV RNA test should be used to distinguish between non-active and active HCV infection. The HCV RNA concentration, together with other markers of active liver disease, needs to be evaluated if antiviral treatment is being considered.[15]

Cross-reactivity in subjects with non-HCV related liver disease

The cross-reactivity of COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 was evaluated with specimens that represented a variety of liver diseases for which active HCV infection was not the underlying cause.

Table 21 below shows the demographic and clinical characteristics for all subjects with non-HCV-related liver disease whose samples were tested with the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0.

Table 21
Demographic and Clinical Characteristics by System
(Subjects with Non-HCV-Related Liver Disease)

Characteristics	Statistic
Total, N	205
Clinical Condition	
HCV RNA Negative, n(%)	
Alcoholic Liver Disease	30 (14.6%)
Autoimmune Hepatitis	30 (14.6%)
Chronic HBV	30 (14.6%)
Fatty Liver Disease	31 (15.1%)
Non-Alcoholic Steatohepatitis (NASH)	51 (24.9%)
Nonspecific Cirrhosis	3 (1.5%)
Primary Billiary Cirrhosis	30 (14.6%)
Age (years)	
Mean ± SD	54 ± 13.1
Median	56
Range	20 - 81
Gender, n(%)	
Male	53 (25.9%)
Female	78 (38.0%)
Unknown	74 (36.1%)
Race, n(%)	
Asian	5 (2.4%)
Black / African-American	12 (5.9%)
White / Caucasian	62 (30.2%)
Other	3 (1.5%)
Unknown	123 (60.0%)
Baby Boomers (Born: 1945-1965), n(%)	
Yes	66 (32.2%)
No	54 (26.3%)
Undisclosed	85 (41.5%)

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

The negative percent agreement of COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 was evaluated with specimens that represented a variety of liver diseases for which active HCV infection was not the underlying cause (Table 22).

Table 22
Number of HCV RNA Negative Samples with Non-HCV-Related Liver Diseases within Test Result Categories by Clinical Condition

Clinical Condition	Number of Valid Tests						Specificity[a] % (95% CI)[b]
	Target Not Detected	< 1.50E+01 IU/mL	1.50E+01 ≤ x < 2.50E+01 IU/mL	2.50E+01 ≤ x ≤ 1.00E+08 IU/mL	> 1.00E+08 IU/mL	Total	
Alcoholic Liver Disease	30	0	0	0	0	30	100.0 (88.4, 100.0)
Autoimmune Hepatitis	30	0	0	0	0	30	100.0 (88.4, 100.0)
Chronic HBV	30	0	0	0	0	30	100.0 (88.4, 100.0)
Fatty Liver Disease	31	0	0	0	0	31	100.0 (88.8, 100.0)
NASH	51	0	0	0	0	51	100.0 (93.0, 100.0)
Nonspecific Cirrhosis	3	0	0	0	0	3	100.0 (29.2, 100.0)
Primary Biliary Cirrhosis	30	0	0	0	0	30	100.0 (88.4, 100.0)
Total	205	0	0	0	0	205	100.0 (98.2, 100.0)

Note: Only valid results from the TaqMan HCV 2 among the HCV Antibody negative specimens (non-HCV-related liver disease) are included in this table.

[a] Clinical Specificity: Percentage of the number of negative results 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; TaqMan HCV 2 = COBAS AmpliPrep/COBAS TaqMan HCV Test, version 2.0.

This study demonstrated the ability of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 to determine the absence of active HCV infection in subjects with a range of liver diseases due to causes other than HCV.

Conclusion

The COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 can quantitate the level of HCV RNA to assess and predict response to antiviral therapy only with peginterferon alfa-2a plus ribavirin. The results of this study demonstrate the clinical utility of this test for determining RVR, EVR, and Week-24 response in the management of patients with chronic HCV infection undergoing peginterferon alfa-2a plus ribavirin.

Additionally, COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 can be used as an aid in the diagnosis of active HCV infection in HCV-antibody-positive patients.

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Document Revision Information	
Doc Rev. 4.0 02/2016	Updated AMPLILINK versions, manuals, terminology and operating system. Please contact your local Roche Representative if you have any questions.
Doc Rev. 5.0 03/2016	Added diagnosis of HCV infection claim to **Intended Use** section. Added Clinical Utility study data for diagnosis of HCV infection. Included hazardous substance information in the **REAGENTS** and **WARNINGS AND PRECAUTIONS** sections. Please contact your local Roche Representative if you have any questions.
Doc Rev. 6.0 05/2016	Corrected storage conditions in **SPECIMEN COLLECTION, TRANSPORT AND STORAGE** section. Please contact your local Roche Representative if you have any questions.

Roche Molecular Systems, Inc.
1080 US Highway 202 South
Branchburg, NJ 08876 USA

Distributed by

Roche Diagnostics
9115 Hague Road
Indianapolis, IN 46250-0457 USA
(For Technical Assistance call the
Roche Response Center
toll-free: 1-800-526-1247)

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The following symbols are now used in labeling for Roche PCR diagnostic products.

	Ancillary Software
	Authorized Representative in the European community
	Barcode Data Sheet
	Consult instructions for use
	Contains Sufficient for <*n*> tests
	Contents of kit
	Distributed by
	*In Vitro* Diagnostic Medical Device
	Test Definition File
	Upper Limit of Assigned Range
	Batch code
	Biological Risks
	Catalogue number
	For IVD Performance Evaluation Only
	Lower Limit of Assigned Range
	Manufacturer
	Store in the dark
	Temperature Limit
	Use-by date
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