

SUMMARY OF SAFETY AND EFFECTIVENESS

1. GENERAL INFORMATION

Device Generic Name:	In vitro nucleic acid amplification test for the quantitation of HCV RNA in human plasma or EDTA-serum.
Device Trade Name:	COBAS® AmpliPrep/COBAS® TaqMan® HCV Test
Applicant's Name and Address:	Roche Molecular Systems, Inc. (RMS) 4300 Hacienda Drive Pleasanton, CA 94588
Premarket Approval Application (PMA) Number:	P060030
Date of Panel Recommendation:	None
Date of Notice of Approval:	October 30, 2008

2. INDICATIONS FOR 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 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

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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.

3. CONTRAINDICATIONS

None Known.

4. WARNINGS AND PRECAUTIONS:

For In-Vitro Diagnostic Use Only

The warnings and precautions for the COBAS AmpliPrep/COBAS TaqMan HCV Test are stated in the respective product labeling.

5. DEVICE DESCRIPTION

The COBAS AmpliPrep/COBAS TaqMan HCV Test is an in vitro nucleic acid amplification test for the quantitation of Hepatitis C Virus (HCV) RNA in human plasma or serum using the COBAS AmpliPrep (CAP) Instrument for automated specimen processing and the COBAS TaqMan (CTM) Analyzer or the COBAS TaqMan 48 Analyzer for automated amplification and detection.

5.1. Kit Configuration and Components

The COBAS AmpliPrep/COBAS TaqMan HCV Test consists of the following kits:

- COBAS AmpliPrep/COBAS TaqMan HCV Test
- COBAS AmpliPrep/COBAS TaqMan Wash Reagent

Each kit contains labeled reagents assembled according to storage temperature requirements and controlled room temperature.

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5.2. COBAS AmpliPrep/COBAS TaqMan HCV Test Kit

COBAS AmpliPrep/COBAS TaqMan HCV Test Kit contains reagents and controls required for sample preparation, amplification and detection. All reagents are packaged in one of four barcoded reagent cassettes which are loaded directly on to the CAP instrument along with specimens and controls. User is not required to manually add any reagent prior to amplification and detection on the CTM Analyzer. The following is a list of the reagents and controls provided in the CAP/CTM HCV Test Kit:

- | | |
|---|---------------------|
| (1) HCV CS1 (HCV Magnetic Glass Particles Reagent Cassette) | 1x48 Tests |
| Magnetic glass particles resuspended in a 93% (w/w) isopropanol solution | |
| (2) HCV CS2 (HCV Lysis Reagent Cassette) | 1x48 Tests |
| A sodium citrate buffered solution containing guanidine thiocyanate, polydocanol and dithiothreitol. | |
| (3) HCV CS3 (HCV Multi-Reagent Cassette) | 1 x 48 Tests |
| Pase (Proteinase Solution) | 1 x 3.8 mL |
| A Tris buffered solution containing EDTA, calcium chloride, calcium acetate, proteinase and glycerol. | |
| EB (Elution Buffer) | 1 x 7.0 mL |
| A tris buffer containing methylparaben. | |
| (4) HCV CS4 (HCV Test-Specific Reagent Cassette) | 1 x 48 Tests |
| HCV QS (HCV Quantitation Standard) | 1 x 3.6 mL |
| A Tris buffered solution containing: | |
| 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), and | |
| 0.05% sodium azide | |
| HCV MMX (HCV Master Mix) | 1 x 2.5 mL |

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<p>A Tricine buffered solution containing:</p> <p>potassium acetate,</p> <p>potassium hydroxide,</p> <p>< 20% dimethylsulfoxide,</p> <p>Glycerol,</p> <p>< 0.04% dATP, dCTP, dGTP, dUTP, dTTP,</p> <p>< 0.002% Upstream and downstream primers to the UTR region of HCV,</p> <p>< 0.001% Fluorescent-labeled oligonucleotide probes specific for HCV and the HCV QS,</p> <p>< 0.001% Oligonucleotide aptamer,</p> <p>< 0.05% Z05 DNA Polymerase (microbial)</p> <p>< 0.1% AmpErase (uracil-N-glycosylase) enzyme (microbial), and</p> <p>0.09% Sodium azide</p> <p>CAP/CTM Mn²⁺ (CAP/CTM Manganese Solution)</p> <p>A solution containing:</p> <p>< 0.5% manganese acetate,</p> <p>glacial acetic acid, and</p> <p>0.09% sodium azide.</p>		1 x 19 mL
(5) HCV H(+)/C (HCV High Positive Control)	<p>< 0.001% Armored HCV RNA construct containing HCV sequences (non-infectious RNA in MS2 bacteriophage)</p> <p>Negative Human Plasma, non-reactive by 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</p> <p>0.1% ProClin 300</p>	4 x 1.0 mL
(6) HCV L(+)/C (HCV Low Positive Control)	<p>< 0.001% Armored HCV RNA construct containing HCV sequences (non-infectious RNA in MS2 bacteriophage)</p> <p>Negative Human Plasma, non-reactive by 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</p> <p>0.1% ProClin 300</p>	4 x 1.0 mL
(7) CTM (-) C [COBAS TaqMan Negative Control (Human Plasma)]	<p>Negative Human Plasma, non-reactive by 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</p> <p>0.1% ProClin 300</p>	4 x 1.0 mL
(8) HCV H(+)/C Clip (HCV High Positive Control Barcode Clip)		1 x 4 Clips
(9) HCV L(+)/C Clip (HCV Low Positive Control Barcode Clip)		1 x 4 Clips
(10) HCV (-)C Clip (HCV Negative Control Barcode Clip)		1 x 4 Clips

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5.3. COBAS AmpliPrep/COBAS TaqMan Wash Reagent (PG WR)

The CAP/CTM Wash Reagent (PG WR) is a generic system fluid using for various washing steps performed on the COBAS AmpliPrep instrument during sample preparation. The ingredients in PG WR are listed below:

- | | |
|---|------------------|
| (1) PG WR (COBAS AmpliPrep/COBAS TaqMan Wash Reagent) | 1 x 5.1 L |
| A sodium citrate dehydrate solution containing < 0.1% Methylisothiazolone-HCl | |

5.4. Assay Principle and Format

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 amplified c-DNA (amplicon) using cleaved dual fluorescent dye-labeled oligonucleotide detection probes.

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

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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.

5.4.1. Fundamentals of COBAS TaqMan Test Quantitation:

The COBAS AmpliPrep/COBAS TaqMan HCV Test is inherently quantitative over a very 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, while 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-log₁₀ range. As the concentration of the virus increases, the growth curves shift to earlier cycles. Therefore, the

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leftmost growth curve corresponds to the highest viral titer level, whereas, the rightmost growth curve corresponds to the lowest viral titer level.

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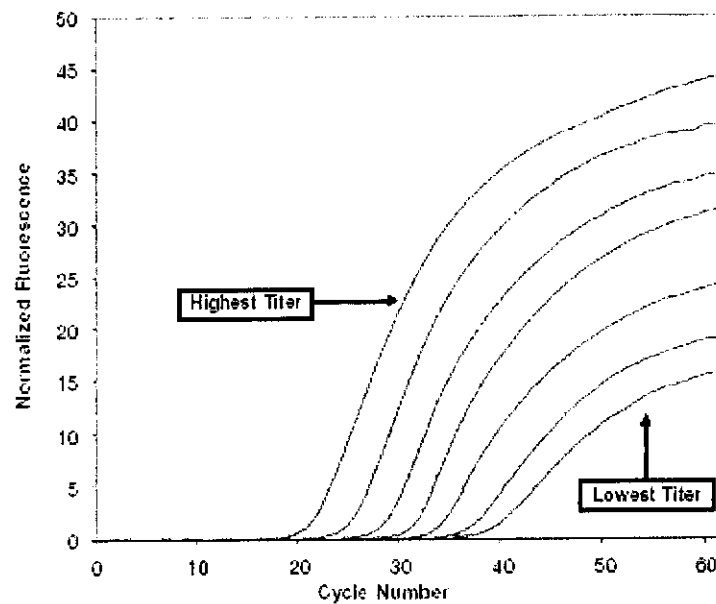


Figure 1: Target Growth Curves for a Dilution Series Spanning a 5-Log₁₀ Range

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.

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

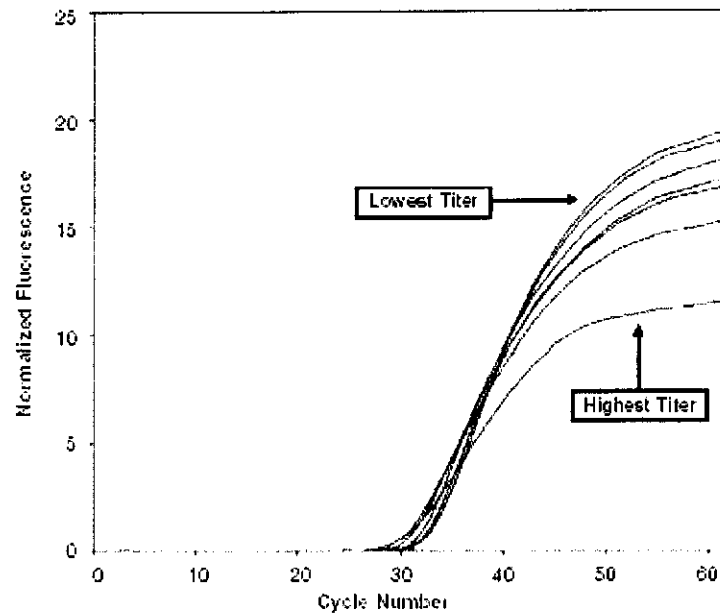
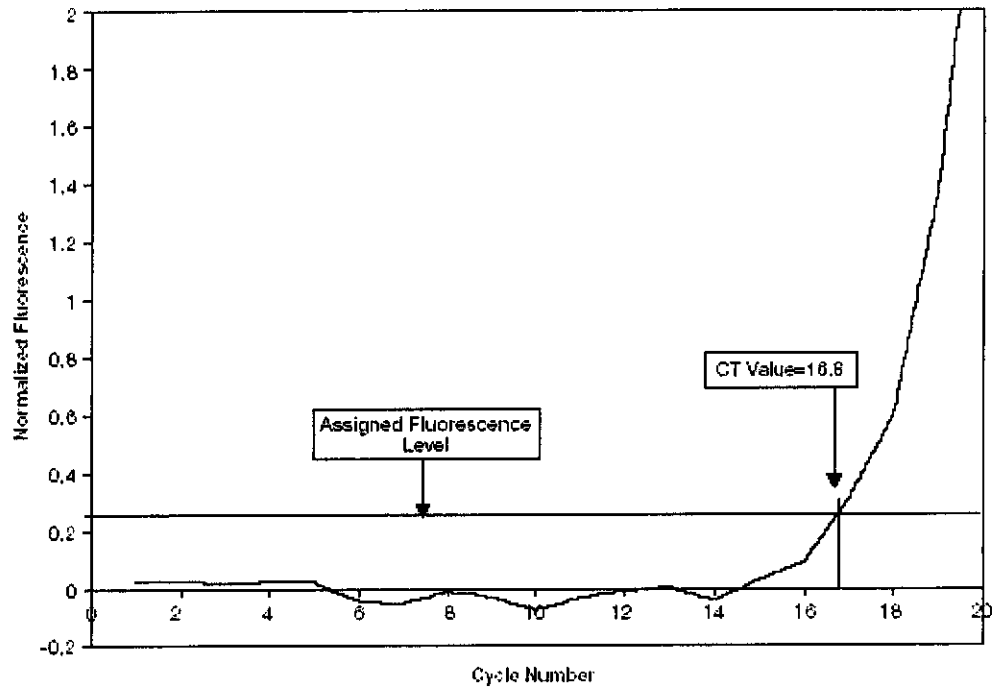


Figure 3 provides an example of how the fluorescence values at every cycle are normalized for each growth curve. The fractional cycle number (Ct) is calculated where the fluorescence signal crosses the Assigned Fluorescence Level.

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



5.4.2. 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

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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)^{1,2}.

5.5. 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.**

5.5.1. 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.

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5.5.2. Batch Validation

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 Positive 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

5.6. 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.

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6. ALTERNATIVE PRACTICES AND PROCEDURES

There are currently a variety of commercially available direct and indirect methods for the detection and quantitation of Hepatitis C in clinical specimens.

Some of these are listed below:

- ELISA, EIA and immunoblot procedures for measuring HCV antibody production
- Nucleic acid probe technologies for direct detection and quantitation of circulating viral particles

7. MARKETING HISTORY

The COBAS AmpliPrep/COBAS TaqMan HCV Test has not been withdrawn from the following markets for reasons related to safety or effectiveness. The test is currently available in the following countries:

Argentina	Greece	Norway
Australia	Hungary	Poland
Austria	Iceland	Portugal
Belgium	Indonesia	Romania
Bulgaria	Ireland	Russia
Canada	Italy	Slovakia
China	Japan	Slovenia
Cyprus	Korea	Spain
Czech Republic	Latvia	Sweden
Denmark	Liechtenstein	Switzerland
Estonia	Lithuania	Taiwan
Finland	Luxembourg	Thailand
France	Malta	Turkey
Germany	Netherlands	United kingdom

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8. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Since the COBAS AmpliPrep/COBAS TaqMan HCV Test is for *in vitro* diagnostic use, there is no direct adverse effect on the health of the patient. However, failure of the product to perform as indicated or human error in use of the product may lead to a false result.

9. SUMMARY OF NONCLINICAL STUDIES

9.1. Laboratory Studies

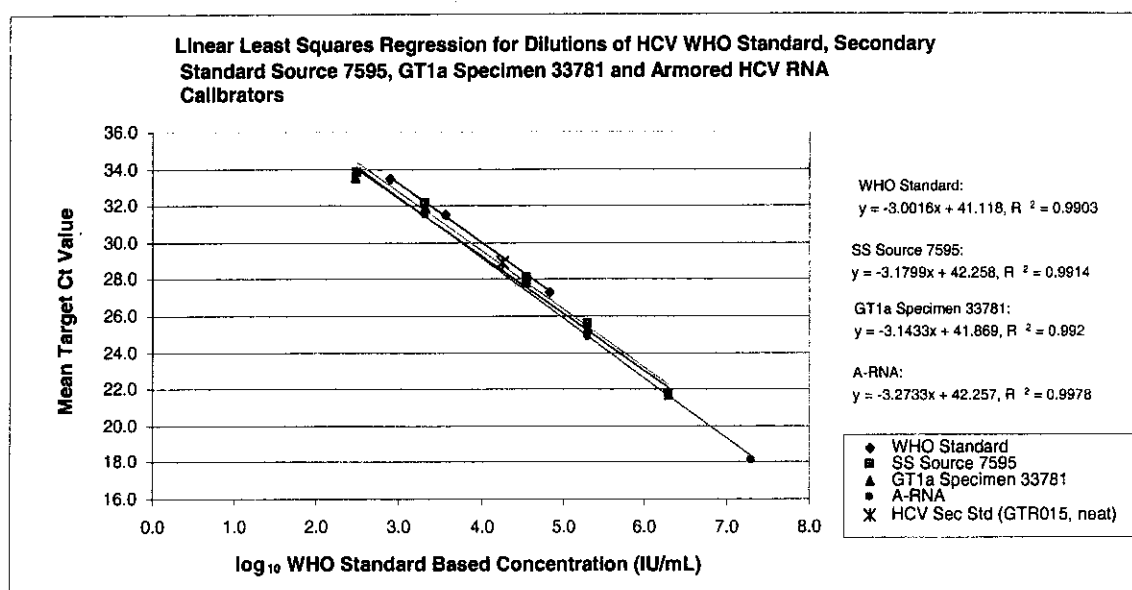
9.1.1. Traceability to the WHO Standard

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.

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Figure 4: Traceability of the COBAS AmpliPrep/COBAS TaqMan HCV Test to the HCV WHO Standard



9.1.2. 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 1

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and Table 2 below). The difference between serum and EDTA plasma was not statistically significant.

Table 1: 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 2: 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]		

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9.1.3. 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 3 below. The overall LoD for this assay is defined as 18 IU/mL.

Table 3: 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%

9.1.4. 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.³ 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

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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 6.90×10^7 HCV RNA IU/mL with maximum observed deviation of $0.2 \log_{10}$ from linearity.

Figure 5 and Figure 6 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 6.9×10^7 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 6.9×10^9 IU/mL.

Figure 5: Linear Range Determination for the COBAS AmpliPrep/COBAS TaqMan HCV Test in EDTA Plasma Specimens

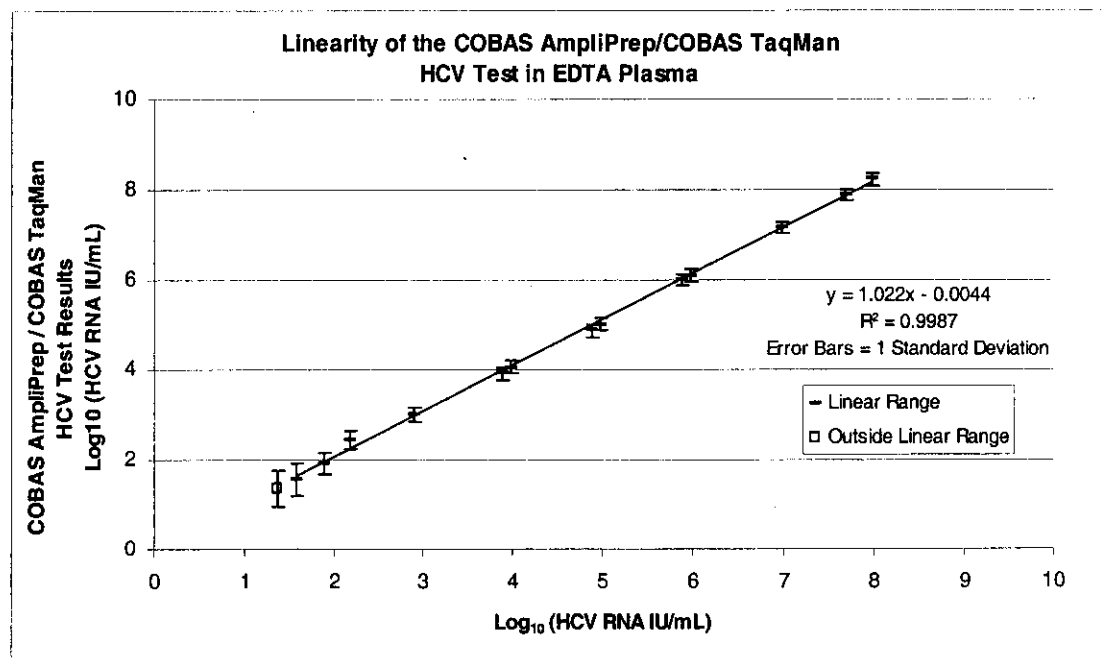
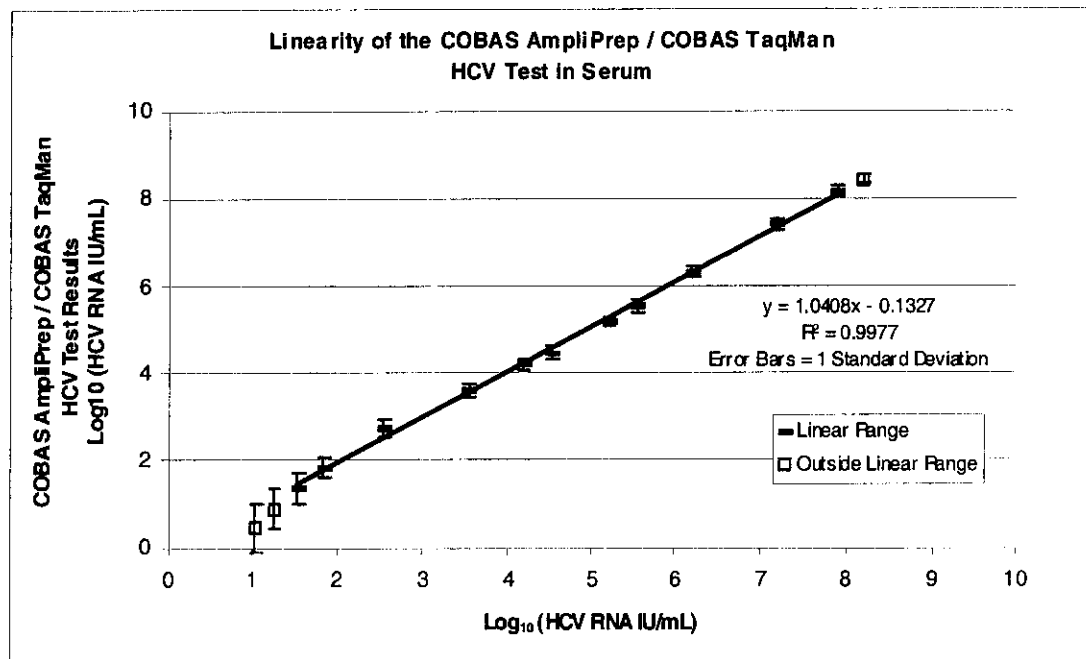


Figure 6: Linear Range Determination for the COBAS AmpliPrep/ COBAS TaqMan HCV Test in Serum Specimens



9.1.5. 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.⁴ 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

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COBAS® AmpliPrep/COBAS TaqMan® HCV Test reagents, and the results are shown in Table 4 through Table 7.

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**Table 4: 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

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

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**Table 5: 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

SUMMARY OF SAFETY AND EFFECTIVENESS

**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

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

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Table 7: 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

9.1.6. 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 log₁₀ titer of clinical specimens of

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HCV genotypes 1-5 to the log₁₀ titer obtained for the COBAS® AMPLICOR HCV MONITOR Test, v2.0 and the VERSANT® HCV RNA 3.0.

9.1.6.1. 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 8.

Table 8: 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

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

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COBAS AmpliPrep/COBAS TaqMan HCV Test is 100% (one-sided lower 95% confidence limit: $\geq 99.6\%$).

9.1.8. 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 9). Stocks of non-HCV viruses as well as bacteria and yeast were diluted to a level of approximately $5E+04$ 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 $5E+04$ copies/mL. None of the non-HCV pathogens showed a false positive result in the COBAS AmpliPrep/COBAS TaqMan HCV Test.

Table 9: 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	

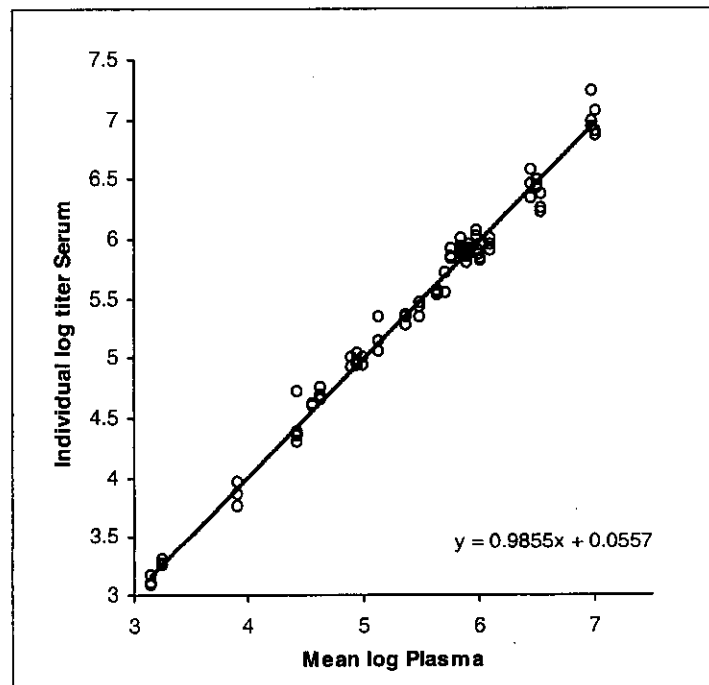
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9.1.9. Matrix Equivalency — Serum versus EDTA Plasma

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 individual log titer for serum to the corresponding individual 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 7. 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 10.

Figure 7: Deming Regression Analysis of Matched Serum — EDTA Plasma Samples (n=29)



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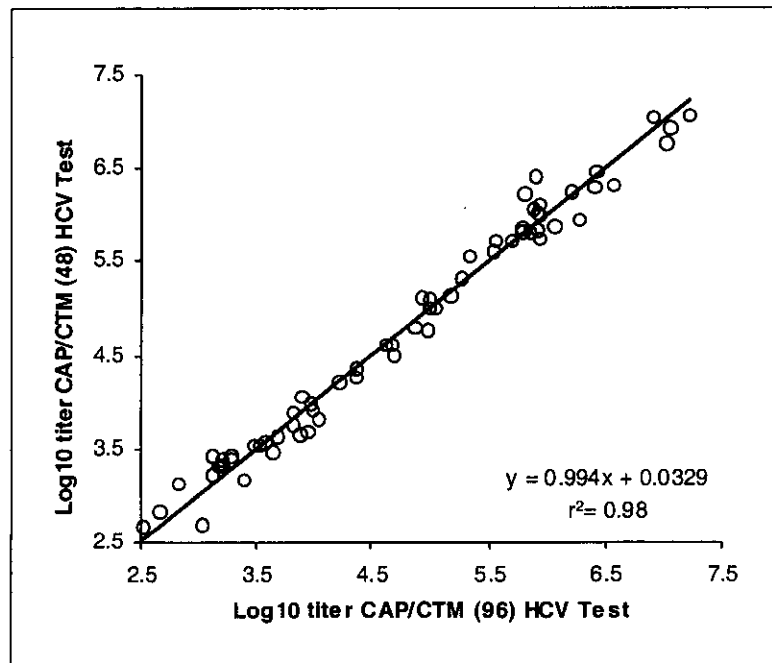
Table 10: Pooled Standard Deviation Estimates for EDTA Plasma and Serum Specimens in the Matrix Equivalency Study

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

9.1.10. Platform Equivalency – COBAS TaqMan Analyzer & 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 8 and Table 11. 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 8: Platform Equivalency – CTM96 Analyzer vs. CTM48 Analyzer



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Table 11: Results of Deming Regression

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

9.1.11. 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) were tested and did not 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

9.2. Recommended Storage Stability

Whole kit stability was determined by a functional real time stability study conducted at 2°C – 8°C, measured over multiple time points using three kit lots of the COBAS AmpliPrep/COBAS TaqMan HCV test kits. Based on these studies, the COBAS AmpliPrep/COBAS TaqMan HCV Test Kit reagents are stable for 24 months from the date of manufacture.

9.3. Sample Handling and Collection

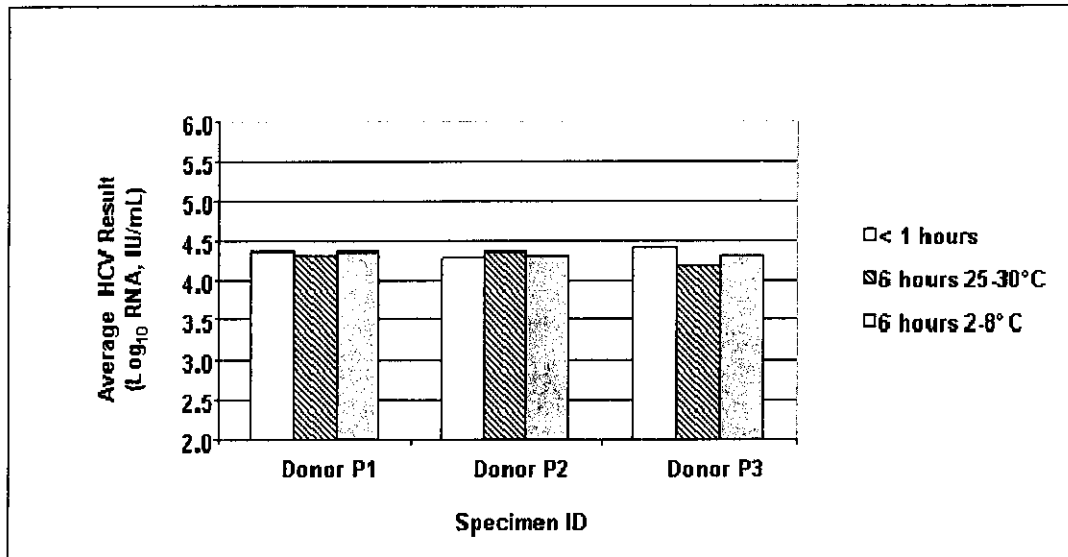
9.3.1. Specimen Collection

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 9 and Figure 10 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 $\pm 0.14 \log_{10}$.

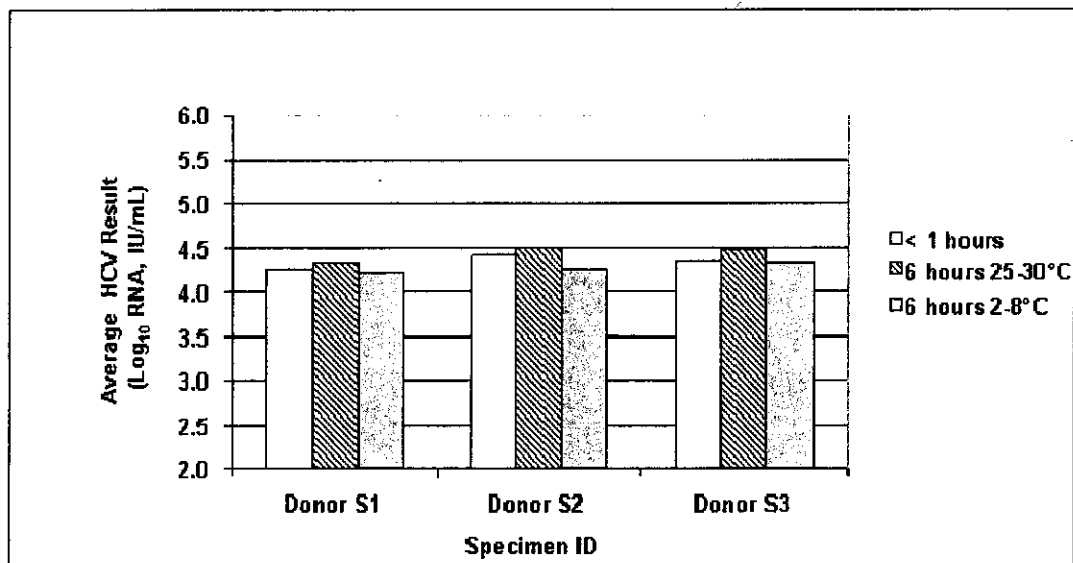
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Figure 9: HCV Stability in Whole Blood with EDTA Anticoagulant



Note: There were four replicates for each time point.

Figure 10: HCV Stability in Whole Blood without Anticoagulant



Note: There were four replicates for each time point.

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9.3.2. Specimen Transport

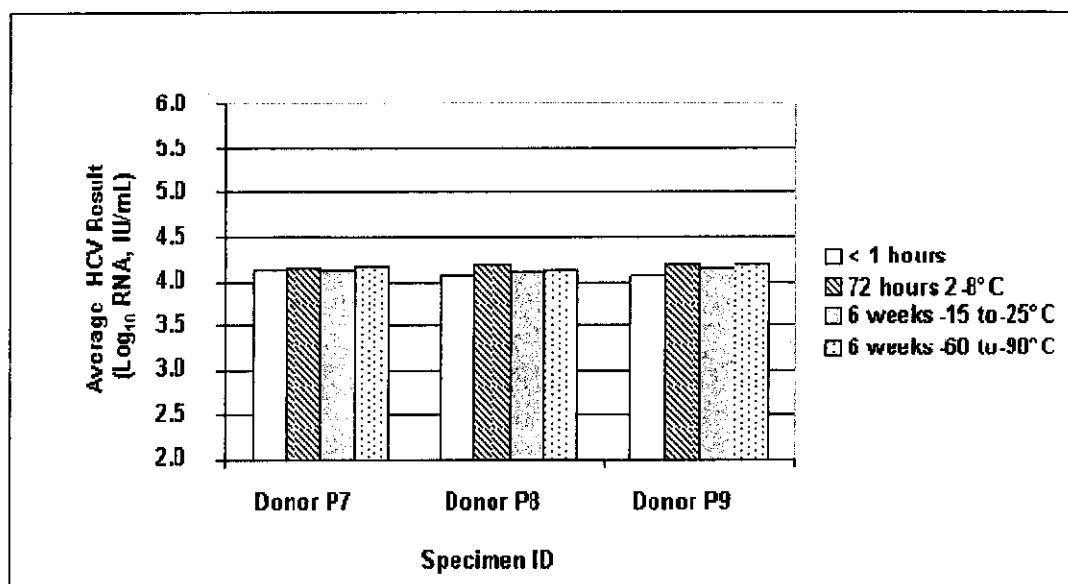
Transportation of whole blood, serum or plasma must comply with country, federal, state, and local regulations for the transport of etiologic agents.⁵ Whole blood must be transported at 2-25°C and centrifuged within 6 hours of collection. Plasma or serum may be transported at 2-8°C or frozen at -70°C or colder, within the defined specimen storage period.

9.3.3. Specimen Stability

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 11 and Figure 12 show specimen stability data from specimen storage studies. Studies were performed using the COBAS AmpliPrep/COBAS TaqMan HCV Test.

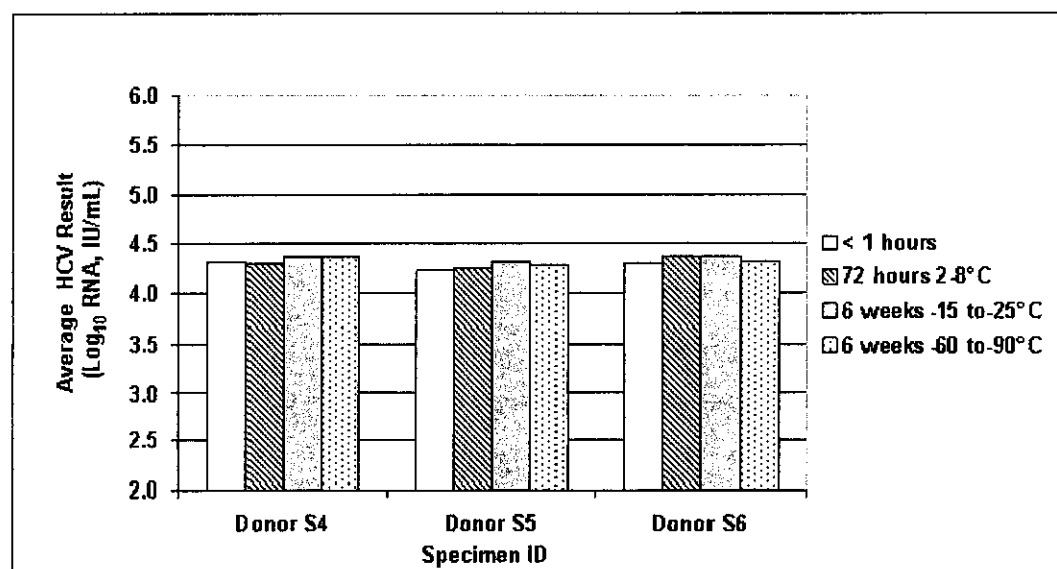
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Figure 11: HCV Stability in EDTA Plasma



Note: There were four replicates for each time point.

Figure 12: HCV Stability in Serum

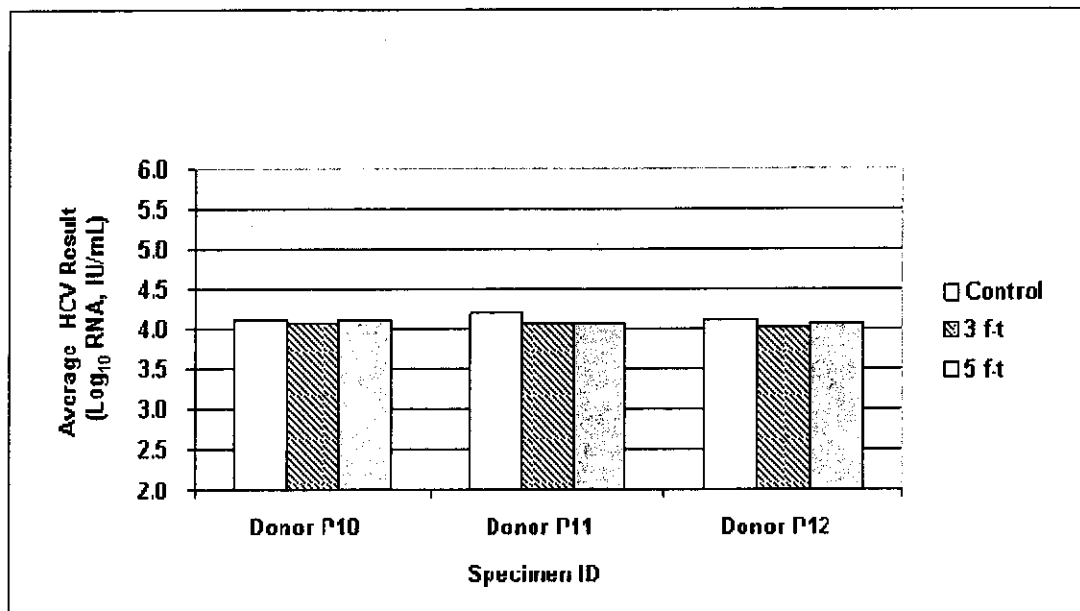


Note: There were four replicates for each time point.

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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 12 and Figure 13 show the data from freeze-thaw studies performed using the COBAS AmpliPrep/COBAS TaqMan HCV Test.

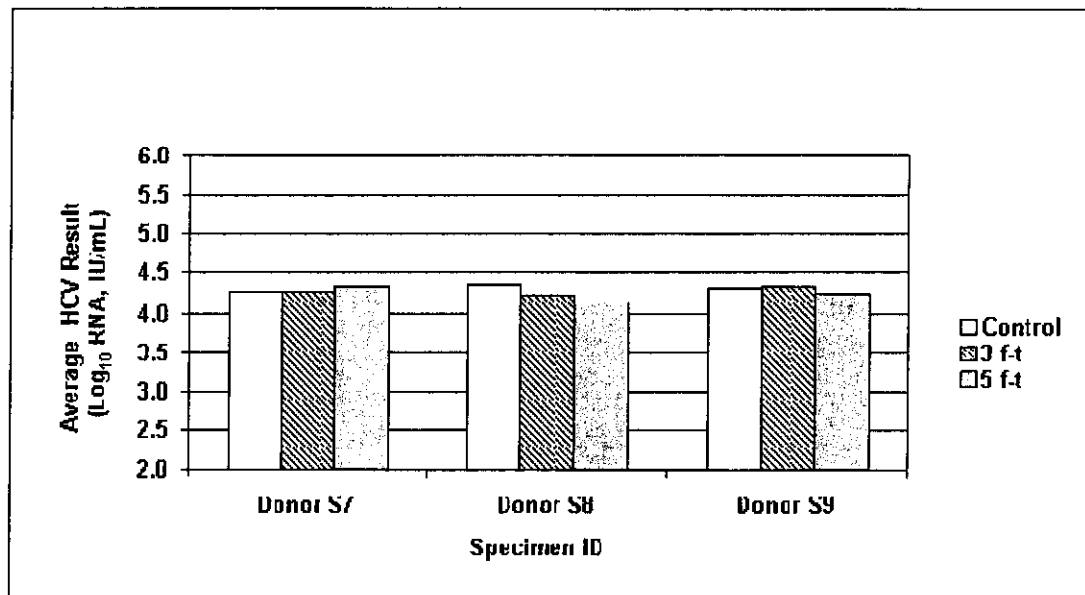
Figure 13: HCV EDTA Plasma Freeze/Thaw Stability



Note: There were four replicates for each time point.

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Figure 14: HCV Serum Freeze/Thaw Stability



Note: There were four replicates for each time point.

10. SUMMARY OF CLINICAL STUDIES

10.1. Reproducibility

Note: As seen below, reproducibility studies were conducted using several levels of each of the 6 genotypes of HCV. These studies were repeated using each of the two platforms. When the results for the two platforms were compared as reported in log10 units, the results were similar for both platforms.

10.1.1. Reproducibility – COBAS TaqMan Analyzer (TaqMan 96)

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 12 and Table 13 below.

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**Table 12: Standard Deviation Components HCV RNA Concentration (log₁₀ IU/mL)
EDTA Plasma**

Geno- type	No. of Tests ¹	Lot	Site/ Instru- ment	Operator	Day/Run	Within- Run	Total Standard Deviation of log ₁₀ HCV RNA Concentration
1	163	0.068	0.085	0.000	0.052	0.176	0.214
	163	0.062	0.000	0.007	0.040	0.094	0.120
	163	0.027	0.014	0.002	0.006	0.075	0.081
	161	0.049	0.010	0.000	0.033	0.066	0.089
	163	0.026	0.024	0.015	0.022	0.069	0.082
	161	0.030	0.028	0.000	0.032	0.062	0.080
2*	157	0.156	0.024	0.038	0.000	0.188	0.248
	154	0.080	0.036	0.000	0.000	0.075	0.115
	151	0.077	0.052	0.000	0.000	0.115	0.148
	156	0.068	0.069	0.000	0.031	0.056	0.116
	156	0.078	0.073	0.000	0.018	0.075	0.132
	157	0.082	0.058	0.000	0.033	0.074	0.129
3	159	0.130	0.000	0.000	0.057	0.121	0.186
	159	0.073	0.032	0.020	0.032	0.063	0.109
	158	0.069	0.079	0.000	0.036	0.088	0.141
	158	0.060	0.087	0.003	0.037	0.086	0.142
	161	0.112	0.094	0.000	0.043	0.066	0.166
	156	0.120	0.106	0.000	0.043	0.080	0.184
4	158	0.192	0.000	0.028	0.113	0.174	0.284
	159	0.179	0.000	0.000	0.123	0.077	0.230
	158	0.169	0.035	0.000	0.108	0.082	0.220
	153	0.163	0.044	0.009	0.132	0.080	0.230
	158	0.179	0.062	0.000	0.124	0.111	0.252
	156	0.204	0.075	0.040	0.178	0.122	0.308
5	154	0.167	0.037	0.000	0.049	0.109	0.209
	157	0.126	0.013	0.021	0.025	0.085	0.156
	158	0.096	0.030	0.000	0.029	0.066	0.123
	154	0.114	0.044	0.022	0.030	0.073	0.147
	157	0.099	0.078	0.000	0.033	0.074	0.149
	158	0.120	0.089	0.000	0.031	0.106	0.186

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Geno- type	No. of Tests ¹	Lot	Site/ Instru- ment	Operator	Day/Run	Within- Run	Total Standard Deviation of log10 HCV RNA Concentration
6	160	0.044	0.036	0.000	0.032	0.155	0.168
	165	0.038	0.000	0.000	0.023	0.061	0.075
	168	0.019	0.034	0.000	0.000	0.078	0.087
	163	0.000	0.040	0.000	0.024	0.089	0.101
	165	0.012	0.038	0.000	0.014	0.092	0.101
	163	0.017	0.041	0.000	0.018	0.082	0.095

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**Table 13: 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 (log10 IU/mL)	No. of Tests ¹	Total Precision Variance of log10 HCV RNA Concentration	Total Precision Standard Deviation of log10 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

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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
	24,379	4.36	157	0.022	0.15	35
	253,987	5.37	158	0.034	0.19	45
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 14 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 14: 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

SUMMARY OF SAFETY AND EFFECTIVENESS

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
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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.

10.2. Reproducibility - COBAS TaqMan 48 Analyzer

The reproducibility of the COBAS AmpliPrep/COBAS TaqMan HCV Test was evaluated for each genotype by two operators at each of three clinical sites. Each operator performed three days of testing on each of three lots of reagents with each genotype panel. Each run comprised a single genotype panel with each panel member tested in triplicate.

The results of the reproducibility study are summarized in Table 15 and Table 16 below.

**Table 15: 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/Instrument	Operator	Day/Run	Within-Run	Total Standard Deviation of log ₁₀ HCV RNA Concentration
1	133	2.01 ^a	159	0.000	0.058	0.000	0.052	0.306	0.316
	1,488	3.16	159	0.063	0.000	0.010	0.000	0.104	0.122
	10,145	3.99	161	0.056	0.000	0.000	0.028	0.090	0.109
	76,432	4.87	154	0.043	0.000	0.000	0.024	0.082	0.096
	726,306	5.85	159	0.013	0.000	0.000	0.037	0.095	0.103
	1,264,581	6.05	159	0.060	0.012	0.000	0.118	0.175	0.220
2	108	1.93 ^b	132	0.067	0.000	0.000	0.000	0.283	0.291
	1,428	3.11	131	0.078	0.000	0.000	0.074	0.165	0.196
	9,839	3.95	131	0.047	0.000	0.000	0.000	0.178	0.184
	72,711	4.84	132	0.018	0.000	0.017	0.062	0.101	0.121
	477,320	5.66	134	0.028	0.040	0.000	0.001	0.117	0.127
	5,583,514	6.71	133	0.056	0.000	0.000	0.150	0.083	0.180
3	105	1.88 ^c	157	0.275	0.094	0.000	0.070	0.182	0.349
	8,253	3.86	160	0.186	0.087	0.000	0.024	0.097	0.228

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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/Instrument	Operator	Day/Run	Within-Run	Total Standard Deviation of \log_{10} HCV RNA Concentration
	774,131	5.79	160	0.187	0.111	0.036	0.132	0.124	0.286
	12,215,944	7.02	160	0.158	0.095	0.000	0.096	0.110	0.235
4	713	2.62	163	0.373	0.104	0.000	0.107	0.199	0.448
	4,428	3.49	162	0.332	0.102	0.000	0.066	0.112	0.371
	20,340	4.21	165	0.244	0.075	0.025	0.032	0.134	0.291
	213,356	5.23	164	0.246	0.110	0.029	0.068	0.106	0.299

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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
5	219	2.21 ^d	156	0.243	0.088	0.022	0.091	0.194	0.337
	1,570	3.08	157	0.231	0.111	0.021	0.077	0.164	0.315
	13,760	4.07	159	0.186	0.071	0.029	0.063	0.109	0.238
	98,283	4.94	158	0.157	0.088	0.033	0.028	0.116	0.218
6	106	1.96	158	0.103	0.044	0.000	0.000	0.212	0.240
	5,271	3.67	159	0.165	0.026	0.040	0.073	0.101	0.212
	39,349	4.55	161	0.114	0.049	0.040	0.000	0.143	0.194
	495,696	5.65	160	0.110	0.087	0.039	0.053	0.135	0.206

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) were additionally tested in the study. Seven fewer Genotype 2 panels than the number of panels/genotype planned were tested.

Note: One result above the linear range (>6.90E+7 IU/mL) from a Genotype 1 panel was excluded from this analysis.

¹ Number of tests with detectable viral load. In total, 162 tests/panel member were performed for Genotypes 1, 3, and 5, 141 tests/panel member were performed for Genotype 2, and 165 tests/panel member were performed for Genotypes 4 and 6. Invalid tests were not repeated.

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

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

^c Five <1.80E+1 IU/mL results were observed for this panel member.

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

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**Table 16: 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	133	2.01 ^a	159	0.100	0.32	84
	1,488	3.16	159	0.015	0.12	29
	10,145	3.99	161	0.012	0.11	26
	76,432	4.87	154	0.009	0.10	22
	726,306	5.85	159	0.011	0.10	24
	1,264,581	6.05	159	0.048	0.22	54
2	108	1.93 ^b	132	0.085	0.29	75
	1,428	3.11	131	0.039	0.20	48
	9,839	3.95	131	0.034	0.18	44
	72,711	4.84	132	0.015	0.12	28
	477,320	5.66	134	0.016	0.13	30
	5,583,514	6.71	133	0.032	0.18	43
3	105	1.88 ^c	157	0.122	0.35	95
	8,253	3.86	160	0.052	0.23	56
	774,131	5.79	160	0.082	0.29	74
	12,215,944	7.02	160	0.055	0.23	58
4	713	2.62	163	0.201	0.45	138
	4,428	3.49	162	0.138	0.37	104
	20,340	4.21	165	0.085	0.29	75
	213,356	5.23	164	0.089	0.30	78
5	219	2.21 ^d	156	0.113	0.34	91
	1,570	3.08	157	0.099	0.31	83
	13,760	4.07	159	0.056	0.24	59
	98,283	4.94	158	0.048	0.22	54

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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	106	1.96	158	0.058	0.24	60
	5,271	3.67	159	0.045	0.21	52
	39,349	4.55	161	0.038	0.19	47
	495,696	5.65	160	0.042	0.21	50

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) were additionally tested in the study. Seven fewer Genotype 2 panels than the number of panels/genotype planned were tested.

Note: One result above the linear range (>6.90E+7 IU/mL) from a Genotype 1 panel was excluded from this analysis.

¹ Number of tests with detectable viral load. In total, 162 tests/panel member were performed for Genotypes 1, 3, and 5, 141 tests/panel member were performed for Genotype 2, and 165 tests/panel member were performed for Genotypes 4 and 6. Invalid tests were not repeated.

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

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

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

^c Five <1.80E+1 IU/mL results were observed for this panel member.

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

Table 17 summarizes the results for the HCV negative panel members from the reproducibility study. There were no false positive results in 932 tests. Specificity was 100% [95% CI = (0.996, 1.000)].

Table 17: 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	932	932	0	0	0

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.

10.3. 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 chronic hepatitis C (CHC) by estimating the negative predictive value (NPV) and positive predictive value (PPV) for achieving an sustained virologic response (SVR) at established clinically relevant time points during antiviral treatment (Week 4/Rapid virologic response (RVR), Week 12/early virologic response (EVR), and Week 24).

10.3.1. 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.⁷

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, 3 sites used the COBAS TaqMan 48 Analyzer and 2 used the COBAS TaqMan Analyzer 96.

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

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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 18.

Table 18: 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	≤ 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

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Characteristic	Category	Summary Statistics	Combined Over All Four Treatment Arms
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¹ 2,000,000 copies/mL = 7.40E+5 IU/mL = 5.87 log₁₀ IU/mL.

² ULN = Upper Limit of Normal Range.

³ SGPT, serum glutamic pyruvic transaminase.

10.3.2. Predictability Analysis

10.3.2.1. 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 19. 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 21. These results demonstrate that genotype non-1 and low baseline viral load for genotype 1 (defined as <7.40E⁵) are the two most significant positive predictors of SVR.

Table 19: 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)

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Characteristic ¹	Category	N (%)	Percent with SVR	Odds Ratio (95% CI) Using Univariate Analysis
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 ⁵	≤ 3*ULN ⁵	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.

⁵ SGPT, serum glutamic pyruvic transaminase; ULN, upper limit of normal range.

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10.3.2.2. 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-log₁₀ 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).

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10.3.2.3. 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/mL⁶. No significant differences were noted in either PPV or NPV when comparing the two prediction rules.

Table 20 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.

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**Table 20: NPV and PPV at Week 4 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

10.3.2.4. Predictive Values at Week 12 of Antiviral Therapy (EVR Analysis)

The NPV is higher for genotype 1 patients than non-1 patients. Additionally, the PPV is less predictive and significantly different for genotype 1 and non-1 patient groups.

Table 21 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.

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**Table 21: NPV and PPV at Week 12 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 ($> 3^* \text{ULN}$ vs $\leq 3^* \text{ULN}$) 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.

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10.3.2.5. Predictive Values at Week 24 of Antiviral Therapy

The Week 24 analysis is performed using the prediction rule of defining <18 IU/mL (the LOD of the test) as response at Week 24. The NPV at Week 24 for all patients is extremely high (>0.98), regardless of genotype. However, the PPV at Week 24 is less predictive of SVR and varies by genotype. 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 22 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.

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**Table 22: 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	NA	NA
	Non-1	<18 IU/mL ³	1.00 (0.16, 1.00)	2/2	0.89 (0.82, 0.94)	110/124	>19.1 (2.7, 816.6) ⁴	>13.5 (1.6, 111.2) ⁴

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

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

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 covariate 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 in Non-1 cannot be calculated.

SUMMARY OF SAFETY AND EFFECTIVENESS

10.3.3. Within-Subject Variability in Absence of Treatment

The objective of this analysis is to estimate the change in viral load (in log units) between two successive measurements of patients not receiving anti-viral therapy.

Baseline and screening serum sample results were available from 196 subjects enrolled in the clinical study to evaluate the effect of pegylated-interferon 2b treatment duration and Ribavirin dose. The screening samples were obtained 2 to 56 days before the collection of the baseline samples with an average of 38 days between collections of the two samples. Out of 196 subjects, 139 were genotype 1 patients and 57 were non-1 genotype. These two results were used to estimate within subject variability, which includes biological variability as well as total assay variability. The within subject variability from these results was estimated to be $0.62 \log_{10}$ IU/mL for genotype 1 patients and $0.59 \log_{10}$ IU/mL for non-1 genotype patients. To obtain an estimate biological variability, total assay variability is subtracted from within subject variability. Biological variability for genotype 1 patients is $0.60 \log_{10}$ IU/mL and $0.54 \log_{10}$ IU/mL for non-1 genotype patients. The mean change of viral load within a subject was estimated to be $0.67 \log_{10}$ IU/mL for genotype 1 patients and $0.39 \log_{10}$ IU/mL for genotype non-1 patients. Viral load between two visits varied as noted in the table below.

Table 23: 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

11. CONCLUSIONS AND DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

11.1. Risk/Benefit Analysis

As a diagnostic test, the COBAS AmpliPrep/COBAS TaqMan HCV Test involves removal of blood from an individual for testing purposes. This test presents no more of a safety hazard to an individual than is presented to an individual who is having their blood drawn for any other diagnostic evaluation. The benefits to chronically HCV-infected individuals undergoing antiviral therapy tested by the assay outweigh any potential adverse event or risk to the patient or user due to assay malfunction or operator error. The potential risks encountered with this in vitro diagnostic test are not unusual in the clinical laboratory setting. Appropriate warnings for these risks are contained in the labeling and package inserts for the device. Standard good laboratory practices are considered sufficient to mitigate the risks to the end user.

11.2. Safety Conclusion

Based on the results of the preclinical and clinical laboratory studies, the COBAS AmpliPrep/COBAS TaqMan HCV Test, when used according to the provided directions and in conjunction with other serological and clinical information, should be safe and pose minimal risk to the patient due to false test results.

11.3. Effectiveness Conclusions

The effectiveness of the COBAS AmpliPrep/COBAS TaqMan HCV has been demonstrated for use in quantitation of Hepatitis C Virus (HCV) RNA in human serum or plasma. A reasonable determination of effectiveness of the COBAS AmpliPrep/COBAS TaqMan HCV for aiding in the management of patients with chronic HCV infection undergoing anti-viral therapy, by measuring HCV RNA levels at baseline and during treatment has been demonstrated.

SUMMARY OF SAFETY AND EFFECTIVENESS

11.4. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use.

The data from the nonclinical studies demonstrated acceptable analytical sensitivity, traceability, linearity, precision, and analytical specificity of the COBAS AmpliPrep/COBAS TaqMan HCV Test when used according to the instructions for use as stated in the labeling, the warnings and precautions, and limitations sections of the labeling.

The clinical studies and the statistical analysis of clinical data in this application has shown that viral response to treatment measured with COBAS AmpliPrep/COBAS TaqMan HCV Test is informative for assessing the effect of treatment in patients with chronic hepatitis C, and that the assay is safe and effective when used according to the directions for use in the labeling.

The results indicate that the performance of the CAP/CTM HCV test as evaluated by the PPV at week 4 and NPV at 12 remains comparable by platform. Additionally, the medical utility for both RVR and EVR are maintained across both platforms as well.

12. PANEL RECOMMENDATIONS

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Microbiology Devices Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

13. CDRH DECISION

CDRH issued an approval order on December 3, 2008.

The applicant's manufacturing facility was inspected on 2/21/07, 12/4/07 and 7/22/08 and the facility was found to be in compliance with the Quality System Regulation (21 CFR 829).

SUMMARY OF SAFETY AND EFFECTIVENESS

14. APPROVAL SPECIFICATIONS

Directions for use: See attached labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions and Adverse Events in the labeling.

Postapproval Requirements and Restrictions: See approval order.

SUMMARY OF SAFETY AND EFFECTIVENESS

15. REFERENCES

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