

COBAS AmpliPrep/COBAS TaqMan HCV Test

Package Insert

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COBAS® AMPLIPREP/COBAS® TAQMAN® HCV TEST

FOR *IN VITRO* DIAGNOSTIC USE

COBAS® AmpliPrep/COBAS® TaqMan® HCV Test	48 Tests	P/N: 03568555 190
COBAS® AmpliPrep/COBAS® TaqMan® Wash Reagent	5.1 Liters	P/N: 03587797 190

INTENDED USE

The COBAS AmpliPrep/COBAS TaqMan HCV Test is an *in vitro* nucleic acid amplification test for the quantitation of hepatitis C viral (HCV) RNA in human 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. Specimens containing HCV genotypes 1 – 6 have been validated for quantitation in the assay.

The COBAS AmpliPrep/COBAS TaqMan HCV Test is intended for use as an aid in the management of HCV-infected individuals undergoing anti-viral therapy. The assay measures HCV RNA levels at baseline and during treatment and can be utilized to predict sustained and non-sustained virological response to HCV therapy. The results from the COBAS AmpliPrep/COBAS TaqMan HCV Test 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. Assay performance for determining the state of HCV infection has not been established.

The COBAS AmpliPrep/COBAS TaqMan HCV Test is not intended for use as a screening test for the presence of HCV in blood or blood products or as a diagnostic test to confirm the presence of HCV infection.

SUMMARY AND EXPLANATION OF THE TEST

HCV infection is the most common chronic blood-borne infection in the United States with an estimated seroprevalence of 1.6%.¹ An estimated 3.2 million Americans suffer from chronic HCV infection making it the leading cause of chronic liver disease.² An estimated 85% of those with acute hepatitis C progress to chronic infection, of which 20%-25% will develop complications cirrhosis within 2 to 3 decades of its onset.³ Of the patients with cirrhosis, a smaller percentage will progress to decompensated liver disease, hepatocellular carcinoma, and death.⁴ In the United States, chronic hepatitis C (CHC) is responsible for an estimated 8000 to 10000 deaths per year and is the leading cause of liver transplantation.² The burden of HCV-associated disease is likely to increase during the next 10 to 20 years as the infected cohort reaches an age at which complications of liver disease typically occur.⁵

The detection and quantitation of HCV RNA by polymerase chain reaction (PCR) nucleic acid amplification offers a measure of active viremia in antibody-positive chronic HCV-infected patients undergoing antiviral therapy. Current guidelines and product information for the current FDA-approved peginterferons support the importance of measuring HCV RNA levels at baseline prior to treatment, at intervals during treatment to assess antiviral response, and after treatment is completed to assess the efficacy of the treatment.^{6,7} Current treatment options of peginterferon plus ribavirin have resulted in a sustained virologic response (SVR) of 40% to 78%, depending on genotype as well as other host and viral factors.^{6,7} In an attempt to improve these response rates and reduce unnecessary exposure to toxic medications, on-treatment HCV-RNA viral load assessment has evolved over the past decade in parallel to evolving treatment options. Initial guidance recommended that treatment be discontinued if HCV RNA was present at 24 weeks due to the high negative predictive value (NPV) for not achieving an SVR.^{8,9} This approach was further refined with the quantification of HCV RNA at 12 weeks to determine if a patient has achieved a 2-log₁₀ drop or is HCV RNA- negative, defined as early virologic response (EVR).^{10,11} A high NPV for not achieving an SVR has also been demonstrated in patients not achieving an EVR and this measurement has been incorporated into all major United States and global HCV treatment guidelines.¹²⁻¹⁵ More recently, achieving an undetectable HCV RNA at 4 weeks, defined as rapid virologic response (RVR), has been associated with a high positive

predictive value (PPV) for SVR.^{16,17} The utility of RVR has primarily focused on the potential for shortening therapy in patients achieving an RVR and extending therapy to prevent relapse in the patients who do not achieve an RVR.¹⁶⁻¹⁸ In summary, the use of HCV RNA for the on-treatment assessment of HCV antiviral therapy has become an increasingly important tool for individualizing treatment and optimizing patient outcomes.

PRINCIPLES OF THE PROCEDURE

The COBAS AmpliPrep/COBAS TaqMan HCV Test is a nucleic acid amplification test for the quantitation of HCV RNA in human serum or 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 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.

The COBAS AmpliPrep/COBAS TaqMan HCV Test permits automated specimen preparation followed by automated reverse transcription, PCR amplification and detection of HCV target RNA and HCV Quantitation Standard (QS) Armored RNA. The Master Mix reagent contains primers and probes specific for both HCV RNA and HCV QS Armored RNA. The Master Mix has been developed to ensure similar quantitation of HCV genotypes 1 through 6. The detection of amplified DNA is performed using a target-specific and a QS-specific dual-labeled oligonucleotide probe that permit independent identification of HCV amplicon and HCV QS amplicon.

The quantitation of HCV viral RNA is performed using the HCV QS. The HCV QS 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 construct that contains 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 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.

Target Selection

Selection of the target RNA sequence for HCV depends on identification of regions within the HCV genome that show maximum sequence conservation among the various HCV genotypes.¹⁹ Generic silica-based specimen preparation is used to capture the HCV RNA and HCV QS RNA and defined oligonucleotides are used as primers in amplification of the HCV RNA and HCV QS RNA. A target-specific and a QS-specific dual-labeled oligonucleotide probe permit independent identification of HCV amplicon and HCV QS amplicon. Accordingly, the appropriate selection of the primers and the dual-labeled oligonucleotide probe is critical to the ability of the test to amplify and detect the HCV genotypes. The COBAS AmpliPrep/COBAS TaqMan HCV Test uses reverse transcription and PCR amplification primers that define a sequence within the highly conserved region of the 5'-untranslated region of the HCV genome.²⁰ The nucleotide sequence of the primers has been optimized to yield comparable amplification of six HCV genotypes.

Specimen Preparation

The COBAS AmpliPrep/COBAS TaqMan HCV Test utilizes automated specimen preparation on the COBAS AmpliPrep Instrument by a generic silica-based capture technique. The procedure processes 850 μ L of plasma or serum. The HCV virus particles are lysed by incubation at elevated temperature with a protease and chaotropic lysis/binding buffer that releases nucleic acids and protects the released HCV RNA from RNases in serum or plasma. Protease and a known number of HCV 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 beads and completing the washing steps, the adsorbed nucleic acids are eluted at elevated temperature with an aqueous solution. The processed specimen, containing the magnetic glass particles as well as 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. The HCV target RNA and the HCV QS RNA are then reverse transcribed, amplified and simultaneously detected by cleavage of a target-specific and a QS-specific dual-labeled oligonucleotide probe.

Reverse Transcription and PCR Amplification

The reverse transcription and PCR amplification reaction is performed with the thermostable recombinant enzyme *Thermus specie* DNA Polymerase (Z05). In the presence of manganese (Mn^{2+}) and under the appropriate buffer conditions, Z05 has both reverse transcriptase and DNA polymerase activity.²¹ 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) in which 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 polymerase extends 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 *Thermus species* Z05 DNA Polymerase (Z05) in the presence of Mn^{2+} and excess deoxynucleotide triphosphates (dNTPs), including deoxyadenosine, deoxyguanosine, deoxycytidine and deoxyuridine (in place of thymidine) triphosphates, 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 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 but not DNA containing deoxythymidine.²² 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 55°C, i.e., throughout the thermal cycling steps, and therefore does not destroy target amplicon formed after PCR reaction.

Detection of PCR Products in a COBAS TaqMan Test

The COBAS AmpliPrep/COBAS TaqMan HCV Test utilizes real-time PCR technology.^{21,23} The use of dual-labeled fluorescent probes allows for real-time detection of PCR product accumulation by monitoring of 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, 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' → 3' nuclease activity of the thermostable Z05 DNA polymerase. 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.

Fundamentals of COBAS TaqMan Test Quantitation

The COBAS AmpliPrep/COBAS TaqMan HCV Test is inherently quantitative over a wide dynamic range since the monitoring of amplicon is performed during the exponential phase of amplification. The higher the HCV titer of a specimen, the earlier the fluorescence of the reporter dye of the HCV probe rises above the baseline fluorescence level (*see* Figure 1). Since the amount of HCV QS RNA is constant between all specimens, the fluorescence of the reporter dye of the HCV QS probe should appear at the same cycle for all specimens (*see* Figure 2). In specimens, where the QS amplification and detection is affected by inhibition or poor specimen recovery, the appearance of fluorescence will be delayed, thereby enabling the calculated titer of HCV target RNA to be adjusted accordingly. The appearance of the specific fluorescent signals is reported as a critical threshold value (Ct). The Ct is defined as the fractional cycle number where reporter dye fluorescence exceeds a predetermined threshold (the Assigned Fluorescence Level), and starts the beginning of an exponential growth phase of this signal (*see* Figure 3). A higher Ct value indicates a lower titer of initial HCV target material. A 2-fold increase in titer correlates with a decrease of 1 Ct for target HCV RNA; a 10-fold increase in titer correlates with a decrease of 3.3 Ct.

Figure 1 depicts the target growth curves for a dilution series spanning a 5- \log_{10} range. As the concentration of the virus increases, the growth curves shift to earlier cycles. Therefore, the leftmost growth curve corresponds to the highest viral titer level, whereas, the rightmost growth curve corresponds to the lowest viral titer level.

Figure 1: Target Growth Curves for a Dilution Series Spanning a 5-Log₁₀ Range

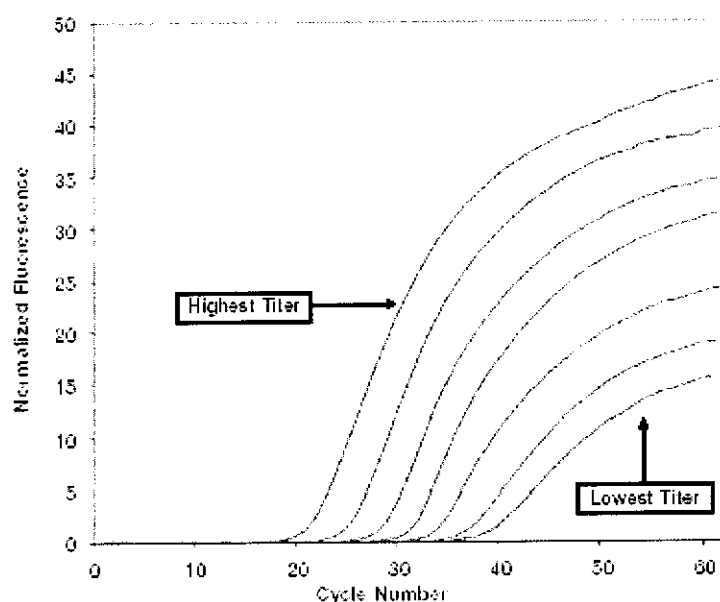


Figure 2 depicts the Quantitation Standard growth curves for specimens from a viral dilution series that spans a 5-log₁₀ range. The amount of Quantitation Standard added to each specimen is constant for each reaction. The Ct value of the Quantitation Standard is similar regardless of the viral titer.

Figure 2: Quantitation Standard Growth Curves for Specimens from a Viral Dilution Series that Spans a 5-Log₁₀ Range

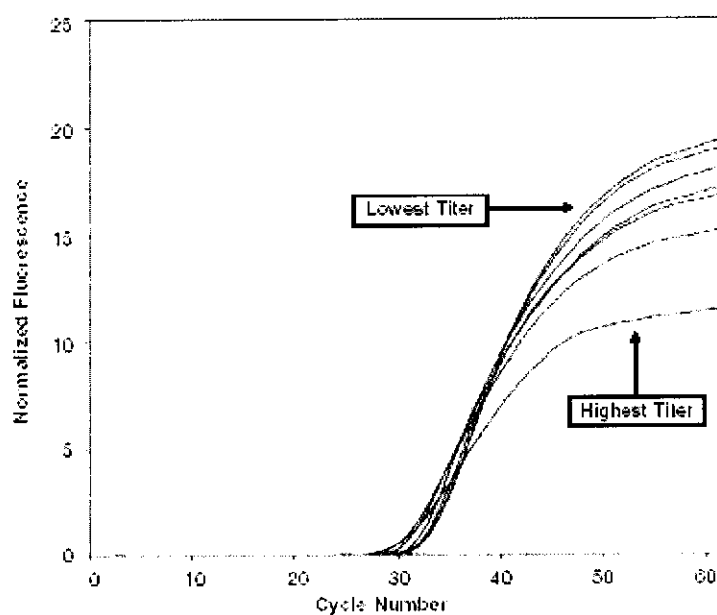
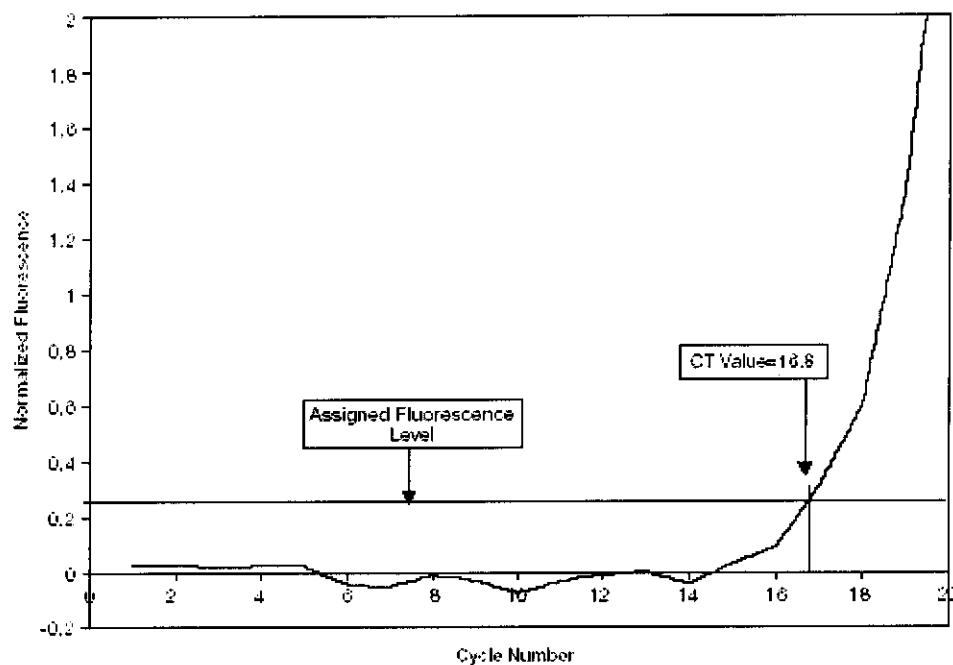


Figure 3 provides an example of how the fluorescence values at every cycle are normalized for each growth curve. The fractional cycle number (Ct) is calculated where the fluorescence signal crosses the Assigned Fluorescence Level.

Figure 3: Fluorescence Values at Every Cycle are Normalized for Each Growth Curve



HCV RNA Quantitation

The COBAS AmpliPrep/COBAS TaqMan HCV Test quantitates HCV viral RNA by utilizing a second target sequence (HCV Quantitation Standard) that is added to each test specimen at a known concentration. The HCV QS is a non-infectious Armored RNA construct, containing fragments of HCV sequences with primer binding regions identical to those of the HCV target sequence. The HCV QS generates an amplification product of the same length and base composition as the HCV target RNA. The detection probe binding region of the HCV QS has been modified to differentiate HCV QS amplicon from HCV target amplicon.

During the annealing 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, and flags are generated when the data lie outside the preset limits. After all Pre-Checks are completed and passed, the fluorescence readings are processed to generate 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 are used to calculate the titer value for the specimens and controls based upon the HCV RNA and HCV QS RNA Ct values. The COBAS AmpliPrep/COBAS TaqMan HCV Test is standardized against the First WHO International Standard for Hepatitis C Virus RNA for Nucleic Acid Amplification Technology Assays (NIBSC code 96/790) and titer results are reported in International Units (IU/mL).^{24,25}

REAGENTS


COBAS AmpliPrep/COBAS TaqMan HCV Test (HCMCAP)	48 Test
(P/N: 03568555 190)	

HCV CS1	1 x 48 Tests
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(HCV Magnetic Glass Particles Reagent Cassette)

Magnetic glass particles

93% Isopropanol

Xi		93% (w/w) Isopropanol
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Irritant

F		93% (w/w) Isopropanol
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Highly

Flammable

HCV CS2

1 x 48 Tests

(HCV Lysis Reagent Cassette)

Sodium citrate dihydrate

42.5% Guanidine thiocyanate

< 14% Polydocanol

0.9% Dithiothreitol

Xn



Harmful

42.5% (w/w) Guanidine thiocyanate

HCV CS3

1 x 48 Tests

*HCV Multi-Reagent Cassette containing:***Pase**

1 x 3.8 mL

(Proteinase Solution)

Tris buffer

< 0.05% EDTA

Calcium chloride

Calcium acetate

≤7.8% Proteinase

Glycerol

Xn



Harmful

≤7.8% (w/w) Proteinase

EB

1 x 7.0 mL

(Elution Buffer)

Tris-base buffer

0.2% Methylparaben

HCV CS4	1 x 48 Tests
<i>HCV Test-Specific Reagent Cassette containing:</i>	
HCV QS	1 x 3.6 mL
(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	
HCV MMX	1 x 2.5 mL
(HCV Master Mix)	
Tricine buffer	
Potassium acetate	
Potassium hydroxide	
< 20% Dimethylsulfoxide	
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 DNA Polymerase (microbial)	
< 0.1% AmpErase (uracil-N-glycosylase) enzyme (microbial)	
0.09% Sodium azide	
CAP/CTM Mn²⁺	1 x 19.8 mL
(CAP/CTM Manganese Solution)	
< 0.5% Manganese acetate	
Glacial acetic acid	
0.09% Sodium azide	

HCV H(+)C (HCV High Positive Control) < 0.001% Armored HCV RNA construct containing HCV sequences (non-infectious RNA in MS2 bacteriophage). Lot specific concentration range is encoded on the COBAS AmpliPrep/COBAS TaqMan HCV Test reagent cassette barcodes. 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	4 x 1.0 mL
HCV L(+)C (HCV Low Positive Control) < 0.001% Armored HCV RNA construct containing HCV sequences (non-infectious RNA in MS2 bacteriophage). Lot specific concentration range is encoded on the COBAS AmpliPrep/COBAS TaqMan HCV Test reagent cassette barcodes. 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	4 x 1.0 mL
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	4 x 1.0 mL
HCV H(+)C Clip (HCV High Positive Control Barcode Clip)	1 x 4 Clips
HCV L(+)C Clip (HCV Low Positive Control Barcode Clip)	1 x 4 Clips
HCV (–) C Clip (HCV Negative Control Barcode Clip)	1 x 4 Clips

COBAS AmpliPrep/COBAS TaqMan Wash Reagent (PG WR)

(P/N: 03587797 190)

PG WR

1 x 5.1 L

(COBAS AmpliPrep/COBAS TaqMan Wash Reagent)

Sodium citrate dihydrate

< 0.1% N-Methylisothiazolone-HCl

WARNINGS AND PRECAUTIONS

- 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 test 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. Material Safety Data Sheets (MSDS) 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 Biosafety in Microbiological and Biomedical Laboratories and in

the CLSI Document M29-A.^{26,27} 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 and HCV H(+)C 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 by FDA licensed tests. 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 should be considered potentially infectious. CTM (–) C, HCV L(+)C and HCV H(+)C should be handled as if infectious using safe laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A20.^{26,27} Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.
- O. HCV QS, CAP/CTM Mn²⁺ 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. Do not allow HCV CS2 and liquid waste from the CAP Instrument, which contain guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. These mixtures can produce a highly toxic gas.

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

STORAGE AND HANDLING REQUIREMENTS

- A. *Do not freeze reagents or controls.*
- B. Store HCV CS1, HCV CS2, HCV CS3 and HCV CS4 at 2-8°C. Unused, these reagents are stable until the expiration date indicated. Once used, these reagents are stable for 28 days at 2-8°C or until the expiration date, whichever comes first. HCV CS1, HCV CS2, HCV CS3 and HCV CS4 can be used for a maximum of 4 instrument cycles, up to a maximum of 64 hours cumulative on board the COBAS AmpliPrep Instrument. Reagents must be stored at 2-8°C between instrument cycles.
- C. Store HCV H(+)C, HCV L(+)C and CTM (-) C at 2-8°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 Clip, HCV L(+)C Clip and HCV (-) C Clip] at 2-30°C.
- E. Store PG WR at 2-30°C. PG WR is stable until the expiration date indicated. Once opened, this reagent is stable for 28 days at 2-30°C or until the expiration date, whichever comes first.

MATERIALS PROVIDED

- A. COBAS AmpliPrep/COBAS TaqMan HCV Test (P/N: 03568555 190)

HCV CS1

(HCV Magnetic Glass Particles Reagent Cassette)

HCV CS2

(HCV Lysis Reagent Cassette)

HCV CS3

(HCV Multi-Reagent Cassette)

HCV CS4

(HCV Test-Specific Reagent Cassette)

HCV H(+)C

(HCV High Positive Control)

HCV L(+)C

(HCV Low Positive Control)

CTM (–) C

[COBAS TaqMan Negative Control (Human Plasma)]

HCV H(+)C Clip

(HCV High Positive Control Barcode Clip)

HCV L(+)C Clip

(HCV Low Positive Control Barcode Clip)

HCV (–) C Clip

(HCV Negative Control Barcode Clip)

- B. COBAS AmpliPrep/COBAS TaqMan Wash Reagent (P/N: 03587797 190)

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
- AMPLILINK Software, Version 3.2 Series
- Data Station for the AMPLILINK software, with printer
- COBAS AmpliPrep Instrument Manual for use with the COBAS TaqMan Analyzer or COBAS TaqMan 48 Analyzer, COBAS AMPLICOR Analyzer and the AMPLILINK Software, Version 3.2 Series
- COBAS TaqMan Analyzer (plus optional Docking Station) Instrument Manual for use with AMPLILINK Software, Version 3.2 Series
- COBAS TaqMan 48 Analyzer Instrument Manual for use with AMPLILINK Software, Version 3.2 Series Application Manual
- AMPLILINK Software, Version 3.2 Series Application Manual For use with the COBAS AmpliPrep Instrument COBAS TaqMan Analyzer, COBAS TaqMan 48 Analyzer and COBAS AMPLICOR Analyzer

Disposables

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

OTHER MATERIALS REQUIRED BUT NOT PROVIDED

- Sample Rack (SK 24 rack)
- Reagent Rack
- SPU rack
- K-tube capper, motorized
- K-tube capper
- K-carrier
- K-carrier Transporter
- K-carrier rack
- Pipettors with aerosol barrier or positive displacement RNase-free tips (capacity 1000 μ L)*
- 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.*

SPECIMEN COLLECTION, TRANSPORT, AND STORAGE

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

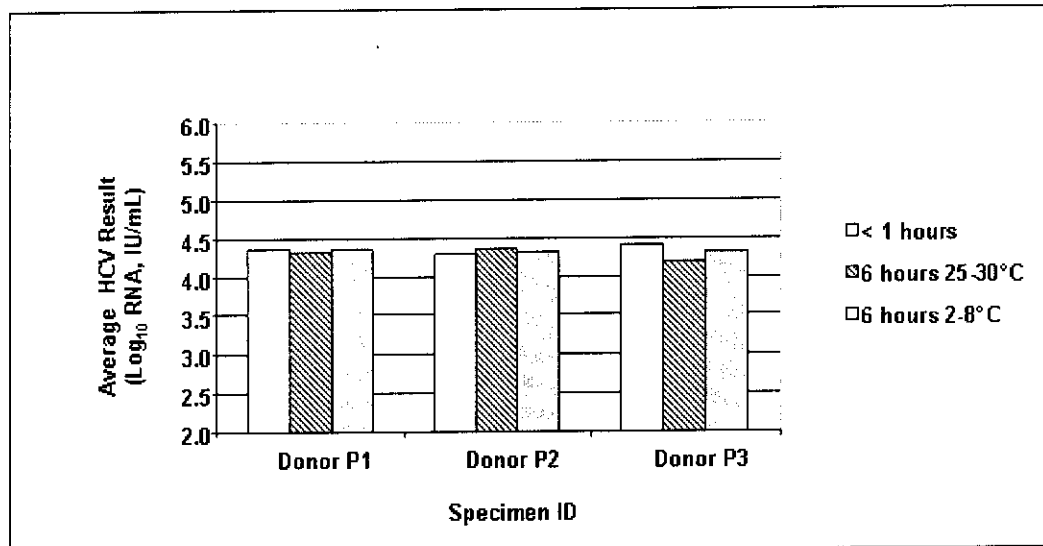
A. Specimen Collection

The COBAS AmpliPrep/COBAS TaqMan HCV Test is for use with serum or plasma specimens. Blood should be collected in SST® Serum Separation Tubes or in sterile tubes using EDTA (lavender top) as the anticoagulant.

Store whole blood at 2-25°C for no longer than 6 hours. Separate serum or plasma from whole blood within 6 hours of collection by centrifugation at 800-1600 x g for 20 minutes at room

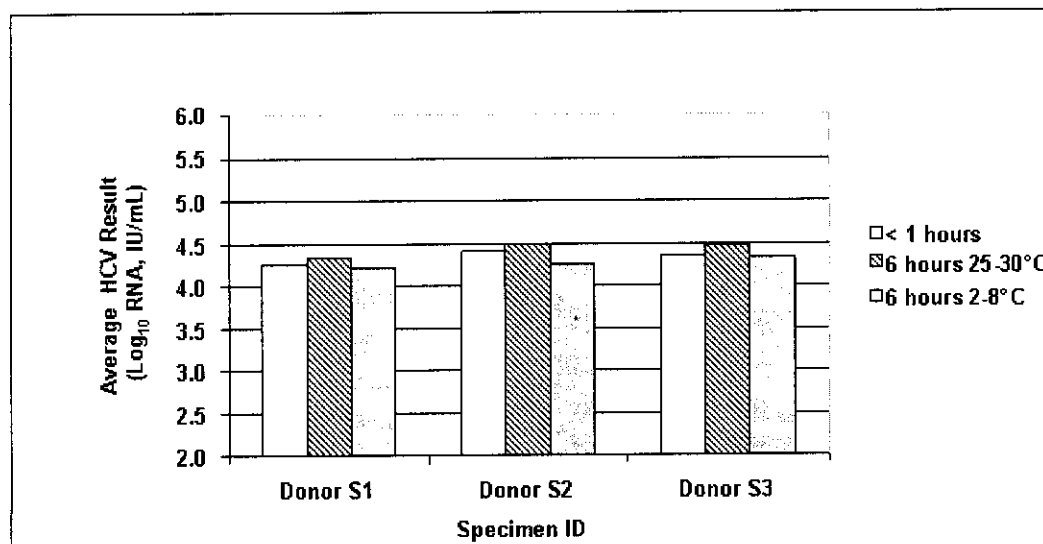
temperature. Transfer serum or plasma to a sterile polypropylene tube. Figure 4 and Figure 5 show specimen stability data from specimen collection studies. Studies were performed using the COBAS AmpliPrep/COBAS TaqMan HCV Test. The largest observed difference between the EDTA plasma conditions was not more than $\pm 0.22 \log_{10}$ and the largest observed difference between the serum conditions was not more than $\pm 0.14 \log_{10}$.

Figure 4: HCV Stability in Whole Blood with EDTA Anticoagulant



Note: There were four replicates for each time point.

Figure 5: HCV Stability in Whole Blood without Anticoagulant



Note: There were four replicates for each time point.

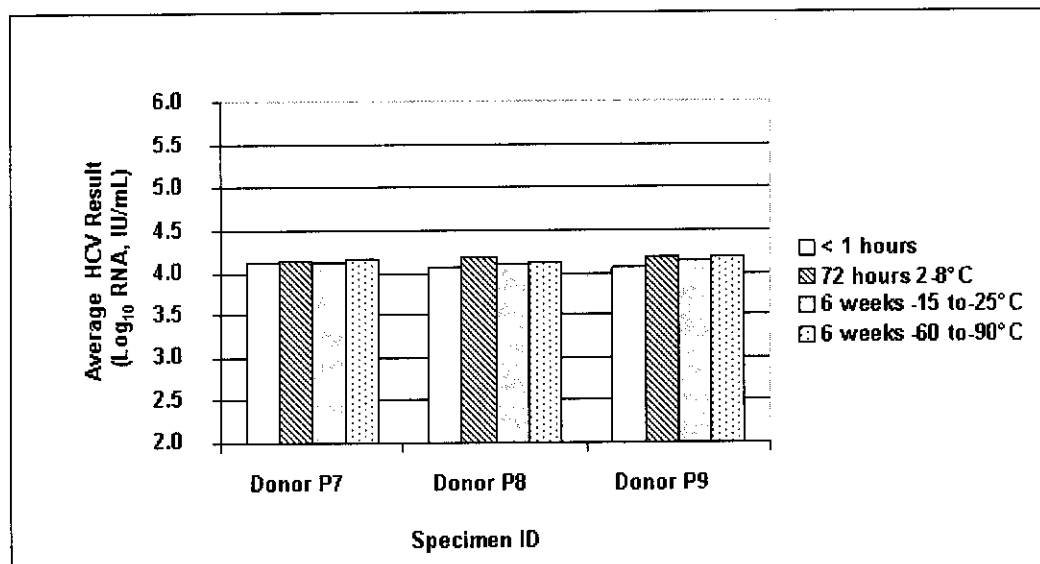
B. Specimen Transport

Transportation of whole blood, serum or plasma must comply with country, federal, state, and local regulations for the transport of etiologic agents.²⁸ Whole blood must be transported at 2-25°C and centrifuged within 6 hours of collection. Plasma or serum may be transported at 2-8°C or frozen at -70°C or colder, within the defined specimen storage period.

C. Specimen Storage

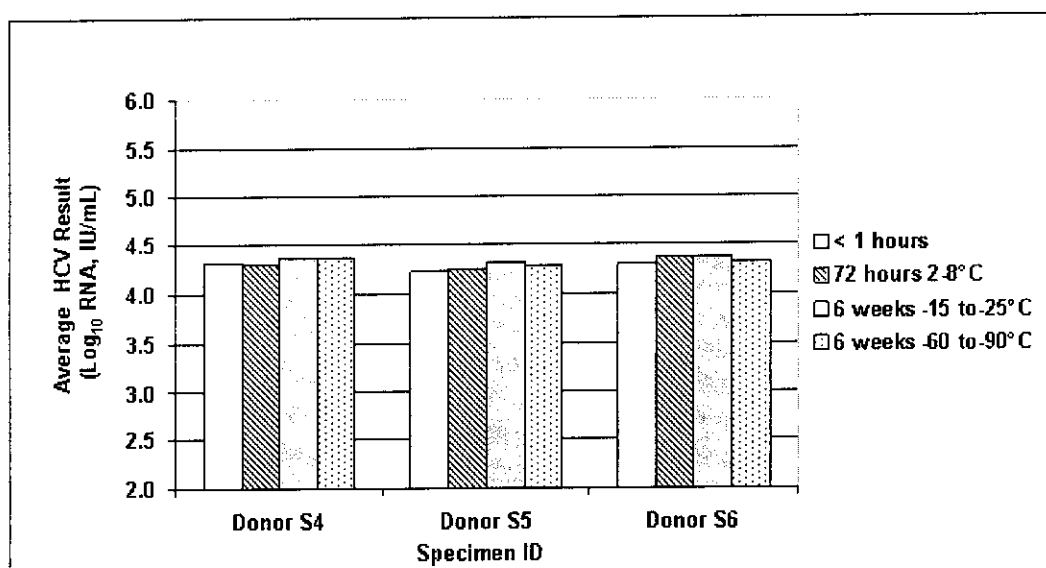
Serum or plasma specimens may be stored at 2-8°C for up to 3 days or frozen at -70°C or colder for up to 6 weeks. The largest observed difference between the EDTA plasma conditions was not more than $\pm 0.13 \log_{10}$ and the largest observed difference between the serum conditions was not more than $\pm 0.06 \log_{10}$ across the tested conditions. It is recommended that specimens be stored in 1100-1200 μL aliquots in sterile, 2.0 mL polypropylene screw-cap tubes (such as Sarstedt 72.694.006). Figure 6 and Figure 7 show specimen stability data from specimen storage studies. Studies were performed using the COBAS AmpliPrep/COBAS TaqMan HCV Test.

Figure 6: HCV Stability in EDTA Plasma



Note: There were four replicates for each time point.

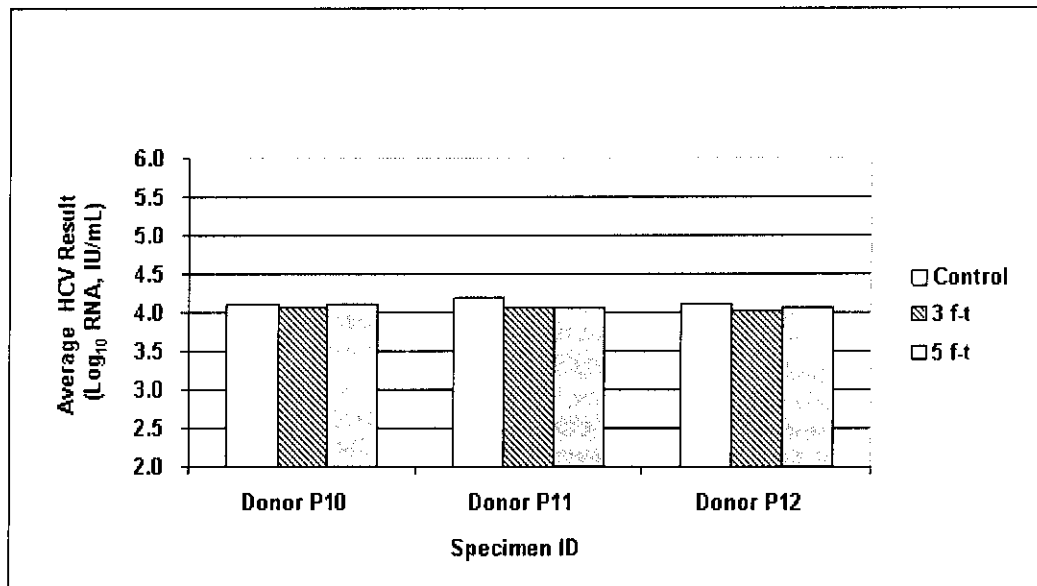
Figure 7: HCV Stability in Serum



Note: There were four replicates for each time point.

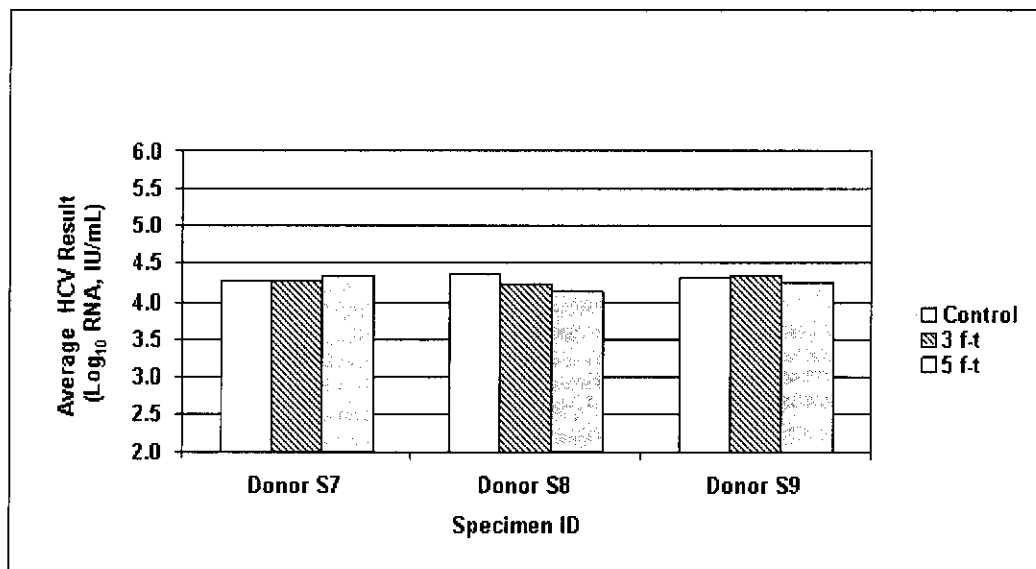
Serum and plasma specimens may be frozen and thawed up to five times without a loss of HCV RNA. The largest observed difference between the EDTA plasma conditions was not more than $\pm 0.11 \log_{10}$ and the largest observed difference between the serum conditions was not more than $\pm 0.22 \log_{10}$. Figure 8 and Figure 9 show the data from freeze-thaw studies performed using the COBAS AmpliPrep/COBAS TaqMan HCV Test.

Figure 8: HCV EDTA Plasma Freeze/Thaw Stability



Note: There were four replicates for each time point.

Figure 9: HCV Serum Freeze/Thaw Stability



Note: There were four replicates for each time point.

INSTRUCTIONS FOR USE

Note: For detailed operating instructions, a detailed descriptions of possible configurations, ordering, reviewing and printing results and interpreting flags, comments and error messages, refer to following manuals:

- (1) COBAS AmpliPrep Instrument Manual for use with the COBAS TaqMan Analyzer or COBAS TaqMan 48 Analyzer, COBAS AMPLICOR Analyzer and the AMPLILINK Software, Version 3.2 Series Application Manual*
- (2) COBAS TaqMan Analyzer (plus optional Docking Station) Instrument Manual for use with AMPLILINK Software, Version 3.2 Series Application Manual*
- (3) COBAS TaqMan 48 Analyzer Instrument Manual For use with AMPLILINK Software, Version 3.2 Series*
- (4) AMPLILINK Software Version 3.2 Series Application Manual For the use with the COBAS AmpliPrep Instrument, COBAS TaqMan Analyzer, COBAS TaqMan 48 Analyzer, and COBAS AMPLICOR Analyzer*

Batch Size

Each kit contains reagents sufficient for 48 tests, which may be performed in batches of 12 to 24 tests. At least one replicate each of CTM (–) C, HCV L(+)C and HCV H(+)C must be included in each batch (see “Quality Control” section).

Workflow

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 2-8°C.

Specimen and Control Preparation

Note: If using frozen specimens, place the specimens at room temperature until completely thawed and vortex for 3-5 seconds before use. Controls should be removed from 2-8°C storage and equilibrated to ambient temperature 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 Data Station for the AMPLILINK software **ON**. Prepare the Data Station as follows:
 - a. Log onto Windows XP.
 - b. Double click the AMPLILINK software icon.
 - c. 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 2-8°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 CS1 onto a reagent rack. Place HCV CS2, HCV CS3, and HCV CS4 onto a separate reagent rack.

- B2. Load the reagent rack containing HCV CS1 onto rack position A of the COBAS AmpliPrep Instrument.
- B3. Load the reagent rack containing HCV CS2, HCV CS3, and HCV CS4 onto rack position B, C, D, or E of the COBAS AmpliPrep Instrument (*see* Table 1 for additional 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. For reference, *see* Table 1 below. 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 (*see* Table 1 for additional information).

- 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. For configurations 3 to 5 using the COBAS TaqMan 48 Analyzer, load K-carriers on K-carrier rack(s) onto rack position M, N, O, or P of the COBAS AmpliPrep Instrument.

Table 1: Possible Configurations for using COBAS AmpliPrep Instrument with COBAS TaqMan Analyzer or COBAS TaqMan 48 Analyzer

	Configuration	Transfer Mode to COBAS TaqMan Analyzer or COBAS TaqMan 48 Analyzer	Racks, Carriers, and Disposables	Position on COBAS AmpliPrep Instrument
1	COBAS AmpliPrep Instrument plus Docking Station plus COBAS TaqMan Analyzer	Automated transfer of K-carrier	K-tubes in full K-tube racks	M – P
			K-tips in full K-tips racks	M – P
			Input S-tubes containing specimens and controls on sample racks	F – H
			SPUs in SPU rack	J – L
			CS1 on Cassette rack	A
			CS2, CS3, CS4 on Cassette rack	B – E
2	COBAS AmpliPrep Instrument plus COBAS TaqMan Analyzer	Manual transfer of K-tubes via sample rack(s) onto COBAS TaqMan Analyzer	K-tubes in full K-tube racks	M – P
			K-tips in full K-tips racks	M – P
			Input S-tubes on sample racks	F – H
			SPUs in SPU rack	J – L
			CS1 on Cassette rack	A
			CS2, CS3, CS4 on Cassette rack	B – E
3, 4	COBAS AmpliPrep Instrument plus 1 – 2 COBAS TaqMan 48 Analyzer(s)	Manual transfer of K-carrier via K-carrier rack(s) onto COBAS TaqMan 48 Analyzer	<u>After specimen processing is finished:</u> K-tubes on sample racks (ready for manual transfer)	Same as above (F – H)
			K-tubes in full K-tube racks	F – H
			K-tubes are placed on the sample rack	M – P
			Input S-tubes on sample racks	F – H
			SPUs in SPU rack	J – L
			CS1 on Cassette rack	A
			CS2, CS3, CS4 on Cassette rack	B – E
			Empty barcoded K-carrier on K-carrier rack	M – P
			<u>After specimen processing is finished:</u> K-tubes in K-carrier on K-carrier rack	Same as above (M – P)

5	Configuration	Transfer Mode to COBAS TaqMan Analyzer or COBAS TaqMan 48 Analyzer	Racks, Carriers, and Disposables	Position on COBAS AmpliPrep Instrument
	COBAS AmpliPrep Instrument plus COBAS TaqMan Analyzer plus COBAS TaqMan 48 Analyzer	Manual transfer of K-tubes on sample rack to COBAS TaqMan Analyzer. Manual transfer of K-carrier via K-carrier rack to COBAS TaqMan 48 Analyzer	Same as configurations 2 and 3	Same as configurations 2 and 3

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 and HCV H(+)C] 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 and HCV H(+)C] for 3 to 5 seconds. Avoid contaminating gloves when manipulating the specimens and controls.
- D6. Transfer 1000 to 1050 µL of each specimen and control [CTM (–) C, HCV L(+)C and HCV H(+)C] 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 in 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 right control to the position with the appropriate control barcode clip. ***Avoid contaminating the upper part of the S-tubes with specimens or controls.***

- D7. For configurations 1 and 2, load the sample rack(s) filled with Input S-tubes onto rack positions F, G, or H of the COBAS AmpliPrep Instrument.
- D8. For configurations 3 to 5 using the COBAS TaqMan 48 Analyzer, 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 Configurations 2–5)

- F1. Check for flags or error messages in the system screen.
- F2. Remove processed specimens and controls from the COBAS AmpliPrep Instrument on either sample racks (for COBAS TaqMan Analyzer without Docking Station) or K-carrier racks (for COBAS TaqMan 48 Analyzer), depending on the configuration (for further details see Part G).
- F3. Remove waste from the COBAS AmpliPrep Instrument.

Note: All 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 2-8°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:

Configuration 1: Automated transfer of K-carrier via docking station to COBAS TaqMan Analyzer. Manual intervention is unnecessary.

Configuration 2 and 5: Manual transfer of K-tubes in sample rack(s) to COBAS TaqMan Analyzer

Configuration 3, 4, and 5: Manual transfer of K-carrier on K-carrier rack(s) to the COBAS TaqMan 48 Analyzer. Manual transfer of K-carriers into COBAS TaqMan 48 Analyzer using the K-carrier Transporter.

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

- H1. Start the COBAS TaqMan Analyzer or COBAS TaqMan 48 Analyzer by one of the options below depending on the configuration used:

Configuration 1: No intervention necessary.

Configuration 2 and 5: Automatic start of the COBAS TaqMan Analyzer after insertion of sample rack(s).

Configuration 3, 4, and 5: Fill K-carrier with empty K-tubes if fewer than 6 K-tubes on the carrier. Filling is guided by the AMPLILINK software. Open thermal cycler cover, load K-carrier into thermal cycler and close lid. Start the COBAS TaqMan 48 Analyzer run.

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 Results Report. Check for flags or error messages in the Result section. Specimens with flags and comments are interpreted as described in the Results section. After acceptance, store data in archive.
- I2. Remove used K-tubes from the COBAS TaqMan Analyzer or COBAS TaqMan 48 Analyzer.

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 (IU)/mL.**

AMPLILINK Software

- Determines the Cycle Threshold value (Ct) 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 and **HCV H(+)**C fall within the lot specific assigned ranges encoded on the COBAS AmpliPrep/COBAS TaqMan HCV Test reagent cassette barcodes supplied with the kit.

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**, HCV H(+)**C** and CTM (–) **C**]. The following results are obtained for a valid batch.

Control	Result	Interpretation
Negative Control	Target Not Detected	Control Within Range
Low Postive Control	A numeric titer X.XXE+XX IU/mL	Control Within Range
High Positive Control	A numeric titer X.XXE+XX IU/mL	Control Within Range

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

Negative Control

Flag	Result	Interpretation
_N_NC_INVALID	Invalid	An invalid result or a "valid" result that was not negative for HCV target

HCV Low Positive Control

Flag	Result	Interpretation
_L_LPCINVALID	<4.3E+01 IU/mL	An invalid result where the Control was below the assay range.
_L_LPCINVALID	Target Not Detected	Control below range
_L_LPCINVALID	A numeric titer X.XXE+XX IU/mL	An invalid result where the Control was out of the assigned control range
_L_LPCINVALID	> 6.90E+07 IU/mL	An invalid result where the Control was above the assay range
_L_LPCINVALID	Invalid	An invalid result

HCV High Positive Control

Flag	Result	Interpretation
_H_HPCINVALID	<4.3E+01 IU/mL	An invalid result where the Control was below the assay range.
_H_HPCINVALID	Target Not Detected	Control below range
_H_HPCINVALID	A numeric titer X.XXE+XX IU/mL	An invalid result where the Control was out of the assigned control range
_H_HPCINVALID	> 6.90E+07 IU/mL	An invalid result where the Control was above the assay range
_H_HPCINVALID	Invalid	An invalid result

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:

Titer Result	Interpretation
Target Not Detected	No Ct value for HCV obtained. Report results as "HCV RNA not detected".
<4.3E+ 01 IU/mL	Below 4.3E+01 IU/mL (lower limit of quantitation, LLoQ); HCV RNA is not quantifiable.
≥ 4.30E+01 IU / mL and ≤ 6.90E+07 IU / mL	Results greater than or equal to 43 IU/mL and less than or equal to 6.90E+07 IU/mL are within the Linear Range of the assay.
> 6.90E+07 IU / mL	Results are above the range of the assay. Report results as "greater than 6.90E+07 HCV RNA IU/mL". If quantitative results are desired, the original specimen should be diluted 1:100 with HCV-negative human serum or EDTA plasma, depending on the matrix of the original specimen, and the test repeated. Multiply the reported result by the dilution factor.

Note: Specimens above the range of the assay may also produce an invalid result with a flag "QS_INVALID." If quantitative results are desired, the original specimen should be diluted 1:100 with HCV-negative human serum or EDTA plasma, depending on the matrix of the original specimen, and the test repeated. Multiply the reported result by the dilution factor.

QUALITY CONTROL

One replicate each of the COBAS TaqMan Negative Control, the HCV Low Positive Control and the 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, HCV H(+)C and CTM (-) C].

Based on results of a carry-over contamination study with alternating high positive HCV samples and HCV negative samples, 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 and HCV H(+)C is specific for each lot of reagents, and is provided on the COBAS AmpliPrep/COBAS TaqMan HCV Test reagent cassette barcodes.

The HCV RNA IU/mL for HCV L(+)C and HCV H(+)C 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.

PROCEDURAL PRECAUTIONS

As with any test procedure, good laboratory technique is essential to the proper performance of this assay.

PROCEDURAL LIMITATIONS

1. This test has been validated for use with only human serum or plasma collected in EDTA anticoagulant. Testing of other specimen types may result in inaccurate results.
2. 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.
3. Though rare, mutations within the highly conserved regions of the viral genome covered by the COBAS AmpliPrep/COBAS TaqMan HCV Test primers and/or probe may result in the under-quantitation of or failure to detect the presence of the virus in this circumstance.
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 in the techniques of PCR.
7. This product can only be used with the COBAS AmpliPrep Instrument and the COBAS TaqMan Analyzer or COBAS TaqMan 48 Analyzer.
8. If another assay was initially used for quantitation of HCV viral RNA in order to assess treatment effect on the patient, it is recommended that prior to switching to the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test users perform method correlation studies in their laboratory to quantify assay differences.

INTERFERING SUBSTANCES

Elevated levels of triglycerides, bilirubin, albumin, hemoglobin and human DNA 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.

The following drug compounds tested at the Peak Plasma Level (C_{max}) and at 3 times the C_{max} have been shown not to interfere with the quantitation of HCV RNA by the COBAS AmpliPrep/COBAS TaqMan HCV Test:

Nucleotide HIV / HBV DNA Polymerase Inhibitors Adefovir dipivoxil Tenofovir	Nucleoside HIV Reverse Transcriptase Inhibitors and DNA Polymerase Inhibitors Lamivudine Zidovudine Stavudine Abacavir Didanosine
HIV Protease Inhibitors Indinavir Saquinavir Ritonavir Nelfinavir Amprenavir Lopinavir/Ritonavir	Non-nucleoside HIV Reverse Transcriptase Inhibitors Nevirapine Efavirenz HIV Fusion Inhibitors Enfurvitide
Immune Modulators Interferon alfa-2a Interferon alfa-2b Peginterferon alfa-2a Peginterferon alfa-2a + Ribavirin Interferon alfa-2b+ Ribavirin	Antidepressants Paroxetine HCl Fluoxetine Sertraline Compounds for Treatment of Herpes Viruses Ganciclovir Valganciclovir Acyclovir

ANALYTICAL PERFORMANCE EVALUATION

The analytical performance characteristics of the COBAS AmpliPrep/COBAS TaqMan HCV Test were determined in a series of studies described below. The limit of detection of the COBAS AmpliPrep/COBAS TaqMan HCV Test was determined by analysis of serial dilutions of the First WHO International Standard for Hepatitis C Virus RNA for Nucleic Acid Amplification Technology Assays (NIBSC code 96/790)18, genotype 1a, obtained from NIBSC, in HCV negative human EDTA plasma or serum as well as with clinical specimens with genotypes 1 through 6.

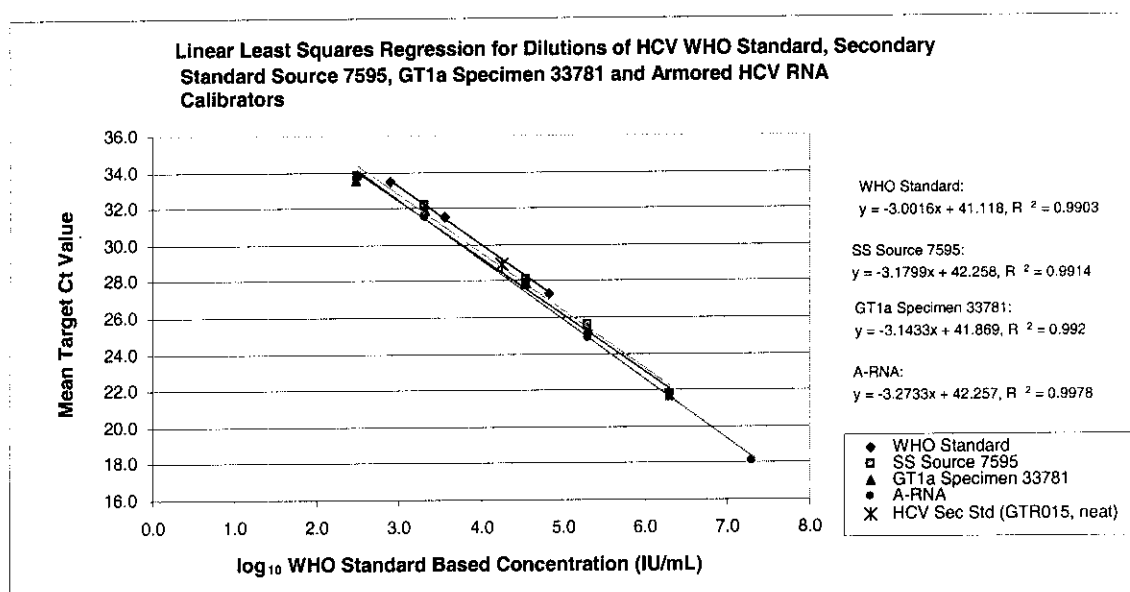
The linearity and precision of the COBAS AmpliPrep/COBAS TaqMan HCV Test were determined by analysis of serial dilutions of clinical HCV specimen in HCV-negative human EDTA-plasma and HCV-negative human serum and serial dilutions of an Armored HCV RNA to validate the high end of the dynamic range. The clinical specimen concentration (stock concentration) and the Armored HCV RNA concentration are traceable to the First WHO International Standard for Hepatitis C Virus RNA for Nucleic Acid Amplification Technology Assays (NIBSC code 96/790).

A. Traceability to the WHO Standard

Several standards and controls have been used during development of this test to provide traceability to the WHO Standard. This includes HCV WHO Standard, RMS HCV Secondary Standard, and RMS Armored HCV RNA calibrators. The HCV WHO Standard, RMS HCV Secondary Standard, RMS HCV Secondary Standard Source Material, RMS Armored HCV RNA Calibration Material and an independent HCV genotype 1a specimen were tested at similar levels. Calibrator ranged from $3.0\text{E}+02$ IU/mL to $2.0\text{E}+07$ IU/mL (2.48 to 7.30 \log_{10}), the HCV WHO Standard ranged from $3.0\text{E}+02$ IU/mL to $3.6\text{E}+04$ IU/mL (2.48 to 4.56) \log_{10} , the HCV Secondary Standard Source Material ranged from $3.0\text{E}+02$ IU/mL to $2.0\text{E}+06$ IU/mL (2.48 to 6.30 \log_{10}), and an HCV genotype 1a specimen ranged from $3.0\text{E}+02$ IU/mL to $2.0\text{E}+06$ IU/mL (2.48 to 6.30 on \log_{10}).

All materials behaved similarly and demonstrated co-linear dilution performance across the linear range.

Figure 10: Traceability of the COBAS AmpliPrep/COBAS TaqMan HCV Test to the HCV WHO Standard



B. Limit of Detection Using the WHO International Standard

The limit of detection of the COBAS AmpliPrep/COBAS TaqMan HCV Test was determined by analysis of serial dilutions of the First WHO International Standard for Hepatitis C Virus RNA for Nucleic Acid Amplification Technology Assays (NIBSC code 96/790), genotype 1a, obtained from NIBSC, in HCV negative human EDTA plasma or serum. First WHO International Standard was freshly diluted into negative human EDTA plasma or serum on three days for each matrix. Each level of each dilution was tested with ten replicates each in three runs for each of two reagent lots for each matrix. A total of three runs were conducted over three days for each reagent lot for each matrix to give a total of 60 replicates for each level for each matrix. These studies demonstrate that the COBAS AmpliPrep/COBAS TaqMan HCV Test can detect HCV RNA in EDTA plasma and serum at concentrations as low as 18 IU/mL with a positivity rate greater than 95%. The concentration of HCV RNA using the First WHO International

Standard in EDTA plasma and serum that can be detected with a positivity rate of greater than 95% as determined by Probit Analysis, is 13.9 IU/mL and 10.5 IU/mL, respectively (*see* Table 2 and Table 3 below). The difference between serum and EDTA plasma was not statistically significant.

Table 2: Limit of Detection in EDTA Plasma of the COBAS AmpliPrep/COBAS TaqMan HCV Test using the First WHO International Standard (Genotype 1a)

WHO Standard Based Concentration (HCV RNA IU/mL)	No. Valid Replicates	No. Positives	Positivity Rate
0.0	56	0	0%
2.5	57	30	53%
5.0	58	41	71%
7.5	59	45	76%
10.0	60	53	88%
15.0	58	58	100%
25.0	56	56	100%
50.0	57	57	100%
Probit 95% Hit Rate	13.9 IU/mL [95% confidence limits of 11.0 – 19.8 IU/mL]		

Table 3: Limit of Detection in Serum of the COBAS AmpliPrep/COBAS TaqMan HCV Test using the First WHO International Standard (Genotype 1a)

WHO Standard Based Concentration (HCV RNA IU/mL)	No. Valid Replicates	No. Positives	Positivity Rate
0.0	59	0	0%
2.5	60	37	62%
5.0	59	43	73%
7.5	60	51	85%
10.0	59	57	97%
15.0	60	60	100%
25.0	58	58	100%
50.0	60	60	100%
Probit 95% Hit Rate	10.5 IU/mL [95% confidence limits of 8.4 – 14.8 IU/mL]		

C. Limit of Detection Using Clinical Specimens across HCV Genotypes

The Limit of Detection for HCV genotypes 1 to 6 was determined by obtaining eight clinical specimens representing genotypes 1, 2, 3, 4, 5, and 6. Original titers for these clinical samples were provided by the vendor and 8 member dilution panels in negative human EDTA plasma were prepared from each sample representing a genotype. The panel members were tested in two runs with 12 replicates per run for a total of 24 replicates for each member. Limit of detection for a genotype is defined as the mean concentration of the lowest panel member at which more than 95% of the results are positive. The results are presented in Table 4 below. The overall LoD for this assay is defined as 18 IU/mL.

Table 4: Limit of Detection for HCV Genotypes

Genotype	Mean Conc. of the Panel Member with >95% Positivity Rate (IU/mL)	Number of Replicates Tested	Number of Positive Results	Positivity Rate
1	7.1	24	23	96%
2	15.3	24	24	100%
3	9.8	24	24	100%
4	5.6	24	23	96%
5	18.3	24	24	100%
6	9.7	24	23	96%

D. Linear Range

The linear range was evaluated in accordance with the methods defined in the CLSI (formerly NCCLS) Guideline EP6A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline.²⁹ Two linearity panels were used to evaluate the linear range of the COBAS AmpliPrep/COBAS TaqMan HCV Test. These panels consisted of dilutions in either EDTA plasma or in serum of a high titer HCV RNA positive clinical specimen for the lower and middle part of the dynamic range and, due to unavailability of very high titer clinical material, of Armored HCV RNA for the high end of the dynamic range. The study was performed for two lots of COBAS AmpliPrep/COBAS TaqMan HCV Test reagents. All 15 panel

members for EDTA plasma and all 14 panel members for serum were tested in 104 to 111 replicates per concentration level.

The COBAS AmpliPrep/COBAS TaqMan HCV Test was found to give a linear response from 43 HCV RNA IU/mL to at least $6.90\text{E}+07$ HCV RNA IU/mL with maximum observed deviation of $0.2 \log_{10}$ from linearity.

Figure 11 and Figure 12 are representative plots from one of the two lots tested.

The analytical measurement range of analyte values that can be directly measured on a sample with out any dilution using the COBAS AmpliPrep/COBAS TaqMan HCV Test is 43 to $6.9\text{E}+07$ IU/mL.

The clinical reportable range of analyte values that can be directly measured on a sample with a maximum dilution of one to one-hundred using the COBAS AmpliPrep/COBAS TaqMan HCV Test is 43 to $6.9\text{E}+09$ IU/mL.

Figure 11: Linear Range Determination for the COBAS AmpliPrep/COBAS TaqMan HCV Test in EDTA Plasma Specimens

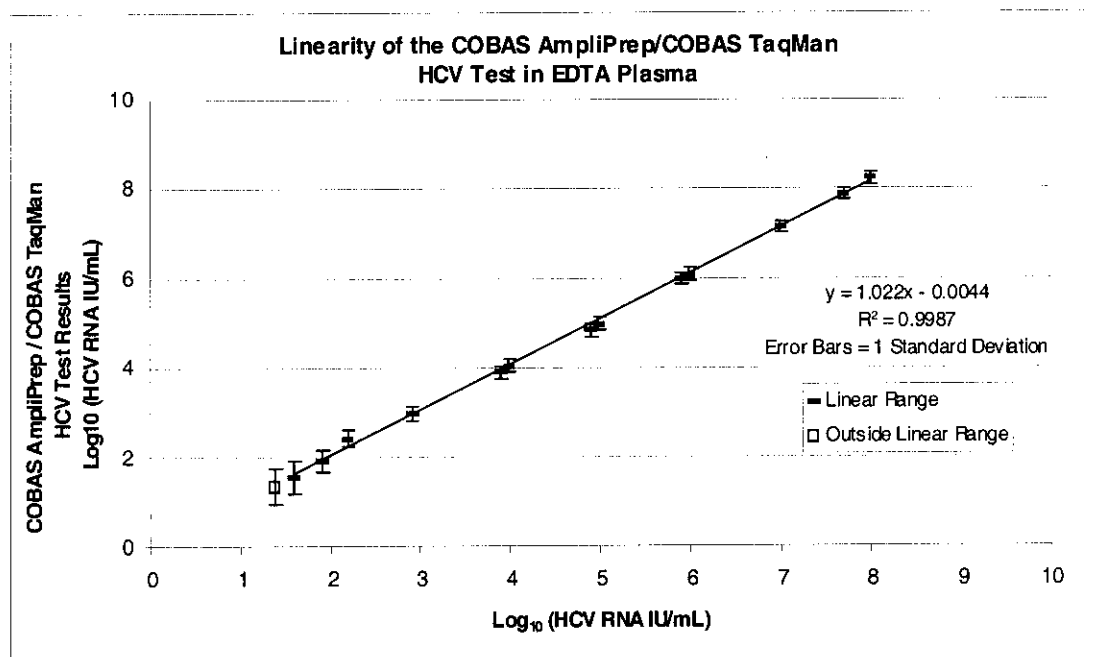
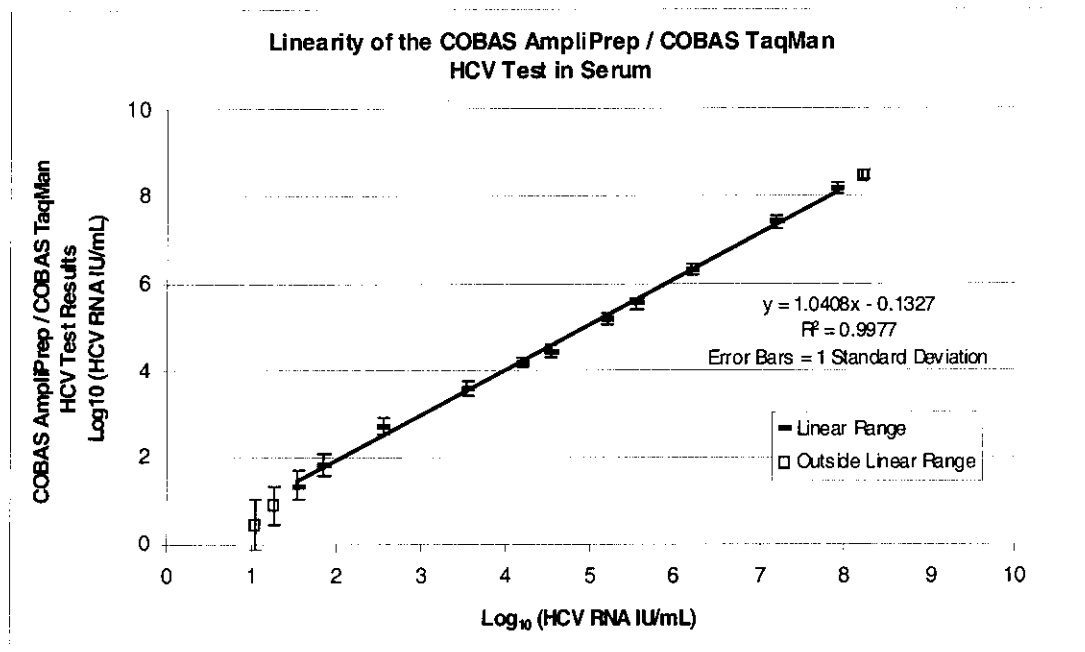


Figure 12: Linear Range Determination for the COBAS AmpliPrep/COBAS TaqMan HCV Test in Serum Specimens



E. Precision – Within Laboratory

Within-Run, Run-to-Run and Total Precision were evaluated in accordance with the methods defined in the NCCLS Guideline EP5-A2, Evaluation of Precision Performance of Clinical Chemistry Devices.³⁰ Precision of the COBAS® AmpliPrep/COBAS TaqMan® HCV Test was determined by analysis of serial dilutions of clinical HCV specimens (Genotype 1) or of Armored HCV RNA in HCV negative human EDTA plasma or in serum.

Six dilution levels of 7 replicates per level were tested in ≥15 runs over ≥15 days. Each sample was carried through the entire COBAS® AmpliPrep/COBAS TaqMan® HCV Test procedure, including specimen preparation, amplification and detection. Therefore, the precision reported here represents all aspects of the test procedure. The study was performed for three lots of COBAS® AmpliPrep/COBAS TaqMan® HCV Test reagents, and the results are shown in Table 5 through Table 9.

**Table 5: Precision of the COBAS AmpliPrep/COBAS TaqMan HCV Test
Using Plasma (in IU/mL)**

Lot	Sample Type	Native HCV RNA			Armored HCV RNA		
1	Titer (IU/mL)	1.42E+02	1.42E+03	1.42E+04	1.48E+05	1.48E+06	1.48E+07
	Within Run CV (%)*	43	28	26	19	19	29
	Run To Run CV (%)*	18	8	7	9	11	11
	Total CV (%)*	47	29	27	21	22	31
	No. Replicates	109	110	110	109	109	109
2	Titer (IU/mL)	1.54E+02	7.72E+03	7.72E+04	9.60E+04	9.60E+05	9.60E+06
	Within Run CV (%)*	43	29	28	35	32	26
	Run To Run CV (%)*	23	9	12	0	6	6
	Total CV (%)*	50	31	30	35	32	27
	No. Replicates	98	98	97	97	97	98
3	Titer (IU/mL)	1.42E+02	1.42E+03	1.42E+04	1.29E+05	1.29E+06	1.29E+07
	Within Run CV (%)*	27	13	13	16	18	15
	Run To Run CV (%)*	7	9	4	4	4	5
	Total CV (%)*	28	16	14	17	18	16
	No. Replicates	98	98	98	98	98	98

* $\%CV = 100 \times \sqrt{10^{\sigma^2 \ln(10)} - 1}$

**Table 6: Precision of the COBAS AmpliPrep/COBAS TaqMan HCV Test
Using Serum (in IU/mL)**

Lot	Sample Type	Native HCV RNA			Armored HCV RNA		
1	Titer (IU/mL)	4.80E+02	4.80E+03	4.80E+04	6.90E+05	6.90E+06	6.90E+07
	Within Run CV (%)*	23	17	16	17	21	26
	Run To Run CV (%)*	29	12	11	26	28	23
	Total CV (%)*	38	21	20	32	35	34
	No. Replicates	104	104	104	104	104	104
2	Titer (IU/mL)	3.50E+02	3.50E+03	3.50E+04	1.56E+05	1.56E+06	1.56E+07
	Within Run CV (%)*	25	16	22	16	15	14
	Run To Run CV (%)*	10	4	10	12	18	13
	Total CV (%)*	27	17	24	20	23	20
	No. Replicates	103	103	102	98	97	98
3	Titer (IU/mL)	1.96E+02	1.96E+03	1.96E+04	1.69E+05	1.69E+06	1.69E+07
	Within Run CV (%)*	31	14	15	16	14	18
	Run To Run CV (%)*	6	3	6	0	8	8
	Total CV (%)*	32	14	16	16	16	19
	No. Replicates	90	91	90	90	90	90

* $\%CV = 100 \times \sqrt{10^{\sigma^2 \ln(10)} - 1}$

**Table 7: Precision of the COBAS AmpliPrep/COBAS TaqMan HCV Test
Using Plasma (in log₁₀ IU/mL)**

Lot	Sample Type	Native HCV RNA			Armored HCV RNA		
1	Titer (log ₁₀ IU/mL)	2.15	3.15	4.15	5.17	6.17	7.17
	Within Run Standard Deviation	0.178	0.118	0.112	0.083	0.082	0.122
	Run To Run Standard Deviation	0.076	0.036	0.03	0.038	0.046	0.047
	Total Standard Deviation (log 10)	0.194	0.123	0.116	0.091	0.094	0.131
	No. Replicates	109	110	110	109	109	109
2	Titer (log ₁₀ IU/mL)	2.19	3.89	4.89	4.98	5.98	6.98
	Within Run Standard Deviation	0.18	0.124	0.119	0.148	0.135	0.11
	Run To Run Standard Deviation	0.096	0.038	0.05	0	0.024	0.027
	Total Standard Deviation (log 10)	0.204	0.129	0.129	0.148	0.137	0.113
	No. Replicates	98	98	97	97	97	98
3	Titer (log ₁₀ IU/mL)	2.15	3.15	4.15	5.11	6.11	7.11
	Within Run Standard Deviation	0.116	0.057	0.057	0.069	0.076	0.063
	Run To Run Standard Deviation	0.031	0.039	0.019	0.017	0.016	0.023
	Total Standard Deviation (log 10)	0.12	0.07	0.06	0.071	0.077	0.068
	No. Replicates	98	98	98	98	98	98

**Table 8: Precision of the COBAS AmpliPrep/COBAS TaqMan HCV Test
Using Serum (in IU/mL)**

Lot	Sample Type	Native HCV RNA			Armored HCV RNA		
1	Titer (IU/mL)	4.80E+02	4.80E+03	4.80E+04	6.90E+05	6.90E+06	6.90E+07
	Within Run CV (%)*	23	17	16	17	21	26
	Run To Run CV (%)*	29	12	11	26	28	23
	Total CV (%)*	38	21	20	32	35	34
	No. Replicates	104	104	104	104	104	104
2	Titer (IU/mL)	3.50E+02	3.50E+03	3.50E+04	1.56E+05	1.56E+06	1.56E+07
	Within Run CV (%)*	25	16	22	16	15	14
	Run To Run CV (%)*	10	4	10	12	18	13
	Total CV (%)*	27	17	24	20	23	20
	No. Replicates	103	103	102	98	97	98
3	Titer (IU/mL)	1.96E+02	1.96E+03	1.96E+04	1.69E+05	1.69E+06	1.69E+07
	Within Run CV (%)*	31	14	15	16	14	18
	Run To Run CV (%)*	6	3	6	0	8	8
	Total CV (%)*	32	14	16	16	16	19
	No. Replicates	90	91	90	90	90	90

* $\%CV = 100 \times \sqrt{10^{\sigma^2 \ln(10)} - 1}$

**Table 9: Precision of the COBAS AmpliPrep/COBAS TaqMan HCV Test
Using Serum (in log₁₀ IU/mL)**

Lot	Sample Type	Native HCV RNA			Armored HCV RNA		
1	Titer (log ₁₀ IU/mL)	2.68	3.68	4.68	5.84	6.84	7.84
	Within Run Standard Deviation	0.122	0.052	0.049	0.112	0.119	0.114
	Run To Run Standard Deviation	0.1	0.072	0.068	0.075	0.088	0.106
	Total Standard Deviation (log 10)	0.158	0.089	0.084	0.134	0.148	0.156
	No. Replicates	104	104	104	104	104	105
2	Titer (log ₁₀ IU/mL)	2.54	3.54	4.54	5.19	6.19	7.19
	Within Run Standard Deviation	0.105	0.07	0.094	0.07	0.064	0.062
	Run To Run Standard Deviation	0.045	0.017	0.042	0.052	0.076	0.058
	Total Standard Deviation (log 10)	0.114	0.072	0.103	0.087	0.099	0.085
	No. Replicates	103	103	102	98	97	98
3	Titer (log ₁₀ IU/mL)	2.29	3.29	4.29	5.23	6.23	7.23
	Within Run Standard Deviation	0.131	0.06	0.064	0.069	0.06	0.077
	Run To Run Standard Deviation	0.024	0.012	0.024	0	0.034	0.033
	Total Standard Deviation (log 10)	0.133	0.061	0.068	0.069	0.068	0.084
	No. Replicates	90	91	90	90	90	90

F. Inclusivity

The performance of the COBAS AmpliPrep/COBAS TaqMan HCV Test on HCV genotypes was evaluated by (1) testing the HCV RNA Genotype Panel for Nucleic Acid Amplification Techniques, NIBSC Code 02/202 and by (2) comparing the \log_{10} titer of clinical specimens of HCV genotypes 1-5 to the \log_{10} titer obtained for the COBAS® AMPLICOR HCV MONITOR Test, v2.0 and the VERSANT® HCV RNA 3.0.

HCV RNA Genotype Panel for Nucleic Acid Amplification Techniques, NIBSC Code 02/202

The HCV RNA Genotype Panel for Nucleic Acid Amplification Techniques, NIBSC Code 02/202 comprises 6 members of HCV genotypes 1 through 6 with a titer of 1000 IU/mL assigned by NIBSC22. The panel was tested in single determination with one lot of COBAS AmpliPrep/COBAS TaqMan HCV Test reagents. The results are presented in Table 10.

**Table 10: HCV RNA Genotype Panel for Nucleic Acid Amplification Techniques,
NIBSC Code 02/202**

Panel Member	HCV Genotype	Titer (IU/mL)	Log ₁₀ Titer NIBSC	Log ₁₀ Titer CAP/CTM HCV	Difference in Titer (CAP/CTM – NIBSC)
NIBSC-1	1	1000	3.0	3.2	0.2
NIBSC-2	2	1000	3.0	3.3*	0.3
NIBSC-3	3	1000	3.0	3.2	0.2
NIBSC-4	4	1000	3.0	3.1	0.1
NIBSC-5	5	1000	3.0	2.9**	- 0.1
NIBSC-6	6	1000	3.0	3.3	0.3

* Mean value of two replicates

** Repeat measurement due to volume error

G. Performance of the COBAS AmpliPrep/COBAS TaqMan HCV Test with HCV Negative Specimens Specimens

The performance of the COBAS AmpliPrep/COBAS TaqMan HCV Test was determined by analysis of HCV RNA-negative EDTA or serum samples from blood donors (all samples were pre-screened by either the Abbott PRISM HCV Test or by the Abbott ARCHITECT Anti-HCV Reagent kit). A total of 808 individual EDTA plasma specimens and a total of 768 individual serum specimens were tested with two lots of COBAS AmpliPrep/COBAS TaqMan HCV Test reagents. All specimens tested negative for HCV RNA. In this panel the specificity of the COBAS AmpliPrep/COBAS TaqMan HCV Test is 100% (one-sided lower 95% confidence limit: $\geq 99.6\%$).

H. Cross Reactivity

The cross reactivity of the COBAS AmpliPrep/COBAS TaqMan HCV Test was evaluated by adding different pathogens (viruses, bacteria, yeast) or isolated cellular DNA (HTLV-II) into HCV negative human EDTA plasma or HCV positive human plasma (*see* Table 11). Stocks of non-HCV viruses as well as bacteria and yeast were diluted to a level of approximately $5\text{E}+04$ particles/mL, except for HTLV-II, which was available as HTLV-II infected cell DNA only. The HTLV-II infected cell DNA was used at approximately $5\text{E}+04$ copies/mL. None of the non-HCV pathogens showed a false positive result in the COBAS AmpliPrep/COBAS TaqMan HCV Test.

Table 11: Cross Reactivity Specimens

Viruses	Non-HCV Flavivirus
Adenovirus type 2	West Nile Virus
Cytomegalovirus	St. Louis Encephalitis Virus
Epstein-Barr virus	Murray Valley Encephalitis Virus
Human Herpes Virus type 6	Dengue Virus Type 1
Herpes simplex virus type 1	Dengue Virus Type 2
Herpes simplex virus type 2	Dengue Virus Type 3
Human T-Cell Lymphotropic virus type 1	Dengue Virus Type 4
Human T-cell Lymphotropic virus type 2	Yellow Fever Virus
Influenza A	Zika Virus
Hepatitis A virus	Banji Virus
Hepatitis B virus	Ilheus
Human Immunodeficiency Virus Type 1B	FSME Virus
	Heptatis G Virus (GBV-C)
Bacteria	Yeast
Staphylococcus aureus	Candida albicans
Propionibacterium acnes	

I. Matrix Equivalency — Serum versus EDTA Plasma

Twenty-nine matched clinical specimen sets (each set is EDTA plasma and serum drawn from a single HCV seropositive individual) with titers ranging from $1.58 \text{ E}+03 \text{ IU/mL}$ to $1.69\text{E}+07 \text{ IU/mL}$ ($3.2 \log_{10}\text{IU/mL}$ to $7.2 \log_{10}\text{IU/mL}$) were tested to demonstrate plasma-serum equivalency. Each specimen was tested in triplicate. The pooled standard deviation is calculated for each matrix (serum as well as plasma). Deming Regression is performed comparing each sample's average log titer for serum to the corresponding average log titer for plasma.

The \log_{10} titer difference (mean log titer serum – mean log titer EDTA plasma) for all 29 matched sets was ≤ 0.3 . The mean difference was $-0.024 \log$ (95% CI: -0.06 and 0.01), indicating that the results between serum and EDTA plasma were not significantly different. The result from the Deming regression analysis is shown in Figure 13. The slope is equal to 0.9855 (95% confidence interval [0.9619 to 1.0090]) with an intercept of $+0.0557$ (95% confidence interval is [-0.0741 to 0.1855]). The pooled standard deviation estimates for the EDTA plasma and serum samples are shown in Table 12.

Figure 13: Deming Regression Analysis of Matched Serum — EDTA Plasma Samples (n=29)

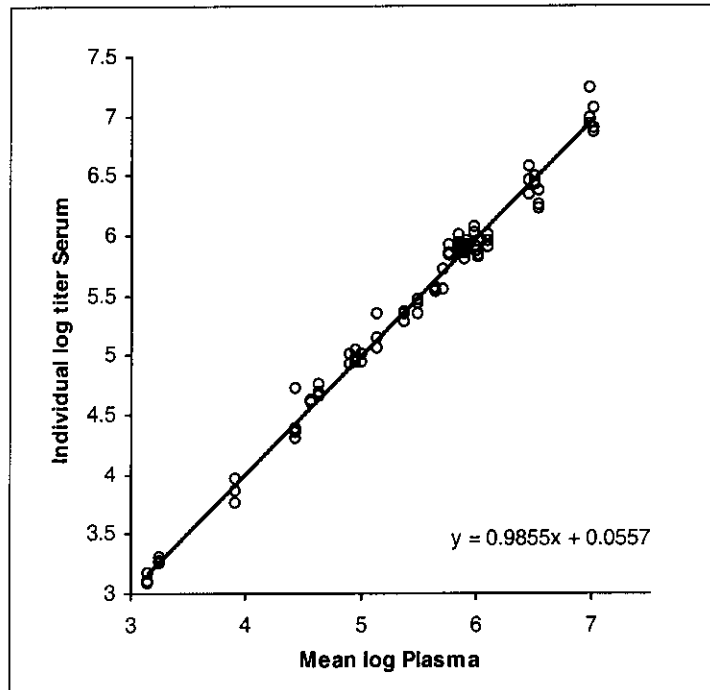


Table 12: Pooled Standard Deviation Estimates for EDTA Plasma and Serum Specimens in the Matrix Equivalency Study

Matrix	Mean log Titer	Pooled SD
EDTA Plasma	5.430	0.0771
Serum	5.406	0.0815

J. Platform Equivalency — COBAS TaqMan Analyzer and COBAS TaqMan 48 Analyzer

Comparison between the COBAS TaqMan Analyzer (CTM96) and COBAS TaqMan 48 Analyzer (CTM48) was assessed using 67 clinical specimens with titer levels ranging from $3.23\text{E}+02$ IU/mL to $1.70\text{E}+07$ IU/mL ($2.5 \log_{10}\text{IU/mL}$ to $7.2 \log_{10}\text{IU/mL}$). The performance was assessed using Deming regression analysis. The results of the analysis are presented in Figure 14 and Table 13. The 95% confidence intervals for slope and intercept include “1” and “0” respectively, indicating that the performance of the two analyzers is equivalent when testing clinical specimens.

Figure 14: Platform Equivalency – CTM96 Analyzer vs. CTM48 Analyzer

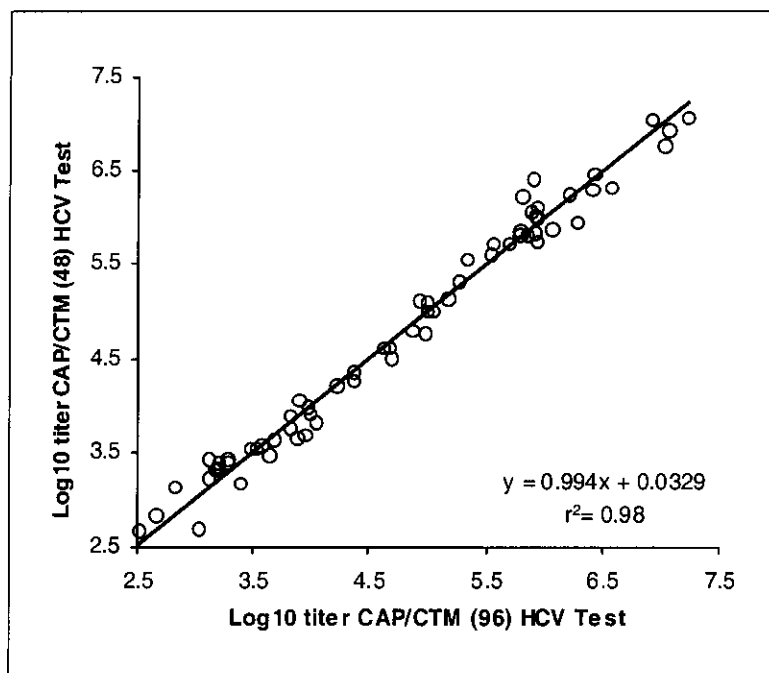


Table 13: Results of Deming Regression

	Coefficient	SE	95% CI	
Intercept	0.0329	0.0822	-0.1312	0.1970
Slope	0.9940	0.0167	0.9607	1.0274

CLINICAL PERFORMANCE STUDIES

A. Reproducibility

The reproducibility of the COBAS AmpliPrep/COBAS TaqMan HCV Test was evaluated for each genotype by 2 operators at each of three clinical sites. Each operator performed 3 days of testing on each of 3 lots of reagents with each genotype panel. 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 14 to Table 15 below.

**Table 14: Standard Deviation Components HCV RNA Concentration (log₁₀ IU/mL)
EDTA Plasma**

Geno- type	Geometric Mean of HCV RNA Concentration (IU/mL)	Mean of HCV RNA Concentration (log ₁₀ IU/mL)	No. of Tests ¹	Lot	Site/ Instru- ment	Operator	Day/Run	Within- Run	Total Standard Deviation of log ₁₀ HCV RNA Concentration
1	320	2.45a	163	0.068	0.085	0.000	0.052	0.176	0.214
	2,902	3.45	163	0.062	0.000	0.007	0.040	0.094	0.120
	21,795	4.33	163	0.027	0.014	0.002	0.006	0.075	0.081
	224,096	5.34	161	0.049	0.010	0.000	0.033	0.066	0.089
	2,384,233	6.37	163	0.026	0.024	0.015	0.022	0.069	0.082
	4,052,668	6.60	161	0.030	0.028	0.000	0.032	0.062	0.080
2*	292	2.40b	157	0.156	0.024	0.038	0.000	0.188	0.248
	2,713	3.42	154	0.080	0.036	0.000	0.000	0.075	0.115
	22,754	4.33	151	0.077	0.052	0.000	0.000	0.115	0.148
	110,171	5.03	156	0.068	0.069	0.000	0.031	0.056	0.116
	840,382	5.90	156	0.078	0.073	0.000	0.018	0.075	0.132
	9,696,516	6.97	157	0.082	0.058	0.000	0.033	0.074	0.129
3	355	2.51	159	0.130	0.000	0.000	0.057	0.121	0.186
	4,764	3.66	159	0.073	0.032	0.020	0.032	0.063	0.109
	32,876	4.49	158	0.069	0.079	0.000	0.036	0.088	0.141
	331,684	5.50	158	0.060	0.087	0.003	0.037	0.086	0.142
	1,318,936	6.09	161	0.112	0.094	0.000	0.043	0.066	0.166
	27,131,043	7.39	156	0.120	0.106	0.000	0.043	0.080	0.184
4	741	2.78	158	0.192	0.000	0.028	0.113	0.174	0.284
	1,847	3.21	159	0.179	0.000	0.000	0.123	0.077	0.230
	5,481	3.68	158	0.169	0.035	0.000	0.108	0.082	0.220
	13,775	4.08	153	0.163	0.044	0.009	0.132	0.080	0.230
	38,721	4.51	158	0.179	0.062	0.000	0.124	0.111	0.252
	106,098	4.92	156	0.204	0.075	0.040	0.178	0.122	0.308
5	254	2.35	154	0.167	0.037	0.000	0.049	0.109	0.209
	943	2.95	157	0.126	0.013	0.021	0.025	0.085	0.156
	3,164	3.48	158	0.096	0.030	0.000	0.029	0.066	0.123
	8,124	3.88	154	0.114	0.044	0.022	0.030	0.073	0.147
	24,379	4.36	157	0.099	0.078	0.000	0.033	0.074	0.149
	253,987	5.37	158	0.120	0.089	0.000	0.031	0.106	0.186

Geno- type	Geometric Mean of HCV RNA Concentration (IU/mL)	Mean of HCV RNA Concentration (log ₁₀ IU/mL)	No. of Tests ¹	Lot	Site/ Instru- ment	Operator	Day/Run	Within- Run	Total Standard Deviation of log ₁₀ HCV RNA Concentration
6	275	2.41 ^c	160	0.044	0.036	0.000	0.032	0.155	0.168
	3,575	3.55	165	0.038	0.000	0.000	0.023	0.061	0.075
	27,274	4.43	168	0.019	0.034	0.000	0.000	0.078	0.087
	279,527	5.43	163	0.000	0.040	0.000	0.024	0.089	0.101
	1,670,245	6.21	165	0.012	0.038	0.000	0.014	0.092	0.101
	3,605,810	6.55	163	0.017	0.041	0.000	0.018	0.082	0.095

Note: Within assay range results are from 18 IU/mL to 6.90E+7 IU/mL (1.26 log₁₀ IU/mL to 7.84 log₁₀ IU/mL), inclusive. The limit of detection (LOD) for the assay is 18 IU/mL. Results <1.80E+1 IU/mL have been imputed as half the limit of detection, 9.0E+0 IU/mL (0.95 log₁₀ IU/mL).

Note: Three extra panels (one Genotype 1 panel and two Genotype 6 panels) than the number of panels/genotype planned were additionally tested in the study. For genotype 2 the results from 3 mis-positioned aliquots are excluded

¹ *Number of tests with detectable viral load. In total, 165 tests/panel member were performed for Genotype 1, 162 tests/panel member were performed for Genotypes 2, 3, 4, and 5, and 168 tests/panel member were performed for Genotype 6. Invalid tests were not repeated.*

^a *Two <1.80E+1 IU/mL results were observed for this panel member.*

^b *Two <1.80E+1 IU/mL results were observed for this panel member.*

^c *One <1.80E+1 IU/mL result was observed for this panel member.*

**Table 15: Reproducibility Results Summary:
Total %CV for HCV Panel Members — EDTA Plasma**

Genotype	Geometric Mean of HCV RNA Concentration (IU/mL)	Mean of HCV RNA Concentration (log ₁₀ IU/mL)	No. of Tests ¹	Total Precision Variance of log ₁₀ HCV RNA Concentration	Total Precision Standard Deviation of log ₁₀ HCV RNA Concentration	lognormal CV (%) ²
1	320	2.45a	163	0.046	0.21	52
	2,902	3.45	163	0.014	0.12	28
	21,795	4.33	163	0.007	0.08	19
	224,096	5.34	161	0.008	0.09	21
	2,384,233	6.37	163	0.007	0.08	19
	4,052,668	6.60	161	0.006	0.08	19
2*	292	2.40b	157	0.061	0.25	62
	2,713	3.42	154	0.013	0.12	27
	22,754	4.33	151	0.022	0.15	35
	110,171	5.03	156	0.014	0.12	27
	840,382	5.90	156	0.017	0.13	31
	9,696,516	6.97	157	0.017	0.13	30
3	355	2.51	159	0.035	0.19	45
	4,764	3.66	159	0.012	0.11	25
	32,876	4.49	158	0.020	0.14	33
	331,684	5.50	158	0.020	0.14	33
	1,318,936	6.09	161	0.028	0.17	40
	27,131,043	7.39	156	0.034	0.18	44
4	741	2.78	158	0.081	0.28	73
	1,847	3.21	159	0.053	0.23	57
	5,481	3.68	158	0.048	0.22	54
	13,775	4.08	153	0.053	0.23	57
	38,721	4.51	158	0.063	0.25	63
	106,098	4.92	156	0.095	0.31	81
5	254	2.35	154	0.044	0.21	51
	943	2.95	157	0.024	0.16	37
	3,164	3.48	158	0.015	0.12	29
	8,124	3.88	154	0.022	0.15	35
	24,379	4.36	157	0.022	0.15	35
	253,987	5.37	158	0.034	0.19	45

Genotype	Geometric Mean of HCV RNA Concentration (IU/mL)	Mean of HCV RNA Concentration (log ₁₀ IU/mL)	No. of Tests ¹	Total Precision Variance of log ₁₀ HCV RNA Concentration	Total Precision Standard Deviation of log ₁₀ HCV RNA Concentration	lognormal CV (%) ²
6	275	2.41 ^c	160	0.028	0.17	40
	3,575	3.55	165	0.006	0.08	17
	27,274	4.43	168	0.008	0.09	20
	279,527	5.43	163	0.010	0.10	23
	1,670,245	6.21	165	0.010	0.10	24
	3,605,810	6.55	163	0.009	0.10	22

Note: Within assay range results are from 18 IU/mL to 6.90E+7 IU/mL (1.26 log₁₀ IU/mL to 7.84 log₁₀ IU/mL), inclusive. The limit of detection (LOD) for the assay is 18 IU/mL. Results <1.80E+1 IU/mL have been imputed as half the limit of detection, 9.0E+0 IU/mL (0.95 log₁₀ IU/mL).

Note: Three extra panels (one Genotype 1 panel and two Genotype 6 panels) than the number of panels/genotype planned were additionally tested in the study.

** For genotype 2 the results from 3 mis-positioned aliquots are excluded.*

¹ *Number of tests with detectable viral load. In total, 165 tests/panel member were performed for Genotype 1, 162 tests/panel member were performed for Genotypes 2, 3, 4, and 5, and 168 tests/panel member were performed for Genotype 6. Invalid tests were not repeated*

$$^2 \%CV_{\log} = 100 \times \sqrt{10^{\sigma^2 \ln(10)} - 1}$$

^a *Two <1.80E+1 IU/mL results were observed for this panel member.*

^b *Two <1.80E+1 IU/mL results were observed for this panel member.*

^c *One <1.80E+1 IU/mL result was observed for this panel member.*

Table 16 summarizes the results for the HCV negative panel members from the reproducibility study. There were 2 false positive results in 961 tests. Specificity was 99.8% [95% CI = (0.99, 1.00)].

Table 16: HCV Negative Panel Member Summary

Expected HCV RNA Concentration	Total Valid Results	Target Not Detected	Target Detected but Below LOD ¹	>=18 and <43 IU/mL ²	Within Linear Range ³
Negative	961	959	1	0	1

¹ The limit of detection (LOD) for the assay is 18 IU/mL. Results <1.80E+1 IU/mL are below the LOD.

² Results 18 IU/mL to <43 IU/ml are above the LOD, but below the linear range.

³ Within linear range results are from 43 IU/mL to 6.90E+7 IU/mL, inclusive.

B. Clinical Utility

The use of HCV RNA for the on-treatment assessment of HCV antiviral therapy has become an increasingly important tool for individualizing treatment and optimizing patient outcomes. The critical on-treatment time points for evaluating therapy for customization or discontinuation are at Week 4, Week 12, and Week 24

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 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.³¹

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 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 17.

Table 17: 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 18. 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 in Table 17. These results demonstrate that genotype non-1 and low baseline viral load for genotype 1 (defined as $<7.40\text{E}^{+5}$) are the two most significant positive predictors of SVR.

Table 18. 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 log₁₀ IU/mL, based on the AASLD Practice Guideline.

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)

The RVR analysis in the current study has been performed using the prediction rule of HCV RNA <18 IU/mL, the established LOD for the test. These results demonstrate a high PPV for all patients at 4 weeks (greater than 0.87) independent of genotype. The NPV for not achieving SVR is less than 0.63 for all subgroups and is less useful for predicting NO SVR, especially in the non-1 genotype population due to the high response rate in this population. The analysis was also performed using a prediction rule of <50 IU/mL³. No significant differences were noted in either PPV or NPV when comparing the two prediction rules.

Table 19 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 19: NPV and PPV at Week 4 (RVR) and Corresponding Odds Ratios:
Treatment Arms A through D**

Treatment Arm ¹	Genotype	Prediction Rule	Negative Predictive Value (NPV)		Positive Predictive Value (PPV)		Odds Ratio (95% CI)	
			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 ($\leq 7.40 \text{ E}+5 \text{ IU/mL}$ vs $> 7.40 \text{ E}+5 \text{ IU/mL}$), liver disease (non-cirrhotic vs cirrhotic), baseline SGPT ($> 3 \times \text{ULN}$ vs $\leq 3 \times \text{ULN}$) 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 CAP/CTM HCV Test is 18 IU/mL.

Table 20 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 20: NPV and PPV at Week 12 (EVR) and Corresponding Odds Ratios:
Treatment Arms A through D**

Treatment Arm ¹	Genotype	Prediction Rule	Negative Predictive Value (NPV)		Positive Predictive Value (PPV)		Odds Ratio (95% CI)	
			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 ($\leq 7.40 \text{ E}+5 \text{ IU/mL}$ vs $> 7.40 \text{ E}+5 \text{ IU/mL}$), liver disease (non-cirrhotic vs cirrhotic), baseline SGPT ($> 3 \times \text{ULN}$ vs $\leq 3 \times \text{ULN}$) 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 21 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 21: NPV and PPV at Week 24 and Corresponding Odds Ratios:
Treatment Arms A through D**

Treatment Arm ¹	Genotype	Prediction Rule	Negative Predictive Value (NPV)		Positive Predictive Value (PPV)		Odds Ratio (95% CI)	
			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 ($\leq 7.40 \text{ E}+5 \text{ IU/mL}$ vs $> 7.40 \text{ E}+5 \text{ IU/mL}$), liver disease (non-cirrhotic vs cirrhotic), baseline SGPT ($> 3 \times \text{ULN}$ vs $\leq 3 \times \text{ULN}$) 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 CAP/CTM 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 Virologic 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 log₁₀ IU/mL for genotype 1 patients and 0.59 log₁₀ 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 log₁₀ IU/mL and 0.54 log₁₀ IU/mL for non-1 genotype patients. The mean change of viral load within a subject was estimated to be 0.67 log₁₀ IU/mL for genotype 1 patients and 0.39 log₁₀ IU/mL for genotype non-1 patients. Viral load between two visits varied as noted in the table below.

Table 22: 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

Discussion

RVR assessment at Week 4 of anti-viral therapy for HCV continues to emerge as a key patient management time point. Clinical trials have previously demonstrated a high PPV associated with RVR across all genotypes, ranging from 0.73-0.91 in genotype 1 patients and 0.79-0.85 in genotype 2-3 patients.¹⁶⁻¹⁸ Current guidelines have incorporated the PPV associated with RVR assessment to customize duration of therapy.^{13,14} However, RVR is relatively unreliable as a negative predictor of non-SVR with current therapies, and is discouraged from being used as a stopping rule for non-RVR patients. The results of this current analysis with peginterferon alfa-2a plus ribavirin are consistent with the current medical literature, ranging from 0.84-0.95. This supports the clinical utility of the COBAS AmpliPrep/COBAS TaqMan HCV Test for RVR assessment and patient management based on current guidelines. The variably lower NPV observed with our results also supports the continued unreliability of RVR assessment as a stopping rule for current treatment algorithms.

EVR remains the most established and accepted virologic assessment used for early identification of non-responders and discontinuation of therapy. The NPV associated with Week 12 non-response has been evaluated extensively in large registration trials with the currently approved therapies.^{6,7,10,11} The NPV for genotype 1 patients in these studies ranged from 0.95-1.0, establishing the Week 12 assessment as a critical time point to consider discontinuing therapy. The NPV observed with EVR are somewhat lower in non-1 genotype patients, with rates of 0.75 reported in genotype 2-3 patients largely due to the high response rate in this population.³² The PPV associated with EVR is much lower than the NPV and therefore less predictive for clinical decision making. The current study supports the clinical utility of the COBAS AmpliPrep/COBAS TaqMan HCV Test for assessing EVR. The NPV from our study for the genotype 1 population is relatively consistent with those reported in the medical literature. The high response rates at Week 12 in non-1 genotype patients resulted in small sample sizes for NPV associated with EVR. However, EVR assessment is not considered a standard of care in current patient management algorithms and treatment guidelines for genotype non-1 patients. The results of our study support the current approach to EVR assessment in the genotype non-1 patients.

The high NPV associated with a positive HCV-RNA result at Week 24 (0.99-1.0) was initially established with the approval of non-pegylated interferon plus ribavirin.⁸ The high NPV associated with this time point was confirmed with peginterferon plus ribavirin combination therapies and is incorporated as a stopping rule in to the labeling of both FDA approved peginterferon products.^{6,7} The results of our trial support the continued value of this assessment, independent of genotype, treatment duration, or treatment regimen.

The COBAS AmpliPrep/COBAS TaqMan HCV Test can reliably quantitate the level of HCV RNA for on-treatment assessment of antiviral therapy. The results of this study support the clinical utility for determining RVR, EVR, and Week 24 response for use in the current management of patients with chronic HCV.

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