

SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: In vitro polymerase chain reaction (PCR) based assay for HCV RNA detection.

Device Trade Name: Abbott RealTime HCV, Abbott RealTime HCV Amplification Reagent Kit, Abbott RealTime HCV Control Kit, Abbott RealTime HCV Calibrator Kit, and optional UNG Uracil-N-Glycosylase (UNG) for use in conjunction with Abbott RealTime HCV

Applicant's Name and Address: Abbott Molecular Inc.
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Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P100017

Date of FDA Notice of Approval: May 17, 2011

Expedited: Not applicable

II. INDICATIONS FOR USE

Abbott RealTime HCV Amplification Reagent Kit:

The Abbott RealTime HCV assay is an in vitro reverse transcription-polymerase chain reaction (RT-PCR) assay for use with the Abbott *m*Sample Preparation System reagents and with the Abbott *m*2000*sp* and *m*2000*rt* instruments for the quantitation of hepatitis C viral (HCV) RNA in human serum or plasma (EDTA) from HCV-infected individuals. Specimens containing HCV genotypes 1 - 6 have been validated for quantitation in the assay.

The Abbott RealTime HCV assay is intended for use as an aid in the management of HCV-infected patients undergoing antiviral 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 RealTime HCV assay 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 or 2b 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 Abbott RealTime HCV assay is not for screening blood, plasma, serum or tissue donors for HCV, or to be used as a diagnostic test to confirm the presence of HCV infection.

Abbott RealTime HCV Control Kit:

The Abbott RealTime HCV Controls are used to establish run validity of the Abbott RealTime HCV assay when used for the quantitation of Hepatitis C Virus (HCV) RNA in human serum and plasma (EDTA) from HCV infected individuals.

Abbott RealTime HCV Calibrator Kit:

The Abbott RealTime HCV Calibrators are for calibration of the Abbott RealTime HCV assay when used for the quantitative determination of Hepatitis C Virus (HCV) RNA in human serum and plasma (EDTA) from HCV infected individuals.

Uracil-N-Glycosylase (UNG) for use in conjunction with Abbott RealTime HCV:

This kit is to be used in conjunction with Abbott RealTime HCV, List No. 1N30-90, as an optional contamination control for customer laboratories that are currently using or have previously used amplification technologies that incorporate uracil into the amplification product.

III. CONTRAINDICATIONS

The contraindications can be found on the Abbott RealTime HCV's labeling.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the Abbott RealTime HCV's labeling.

V. DEVICE DESCRIPTION

The Abbott RealTime HCV assay uses RT-PCR to generate amplified product from the RNA genome of HCV in clinical specimens. In addition, an RNA sequence that is unrelated to the HCV target sequence is introduced into each specimen at the beginning of sample preparation. This unrelated RNA sequence is simultaneously amplified by RT-PCR and serves as an internal control (IC) to demonstrate that the process has proceeded correctly for each sample. The amount of target sequence that is present at each amplification cycle is measured through the use of fluorescent labeled oligonucleotide probes on the Abbott *m2000rt* instrument. The probes do not generate signal unless they are specifically bound to the amplified product. The amplification cycle at which the HCV-specific fluorescent signal is detected by the Abbott *m2000rt* is proportional to the log of the HCV RNA concentration present in the original sample.

The Abbott *m2000* System integrates sample preparation with nucleic acid amplification and detection to generate assay results. The Abbott *m2000* System consists of the Abbott *m2000sp* and Abbott *m2000rt* instruments.

Application parameters specific to Abbott RealTime HCV are contained on an assay specific application file, housed on a CD-ROM and loaded onto the Abbott *m2000sp* and Abbott *m2000rt* instruments.

The Abbott RealTime HCV assay consists of three kits:

- Abbott RealTime HCV Amplification Reagent Kit (List No. 1N30-90)
- Abbott RealTime HCV Control Kit (List No. 1N30-80)
- Abbott RealTime HCV Calibrator Kit (List No. 1N30-70)

A fourth kit (1N30-66, Uracil-N-Glycosylase (UNG) for use in conjunction with Abbott RealTime HCV) may also be used if it is necessary to remove contamination from external dU-containing amplified product.

Sample Preparation

The purpose of sample preparation is to extract and concentrate the target RNA molecules to make them accessible for amplification and to remove potential inhibitors of amplification from the extract. The Abbott *m2000sp* is an automated sample preparation system designed to use magnetic microparticle processes for the purification of nucleic acids from samples. The Abbott *m2000sp* instrument along with the Abbott *mSample Preparation System* (4 X 24 Preps) processes plasma or serum samples for nucleic acid amplification. Multiple samples can be processed simultaneously. The IC is taken through the entire sample preparation procedure along with the calibrators, controls, and specimens. After capture of nucleic acids onto magnetic microparticles, the microparticles are washed to remove unbound sample components. Next, the bound nucleic acids are eluted from the microparticles and the eluates are transferred to the Abbott 96 Deep-Well Plate.

Reagent Preparation and Reaction Plate Assembly

The Abbott *m2000sp* instrument automates the assembly of the amplification master mix (HCV Oligonucleotide Reagent, Thermostable rTth Polymerase Enzyme, and Activation Reagent) and then transfers aliquots of the master mix to the Abbott 96-Well Optical Reaction Plate. Nucleic acid samples from the Abbott 96 Deep-Well Plate are then transferred into the Abbott 96-Well Optical Reaction Plate by the Abbott *m2000sp*. The plate is sealed by the user with an Abbott Optical Adhesive Cover and placed into the Abbott *m2000rt* instrument for PCR amplification and fluorescence detection.

Amplification

During the amplification reaction on the Abbott *m2000rt*, the target RNA is converted to cDNA by the reverse transcriptase activity of the thermostable rTth DNA polymerase. First, the HCV and IC reverse primers anneal to their respective targets and are extended during a prolonged incubation period. After a denaturation step, in which the temperature of the reaction is raised above the melting point of the double-stranded cDNA:RNA product, a second primer anneals to the cDNA strand and is extended by the DNA polymerase activity of the rTth enzyme to create a double-stranded DNA product.

During each round of thermal cycling, amplification products dissociate to single strands at high temperature, allowing primer annealing and extension as the temperature is lowered. Exponential amplification of the product is achieved through repeated cycling between high and low temperatures, resulting in a billion-fold or greater amplification of target sequences. Amplification of both targets (HCV and IC) takes place simultaneously in the same reaction.

The target sequence for the Abbott RealTime HCV assay is in the 5' *utr* region of the HCV genome. This region is specific for HCV and is highly conserved.¹ The primers are designed to hybridize to the 5' *utr* region with the fewest possible mismatches among HCV genotypes 1, 2, 3, 4, 5, and 6.

The IC target sequence is derived from the hydroxypyruvate reductase gene from the pumpkin plant, *Cucurbita pepo* and is delivered in an Armored RNA[®] particle that has been diluted in HCV-negative human plasma.

Detection

During the read cycles of amplification on the Abbott *m2000rt*, the temperature is lowered further to allow fluorescent detection of amplification products as the HCV and IC probes anneal to their targets (real-time fluorescence detection).

The HCV and IC probes are single-stranded DNA oligonucleotides consisting of a probe sequence with a fluorescent moiety that is covalently linked to the 5' end of the probe and a quenching moiety that is covalently linked to the 3' end of the probe.

In the absence of the HCV or IC target sequences, probe fluorescence is quenched. In the presence of the HCV or IC target sequences, probe hybridization to complementary sequences separates the fluorophore and the quencher and allows fluorescent emission and detection.

The HCV and IC specific probes are each labeled with a different fluorophore, thus allowing for simultaneous detection of both amplified products at each cycle. The amplification cycle at which the HCV probe fluorescent signal is detected by the Abbott *m2000rt* is proportional to the log of the HCV RNA concentration present in the original sample.

Quantitation

A calibration curve is required to quantitate the HCV RNA concentration of specimens and controls. Two assay calibrators are run in replicates of three to generate a calibration curve. The calibration curve slope and intercept are calculated from the assigned HCV RNA concentration and the median observed threshold cycle for each calibrator and are then stored on the instrument. The concentration of HCV RNA in specimens and controls is calculated from the stored calibration curve, and the results are automatically reported on the Abbott *m2000rt* workstation. The Abbott RealTime HCV Negative Control, Low Positive Control, and High Positive Control must be included in each run to verify run validity. The Abbott *m2000rt* verifies that the controls are within the assigned ranges.

Abbott RealTime HCV Amplification Reagent Kit (List No. 1N30-90)

The Abbott RealTime HCV Amplification Reagent Kit consists of:

- Abbott RealTime HCV Internal Control
- Abbott RealTime HCV Amplification Reagent Pack, which contains:
 - Thermostable rTth Polymerase Enzyme
 - HCV Oligonucleotide Reagent
 - Activation Reagent

The Abbott RealTime HCV Amplification Reagent Kit contains sufficient reagents to process 96 tests (4 packs, 24 tests per pack).

Abbott RealTime HCV Internal Control

The Abbott RealTime HCV Internal Control consists of noninfectious Armored RNA with a RNA sequence unrelated to HCV in negative human plasma containing the preservatives ProClin[®] 300 and ProClin 950.

The Abbott RealTime HCV Internal Control contains negative human plasma that is tested and found to be nonreactive by FDA licensed tests for antibody to HCV, antibody to HIV-1, antibody to HIV-2, and HBsAg. The material is also tested and found to be negative by FDA licensed PCR methods for HIV-1 RNA and HCV RNA.

The Internal Control is introduced into each specimen, calibrator, and control at the beginning of sample preparation and measured on the Abbott *m2000rt* instrument to demonstrate proper sample processing and assay validity.

Abbott RealTime HCV Amplification Reagent Pack

The Abbott RealTime HCV Amplification Reagent Pack consists of the Thermostable rTth Polymerase Enzyme, the HCV Oligonucleotide Reagent, and the Activation Reagent.

DNA Polymerase

Each vial contains Thermostable rTth Polymerase Enzyme in a buffered solution with stabilizers. The recombinant *Thermus thermophilus* thermostable DNA polymerase enzyme has a dual function as a reverse transcriptase transcribing cDNA from RNA target sequence and as a DNA polymerase in PCR amplification.

HCV Oligonucleotide Reagent

Each vial of HCV Oligonucleotide Reagent contains two sets of oligonucleotide primers and two probes, one set for amplifying and detecting HCV RNA and the other specific for amplifying and detecting Internal Control RNA. The reagent also contains dNTPs and ROX[™] passive reference dye. The reagent is formulated in a potassium acetate-bicine buffer with the preservatives ProClin 300 and ProClin 950.

Activation Reagent

Each vial of Activation Reagent contains a 30 mM manganese chloride solution and the preservatives ProClin 300 and ProClin 950. Manganese chloride is a cofactor of recombinant *Thermus thermophilus* thermostable DNA polymerase.

Abbott RealTime HCV Control Kit (List No. 1N30-80)

The Abbott RealTime HCV Control Kit consists of:

- Abbott RealTime HCV Negative Control
- Abbott RealTime HCV Low Positive Control
- Abbott RealTime HCV High Positive Control

The Abbott RealTime HCV Control Kit contains three controls (eight vials of Abbott RealTime HCV Negative Control, eight vials of Abbott RealTime HCV Low Positive Control, and eight vials of Abbott RealTime HCV High Positive Control) that are used to establish the run validity of the Abbott RealTime HCV assay.

The Abbott RealTime HCV Negative Control contains negative human plasma that is tested and found to be nonreactive by FDA licensed tests for antibody to HCV, antibody to HIV-1, antibody to HIV-2, and HBsAg. The material is also tested and found to be negative by FDA licensed PCR methods for HIV-1 RNA and HCV RNA, and contains the preservatives ProClin 300 and ProClin 950.

The Abbott RealTime HCV Low Positive Control and High Positive Control contain noninfectious armored RNA with HCV sequences in negative human plasma. The negative human plasma used in the Abbott RealTime HCV Low Positive Control and High Positive Control is tested and found to be nonreactive by FDA licensed tests for antibody to HCV, antibody to HIV-1, antibody to HIV-2, and HBsAg. The material is also tested and found to be negative by FDA licensed PCR methods for HIV-1 RNA and HCV RNA, and contains the preservatives ProClin 300 and ProClin 950.

Abbott RealTime HCV Calibrator Kit (List No. 1N30-70)

The Abbott RealTime HCV Calibrator Kit consists of:

- Abbott RealTime HCV Calibrator A
- Abbott RealTime HCV Calibrator B

The Abbott RealTime HCV Calibrator Kit contains two calibrators (12 vials of Abbott RealTime HCV Calibrator A and 12 vials of Abbott RealTime HCV Calibrator B) that are used to generate a calibration curve for the quantitative determination of HCV in human plasma or serum.

The Abbott RealTime HCV Calibrator A and Calibrator B contain noninfectious armored RNA with HCV sequences in negative human plasma. The negative human plasma used in the Abbott RealTime HCV Calibrator A and HCV Calibrator B is tested and found to be nonreactive by FDA licensed tests for antibody to HCV, antibody to HIV-1, antibody

to HIV-2, and HBsAg. The material is also tested and found to be negative by FDA licensed PCR methods for HIV-1 RNA and HCV RNA and contains the preservatives ProClin 300 and ProClin 950.

Uracil-N-Glycosylase (UNG, List No. 1N30-66) for use in conjunction with Abbott RealTime HCV

The Uracil-N-Glycosylase (UNG) for use in conjunction with Abbott RealTime HCV kit contains one vial of UNG enzyme. This kit is to be used in conjunction with the Abbott RealTime HCV assay as an optional contamination control for customer laboratories that are currently using or have previously used amplification technologies that incorporate uracil into the amplification product.

Assay Calibration

A calibration curve is required to quantitate HCV RNA concentration of specimens and controls. Two assay calibrators are run in replicates of three to generate a calibration curve (log HCV concentration versus the threshold cycle [C_T] at which a reactive level of fluorescent signal is detected). The lot specific values for Calibrator A and Calibrator B are specified on each Abbott RealTime HCV Calibrator Kit Card and must be entered into the assay test order when a run is performed. The calibration curve slope and intercept are calculated and stored on the instrument. The concentration of HCV RNA in a sample is calculated from the stored calibration curve. Results are automatically reported on the Abbott *m2000rt* workstation.

The Abbott RealTime HCV Low and High Positive Controls and Negative Control must be included in the calibration run.

Once an Abbott RealTime HCV calibration is accepted and stored, it may be used for 6 months. During this time, all subsequent samples may be tested without further calibration unless:

- An Abbott RealTime HCV Amplification Reagent Kit with a new lot number is used.
- An Abbott *mSample* Preparation System (4 x 24 Preps) with a new lot number is used.
- A new version of the Abbott RealTime HCV application specification file is installed.
- An optical calibration of the Abbott *m2000rt* is performed per the Calibration Procedures section of the Abbott *m2000rt* Operations Manual.

Quality Control Procedures:

Detection of Inhibition

An IC threshold cycle [C_T] assay validity parameter is established during a calibration run. A defined, consistent quantity of IC is introduced into each specimen, calibrator, and control at the beginning of sample preparation and measured on the Abbott *m2000rt* instrument to demonstrate proper specimen

processing and assay validity. The IC is composed of an RNA sequence unrelated to the HCV target sequence. The median amplification cycle at which the IC target sequence fluorescent signal is detected in calibration samples establishes the IC C_T validity range to be met by all subsequent processed specimens using that calibration curve. Specimens whose IC C_T value exceeds the established range must be retested starting with sample preparation.

Negative and Positive Controls

An Abbott RealTime Negative Control, Low Positive Control, and High Positive Control are included in each run to evaluate run validity.

The lot specific values for the Low Positive Control and High Positive Control are specified on each Abbott RealTime HCV Control Kit Card and must be entered into the Abbott *m2000sp* test order when a run is performed.

An error control flag is displayed when a control result is out of range. The user should refer to the Abbott *m2000rt* Operations Manual for an explanation of the error code flag with suggested corrective actions. If negative or positive controls are out of range, all of the specimens and controls from that run must be reprocessed, beginning with sample preparation.

The presence of HCV must not be detected in the Negative Control. HCV detected in the Negative Control is indicative of contamination by other samples or by amplified product introduced during sample preparation or during preparation of the Abbott 96-Well Optical Reaction Plate. To avoid contamination, the user should clean the Abbott *m2000sp* and Abbott *m2000rt* instruments and repeat the sample processing for controls and specimens following the Procedural Precautions.

Results Calculation

The Abbott RealTime HCV assay results and interpretations are reported as follows. An assay result is reported in units of either Log IU/mL or IU/mL and the unit is selected as described in the Operating Instructions section of the *m2000rt* Operations Manual:

Result/Units		Interpretation
Log IU/mL	IU/mL	
Not Detected	Not Detected	Target not detected
< 1.08 Log IU/mL	< 12 IU/mL	Detected ^a
1.08 to 8.00 Log IU/mL ^b	12 to 100,000,000 IU/mL ^b	
> 8.00 Log IU/mL ^c	> 100,000,000 IU/mL ^c	> ULQ

^a Below LLQ (lower limit of quantitation or LLoQ); HCV RNA can be detected but is not quantifiable.

^b A result between 1.08 and 8.00 Log IU/mL (12 to 100,000,000 IU/mL) indicates that HCV RNA was detected and the concentration falls between the LLQ and ULQ.

^c A result of "> 8.00 Log IU/mL" ("> 100,000,000 IU/mL") indicates that target was detected and is greater than ULQ (upper limit of quantitation).

If quantitative results are desired for those specimens with interpretation "> ULQ," the original specimen should be diluted 1:100 with HCV-negative plasma or serum (consistent with the matrix of the original specimen) and the test repeated. The reported result is then multiplied by the dilution factor of 100 to obtain the quantitative result.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

Currently, monitoring of patients undergoing HCV antiviral therapy includes measurement of the complete blood count, serum creatine and alanine aminotransferase (ALT) levels and HCV RNA. Molecular methods currently available for the quantitation of HCV RNA are the Versant HCV RNA 3.0 Assay (bDNA) and the Roche Cobas[®] TaqMan[®] HCV Test.

VII. MARKETING HISTORY

The Abbott RealTime HCV assay received CE certification and was launched in July 2005 outside of the United States under the list number of 4J86. The following countries receive the Abbott RealTime HCV assay with list number 4J86:

Argentina, Australia, Austria, Belgium, Brazil, Canada, Colombia, Costa Rica, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, India, Ireland, Italy, Japan, Latvia, Liechtenstein, Lithuania, Luxembourg, Malta, Mexico, Netherlands, New Zealand, Norway, Poland, Portugal, Slovenia, Slovakia, Spain, Sweden, Thailand, Taiwan, United Kingdom, Yugoslavia, Switzerland.

Under list number 2N26, it is currently being marketed in Korea and Brazil.

The CE-marked version of the assay has identical reagent components in the three kits in this submission, namely, Abbott RealTime HCV Amplification Reagent Kit, Abbott RealTime HCV Control Kit, and Abbott RealTime HCV Calibrator Kit.

This product has not been withdrawn from the market from any country related to safety or effectiveness, or for any other reasons.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Below is a list of the potential adverse effects (e.g., complications) associated with the use of the device.

An erroneous test result too low or too high may occur with the Abbott RealTime HCV assay. An erroneous low test result may lead a clinician to believe that the current therapy is effective when it is not. Consequently, the clinician could fail to implement a more appropriate therapy. An erroneous high test result may lead a clinician to believe that the current therapy is not effective. Consequently, the clinician could implement an inappropriate change in therapy. The possibility of incorrect results can happen with assignable causes such as a technician's error in following the procedures in the package insert or a device malfunction. However, if the appropriate instruction is followed as stated in the package insert, the likelihood of erroneous results are minimal from the use of this device.

The performance of the product in the clinical studies indicates that the benefit to the patient outweighs any potential risk of adverse effect to the patient as a result of its use.

There were no specific adverse events that occurred in the clinical studies.

IX. SUMMARY OF PRECLINICAL STUDIES

A. Laboratory Studies

Traceability to the WHO Standard

The Abbott RealTime HCV assay is standardized to the Second WHO International Standard (WHO IS) for Hepatitis C Virus RNA (NIBSC Code 96/798).² The Abbott RealTime HCV assay uses two calibrators targeted to 3.00 and 7.00 log IU/mL. Primary Calibrators are assigned lot-specific HCV RNA concentrations based on the results of direct testing against the WHO IS. Product Calibrators are, in turn, assigned lot-specific HCV RNA concentrations based on the results of direct testing against Primary Calibrators. The lot-specific quantitation values for each HCV Calibrator, Primary or Product, are entered into the *m2000rt* software when a calibration run is performed.

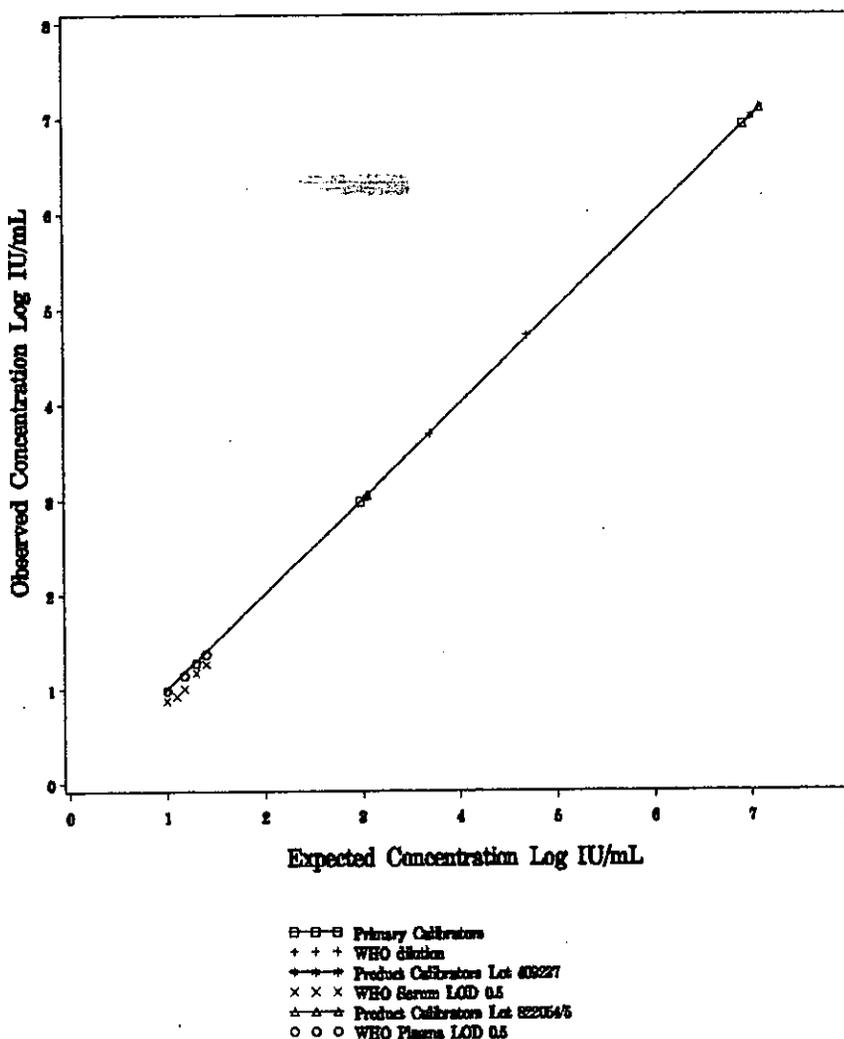
Standardization to the WHO IS was demonstrated by comparison of observed HCV RNA concentrations of dilutions of the WHO IS to expected concentrations based on the WHO IS assigned value of 5.00 log IU/mL. Three comparisons are shown in the figure below. In each comparison, observed HCV RNA concentrations of dilutions of the WHO IS are plotted as a function of the expected concentration. The observed concentrations of the calibrators used for each WHO IS comparison are also plotted against the expected concentrations of the calibrators. Expected concentrations for the calibrators are the lot-

specific values. For calibrators, the observed concentrations are equal to the expected concentrations.

In the first comparison, dilutions of the WHO IS, reconstituted per instructions and diluted 1:2 and 1:20 with negative human plasma to expected concentrations of 4.70 and 3.70 log IU/mL, were quantitated by Primary Calibrators. In the two additional comparisons, WHO IS dilutions from 1.00 to 1.40 log IU/mL (10 to 25 IU/mL), prepared for LoD studies were quantitated by two separate lots of Product Calibrators.

The mean difference between observed and expected concentration for each dilution of the WHO IS ranged from -0.17 to 0.03 log IU/mL. These results demonstrate that the Abbott RealTime HCV assay, using either Primary Calibrators or Product Calibrators, returns quantitation values for the WHO International Standard in good agreement with expected values in a range from half the concentration of the undiluted standard to the lower limit of quantitation (LLQ) of the assay, as shown in the figure below.

Abbott RealTime HCV: Standardization to Second WHO IS

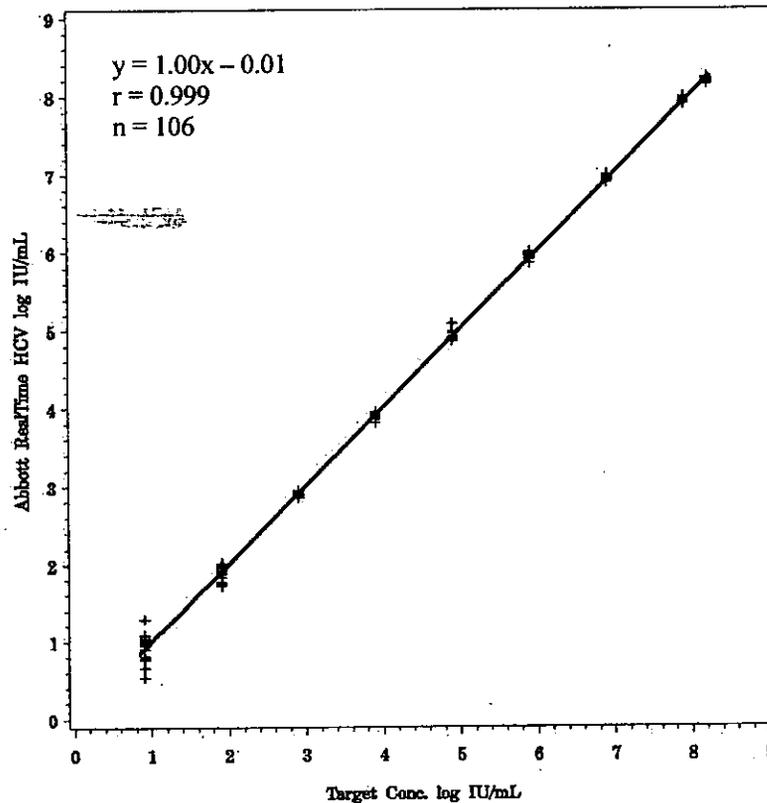


Linear Range

The linearity analysis was performed according to CLSI Guideline EP6-A "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline."³ A 9-member panel prepared by diluting HCV armored RNA (Genotype 1) from 8.21 log IU/mL to 0.91 log IU/mL in HCV negative human plasma was tested. Data from this study demonstrated that the RealTime HCV assay is capable of quantitating HCV in human plasma across the linear range with a deviation of not more than 0.08 log IU/mL. The upper limit of quantitation (ULQ) for the Abbott RealTime HCV assay was determined to be 100,000,000 IU/mL (8.00 log IU/mL) and the lower limit of quantitation was equivalent to LoD (12 IU/mL or 1.08 log IU/mL).

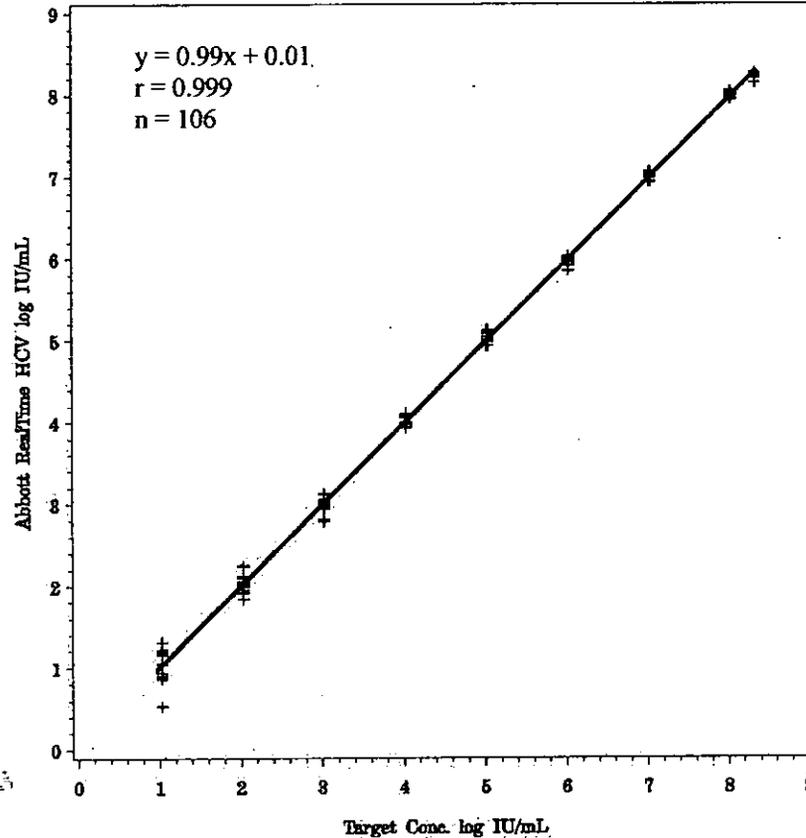
The results demonstrating the Abbott RealTime HCV assay linearity in plasma are shown in the figure below.

Abbott RealTime HCV: Linearity in Plasma



A 9-member panel prepared by diluting HCV armored RNA (Genotype 1) from 8.33 log IU/mL to 1.03 log IU/mL in HCV negative human serum was tested. The analysis for this study is summarized in the figure below. Data from this study demonstrated that the RealTime HCV assay is capable of quantitating HCV in human serum across the linear range with a deviation of not more than 0.10 log IU/mL.

Abbott RealTime HCV: Linearity in Serum



The two studies described above demonstrate that the Abbott RealTime HCV assay was linear in plasma and serum across the range of HCV RNA concentrations tested for Genotype 1.

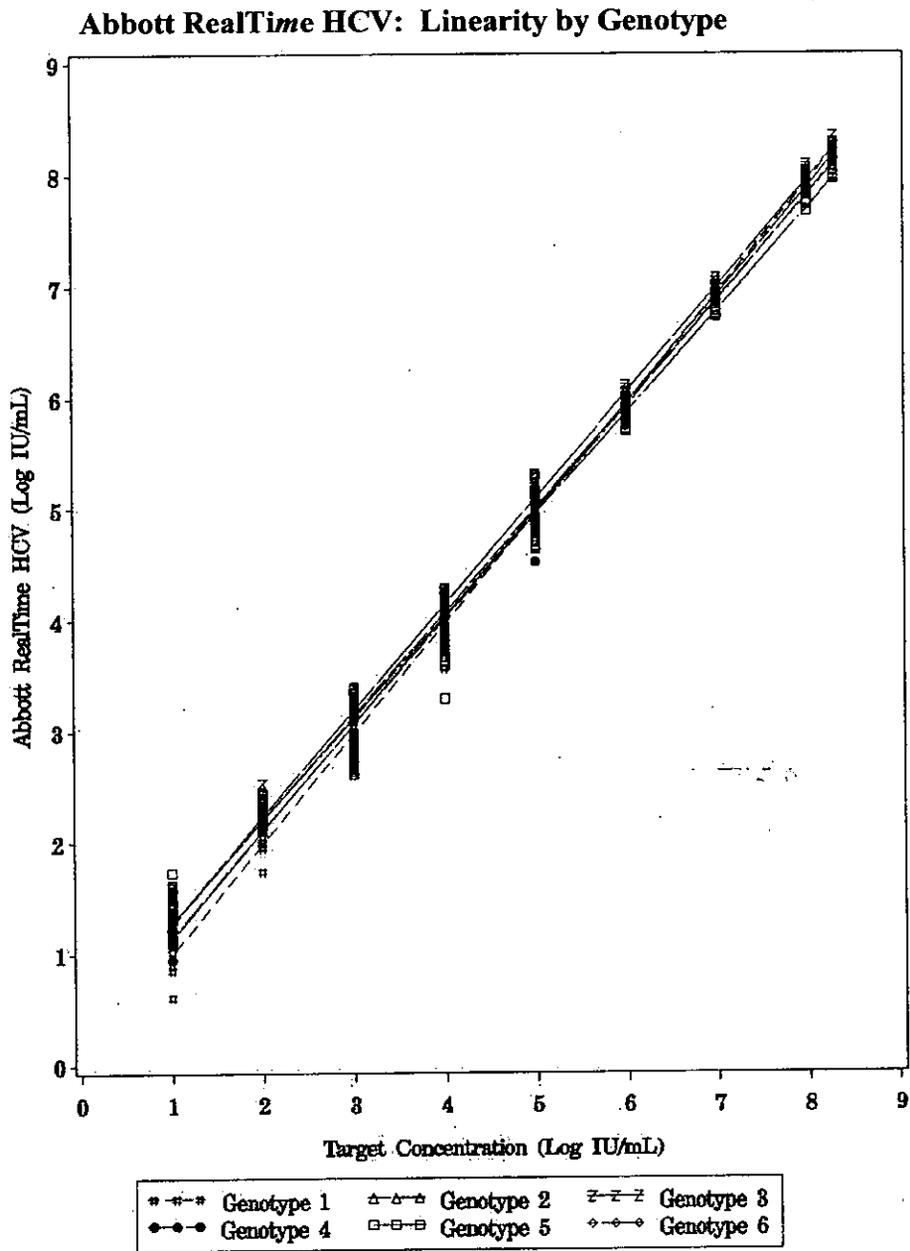
Linearity by HCV Genotype

The linear range for each of Genotypes 1 through 6 was evaluated per the recommendations in the CLSI Guideline EP6A, "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline."³

The linearity of the RealTime HCV assay was demonstrated by evaluating a dilution series for each HCV Genotype 1 through 6 with concentrations ranging from 1.00 log IU/mL to 8.30 log IU/mL. Two linearity panels were used to evaluate the linear range. These panels consisted of dilutions in plasma 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 panel members were tested in replicates of 12. The study was performed using two lots of the Abbott RealTime HCV reagents.

The results of these analyses demonstrated that the RealTime HCV assay is capable of quantitating different HCV genotypes across the linear range with deviation of not more

than 0.28 log IU/mL. The results are summarized in the following figure and table.



Abbott RealTime HCV: Linearity of Assay by HCV Genotypes

Genotype	Linear Equation from Linearity Study	Maximum Difference Between Genotype 1 and Corresponding Genotype (log IU/mL) ^a
1	$Y = 0.99X + 0.01$	NA
2	$Y = 0.93X + 0.33$	0.26
3	$Y = 0.95X + 0.33$	0.28
4	$Y = 0.95X + 0.20$	0.15
5	$Y = 0.92X + 0.37$	0.28
6	$Y = 0.96X + 0.17$	0.13

^a The maximum difference was obtained at either the assay ULQ (ULoQ) or LLQ (LLoQ).

Limit of Detection (LoD) using the Second WHO international Standard

The LoD was determined by testing dilutions of the Second WHO International Standard for Hepatitis C Virus RNA (NIBSC 96/798)² prepared in HCV negative human plasma or serum. The WHO IS consists of HCV Genotype 1. Testing was performed with three lots of amplification reagents on three instrument systems. Probit analysis of the data was used to determine the concentration of the WHO Standard detected with 95% probability. The results for plasma and serum are summarized in the following two tables.

Abbott RealTime HCV: Limit of Detection (LoD) for Plasma (IU/mL)

Concentration (IU/mL)	Number Tested	Number Detected	Percent Detected
25.0	57	57	100
20.0	57	57	100
15.0	57	55	96
12.5	57	53	93
10.0	57	56	98
7.5	57	51	89
5.0	57	46	81
2.5	57	33	58

Probit analysis of the data in the above table determined that the concentration of HCV RNA detected in human plasma with 95% probability was 10.5 IU/mL (95% CI 8.6-14.0 IU/mL).

Abbott RealTime HCV: Limit of Detection (LoD) for Serum (IU/mL)

Concentration (IU/mL)	Number Tested	Number Detected	Percent Detected
25.0	60	60	100
20.0	60	60	100
15.0	60	60	100
12.5	60	60	100
10.0	60	58	97
7.5	60	56	93
5.0	60	53	88
2.5	60	39	65

Probit analysis of the data in the above table determined that the concentration of HCV RNA detected in human serum with 95% probability was 7.2 IU/mL (95% CI 6.0-9.4 IU/mL).

The LoD of the Abbott RealTime HCV assay is determined as 12 IU/mL for plasma and serum.

Limit of Detection (LoD) by Genotype Using Clinical Specimens

The Limits of Detection (LoDs) for HCV Genotypes 1, 2, 3, 4, 5, and 6 were determined by analyses of dilution series of a single patient specimen for each HCV genotype. Dilutions were made in HCV negative human plasma to create an eight-member panel for each genotype. Panel members of each HCV genotype were targeted to concentrations of 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 20.0, and 25.0 IU/mL. Five replicates of each level were tested in each of six runs for a total of 30 replicates per level.

Probit analysis of the data was used to determine the concentration of each HCV genotype detected with 95% probability. The LoDs determined by Probit analysis ranged from 4.4 to 11.0 IU/mL as shown in the table below. Thus, the results of this study demonstrate LoDs for each genotype that are consistent with the LoD of 12 IU/mL (1.08 log IU/mL) determined with the Second WHO International Standard. The LoDs from Probit analysis were consistent with values determined as the lowest concentrations that gave a positivity rate of at least 95%: the latter values ranged from 7.5 to 12.5 IU/mL.

**Abbott RealTime HCV: Limit of Detection by Genotype
Percent Detected**

Genotype	HCV Genotype Concentration (IU/mL)								Probit Analysis	
	25.0	20.0	15.0	12.5	10.0	7.5	5.0	2.5	LoD (IU/mL)	95% CI
1	100%	100%	100%	100%	93%	97%	77%	63%	8.3	(6.5, 12.4)
2	100%	100%	100%	100%	97%	93%	93%	77%	6.4	(4.7, 10.5)
3	100%	100%	100%	100%	97%	100%	87%	57%	6.6	(5.2, 9.6)
4	100%	100%	100%	100%	90%	80%	77%	60%	11.0	(8.5, 16.8)
5	100%	100%	100%	100%	100%	93%	87%	67%	6.7	(5.3, 10.2)
6	100%	100%	100%	100%	100%	97%	93%	90%	4.4	(2.3, 7.8)

Limit of Quantitation

The total analytical error (TAE) was calculated using estimates determined through analysis of data from Limit of Detection (LoD) studies (Genotype 1) and internal precision and external reproducibility studies (Genotypes 1 and 3). The TAE estimates for plasma panel members that had an observed concentration at or near the assay limit of detection (12 IU/mL or 1.08 log IU/mL) are presented in the following table. TAE was estimated by two different methods (see table footnotes).

**Abbott RealTime HCV: Total Analytical Error (TAE) Estimates (Plasma)
(log IU/mL)**

Plasma Panel Member	Genotype	n	Concentration (log IU/mL)		Bias	Total SD	TAE ^c Absolute Bias + (2 x SD)	TAE ^d SQRT(2) x (2 x SD)
			Expected	Observed				
Internal Precision Study 1	3	65	1.00	0.88 ^a	-0.12	0.25 ^b	0.62	0.71
Internal LoD Study 4	1	56	1.00	0.99 ^a	-0.01	0.26	0.53	0.74
5	1	53	1.10	1.11	0.02	0.25	0.51	0.69
6	1	55	1.18	1.14	-0.03	0.25	0.52	0.69
External Reproducibility Study 4	1	269	1.23	1.07 ^a	-0.16	0.21 ^b	0.58	0.59
5	1	242	0.68	0.61 ^a	-0.07	0.25 ^b	0.57	0.71

Plasma Panel Member	Genotype	n	Concentration (log IU/mL)		Bias	Total SD	TAE ^c Absolute Bias + (2 x SD)	TAE ^d SQRT(2) x (2 x SD)
			Expected	Observed				
9	3	270	1.34	1.34	0.00	0.17 ^b	0.34	0.48
10	3	252	0.76	0.73 ^a	-0.03	0.30 ^b	0.63	0.84

^a Panel member is below the LoD (1.08 log IU/mL)

^b Total SD = Within-run component variability + Between-run component variability.

^c Per section 5.1 of EP17-A CLSI guideline.⁴

^d Based upon the difference between two measurements approach.

The TAE estimates for serum panel members that had an observed concentration at or near the assay limit of detection (12 IU/mL or 1.08 log IU/mL) are presented in the table below.

**Abbott RealTime HCV: Total Analytical Error (TAE) Estimates (Serum)
(log IU/mL)**

Serum Panel Member	Genotype	n	Concentration (log IU/mL)		Bias	Total SD	TAE ^b Absolute Bias + (2 x SD)	TAE ^c SQRT(2) x (2 x SD)
			Expected	Observed				
Internal LoD Study								
4	1	58	1.00	0.87 ^a	-0.13	0.21	0.55	0.59
5	1	60	1.10	0.93 ^a	-0.17	0.22	0.61	0.63
6	1	60	1.18	1.01 ^a	-0.17	0.19	0.55	0.54

^a Panel member is below the LoD (1.08 log IU/mL).

^b Per section 5.1 of EP17-A CLSI guideline.⁴

^c Based upon the difference between two measurements approach.

The results of these analyses demonstrated that the Abbott RealTime HCV assay can determine with an acceptable level of accuracy the concentration of HCV RNA in plasma (EDTA) and serum at a concentration of 12 IU/mL (1.08 log IU/mL). At this concentration, the difference between two measurements of more than 1.00 log IU/mL is statistically significant.

Limit of Quantitation by HCV genotype

The total analytical error (TAE) was calculated by analysis of data from the genotype Limit of Detection (LoD) study. HCV Genotypes 1 through 6 were tested in plasma. For each genotype, panel members were targeted to concentrations of 1.00, 1.10, and 1.18 log IU/mL (10.0, 12.5 and 15.0 IU/mL, respectively). The results are summarized in the following table.

**Abbott RealTime HCV: Total Analytical Error (TAE) Estimates by Genotype
(log IU/mL)**

Genotype / LoD Panel Member	n	Concentration (log/IU/mL)		Bias	Total SD	TAE ^b Absolute Bias + (2 x SD)	TAE ^c SQRT(2) x (2 x SD)
		Expected	Observed				
Genotype 1							
4	28	1.00	0.94 ^a	-0.06	0.30	0.66	0.85
5	30	1.10	0.95 ^a	-0.15	0.31	0.77	0.88
6	30	1.18	1.12	-0.06	0.20	0.45	0.56
Genotype 2							
4	29	1.00	0.97 ^a	-0.03	0.28	0.59	0.79
5	30	1.10	1.10	0.00	0.21	0.43	0.61
6	30	1.18	1.19	0.02	0.25	0.51	0.70
Genotype 3							
4	29	1.00	0.99 ^a	-0.01	0.23	0.47	0.64
5	30	1.10	1.12	0.02	0.20	0.42	0.56
6	30	1.18	1.26	0.08	0.16	0.40	0.46
Genotype 4							
4	27	1.00	0.87 ^a	-0.13	0.25	0.63	0.72
5	30	1.10	1.04 ^a	-0.05	0.16	0.37	0.45
6	30	1.18	1.19	0.01	0.20	0.41	0.56
Genotype 5							
4	30	1.00	0.96 ^a	-0.04	0.24	0.53	0.69
5	30	1.10	1.11	0.01	0.20	0.41	0.56
6	30	1.18	1.19	0.01	0.31	0.63	0.87
Genotype 6							
4	30	1.00	1.08	0.08	0.23	0.54	0.64
5	30	1.10	1.15	0.05	0.23	0.52	0.66
6	30	1.18	1.23	0.06	0.22	0.49	0.61

^a Panel member is below the LoD (1.08 log IU/mL)

^b Per section 5.1 of EP17-A CLSI guideline⁴

^c Based upon the difference between two measurements approach.

The results of these analyses demonstrated that, for samples of HCV genotypes 1 through 6, the Abbott RealTime HCV assay can determine with an acceptable level of accuracy the concentration of HCV RNA in plasma (EDTA) and serum at a concentration of 12 IU/mL (1.08 log IU/mL). At this concentration, the difference between two measurements of more than 1.00 log IU/mL is statistically significant.

Precision

Within-Laboratory Precision (Lot-to-Lot)

The precision of the Abbott RealTime HCV assay was evaluated using an 8-member HCV RNA panel. Panel members 1 through 5 were dilutions of an HCV Genotype 3 clinical sample. Panel members 1, 3, and 5 were diluted in HCV negative human plasma and panel members 2 and 4 were diluted in HCV negative human serum. Panel members 6 through 8 were prepared by diluting HCV armored RNA in HCV negative human plasma. Three Abbott *m2000* Instrument Systems were used with a unique lot of amplification reagents assigned to each. One run per day was performed on each instrument pair for five days for a total of 15 runs. Panel members 1 through 8 were run in replicates of four in the first run on each instrument pair and replicates of five in each subsequent run for a total of 72 replicates across the three Abbott *m2000* Instrument Systems. Within-run, between-run, between lot/instrument, and total standard deviations for log IU/mL and %CV for IU/mL were determined. The total SD for the Abbott RealTime HCV assay was found to be less than or equal to 0.10 log IU/mL for all panel members that exceeded the assay limit of detection (12 IU/mL or 1.08 log IU/mL). The results, representative of the precision of the Abbott RealTime HCV assay, are summarized in the following two tables.

**Abbott RealTime HCV: Within-Laboratory Precision Analysis: Lot-to-Lot
(log IU/mL)**

Panel Member	Genotype	n	Mean Concentration (log IU/mL)	Within-Run Component SD ^a	Between-Run Component SD ^a	Between-Lot/Instrument Component SD ^a	Total SD ^{a,b}
1	3	65 ^c	0.88	0.25	0.00	0.13	0.28
2	3	72	1.96	0.08	0.04	0.04	0.10
3	3	72	2.75	0.05	0.03	0.04	0.08
4	3	72	3.95	0.03	0.02	0.03	0.05
5	3	71 ^d	4.79	0.04	0.02	0.04	0.06
6	1	71 ^d	5.97	0.04	0.02	0.02	0.05
7	1	71 ^d	7.00	0.07	0.01	0.02	0.08
8	1	71 ^d	8.04	0.04	0.01	0.05	0.06

^a Standard Deviations (SD) are in log IU/mL.

^b Includes Within-Run, Between-Run, and Between-Lot/Instrument components.

^c HCV RNA was not detected in seven replicates. This level is below the LoD (1.08 log IU/mL).

^d One replicate was not available for the data analysis due to an instrument error.

**Abbott RealTime HCV: Within-Laboratory Precision Analysis: Lot-to-Lot
(IU/mL)**

Panel Member	Genotype	n	Mean Concentration (IU/mL)	Within-Run Component %CV	Between-Run Component %CV	Between-Lot/Instrument Component %CV	Total %CV ^a
1	3	65 ^b	9	53.8	0.0	22.6	58.4
2	3	72	94	18.5	9.6	10.7	23.4
3	3	72	572	12.5	7.1	9.0	17.0
4	3	72	8,901	6.5	5.5	7.5	11.4
5	3	71 ^c	61,792	8.8	5.2	8.8	13.5
6	1	71 ^c	930,544	8.4	5.7	4.6	11.2
7	1	71 ^c	10,199,543	16.5	0.0	7.2	17.9
8	1	71 ^c	110,214,079	8.5	3.3	11.7	14.9

^a Includes Within-Run, Between-Run, and Between-Lot/Instrument components.

^b HCV RNA was not detected in seven replicates. This level is below the LoD (12 IU/mL).

^c One replicate was not available for the data analysis due to an instrument error.

Within-Laboratory Precision (Operator-to-Operator)

The within-run, between-run, and between-technician (operator) precision of the Abbott RealTime HCV Assay was evaluated by testing 84 replicates each of HCV panel members that span the dynamic range of the assay from approximately 1.08 log IU/mL to approximately 8.0 log IU/mL for HCV Genotypes 1 and 3. Panel members 1 through 5 were HCV Genotype 1, and panel members 6 through 10 were HCV Genotype 3. One lot of amplification reagents was run on one *m2000sp* and *m2000rt* instrument pair by three technicians. Each technician completed one run per day for seven days, for a total of 21 runs. Four replicates were run for each panel member.

The SD for between-technician component and total SD for the Abbott RealTime HCV assay were found to be less than or equal to 0.02 log IU/mL and 0.23 log IU/mL, respectively, for all panel members that exceeded the assay limit of detection (1.08 log IU/mL). The results are summarized in the table below.

**Abbott RealTime HCV: Within-Laboratory Precision Analysis: Operator-to-Operator
(log IU/mL)**

Panel Member	Genotype	n	Mean Concentration (log IU/mL)	Within-Run Component SD ^{a,b}	Between-Run Component SD ^{a,c}	Between-Technician Component SD ^{a,d}	Total SD ^{a,e}
1	1a	84	7.94	0.05	0.02	0.00	0.05
2	1a	84	5.09	0.08	0.02	0.00	0.08
3	1a	84	3.16	0.07	0.03	0.01	0.08
4	1a	84	1.09	0.23	0.00	0.00	0.23
5	1a	76 ^f	0.59	0.23	0.03	0.00	0.24
6	3	84	6.89	0.07	0.00	0.00	0.07
7	3a	84	4.43	0.09	0.01	0.00	0.09
8	3a	84	2.60	0.10	0.02	0.02	0.10
9	3a	82 ^g	1.29	0.17	0.04	0.00	0.17
10	3a	70 ^h	0.70	0.19	0.03	0.00	0.20

^a Standard Deviations (SD) are in log IU/ml.

^b Within-Run Component = Intra-Run component

^c Between-Run Component = Inter-Run component

^d Between-Technician component = Inter-Operator component

^e Total SD = Intra-Run component + Inter-Run component + Inter-Operator component

^f One replicate was not included due to an instrument error. Seven replicates generated a result of "Target Not Detected" and were not included in the analysis. This level is below the LoD (1.08 log IU/mL).

^g Two replicates were not included due to an instrument error.

^h Fourteen replicates generated a result of "Target Not Detected" and were not included in the analysis. This level is below the LoD (1.08 log IU/mL).

Reproducibility

The Reproducibility panel tested consisted of a 90-member panel (consisting of 10 unique panel members). The HCV genotypes selected for the Reproducibility panel were genotypes that were recognized as prevalent in the U.S. population. The panel included five concentration levels of each of two HCV genotypes with each level represented nine times. All panel members were diluted in a base matrix of defibrinated human plasma. Panel member 1 consisted of HCV Genotype 1a armored RNA. Panel members 2, 3, 4, and 5 were prepared from a mix of two unique donor units of HCV Genotype 1a. Panel member 6 consisted of HCV Genotype 3 armored RNA. Panel members 7, 8, 9, and 10 were prepared from a mix of two unique donor units of HCV Genotype 3a.

The concentration levels targeted for the Reproducibility panel spanned the linear quantitation range of the assay and also included some members below the lower limit of

quantitation. A total of three Abbott RealTime HCV Amplification reagent lots were used. Each of the three clinical sites tested two of the three Amplification reagent lots for five nonconsecutive days each, resulting in a total of 10 reproducibility runs at each site. The Reproducibility results are summarized in the following two tables.

**Abbott RealTime HCV: Reproducibility
(log IU/mL)**

Panel Member	Genotype	n	Mean Concentration (log IU/mL)	Within-Run Component SD ^a	Between-Run Component SD ^a	Between-Lot Component SD ^a	Between-Site Component SD ^a	Total SD ^{a,b}
1	1a	270	7.98	0.04	0.00	0.02	0.08	0.09
2	1a	269 ^c	5.15	0.05	0.03	0.00	0.05	0.08
3	1a	270	3.17	0.07	0.02	0.00	0.02	0.08
4	1a	269 ^d	1.07 ^j	0.21	0.00	0.03	0.04	0.22
5	1a	242 ^e	0.61 ^j	0.25	0.03	0.05	0.07	0.27
6	3	266 ^f	6.96	0.06	0.02	0.02	0.06	0.09
7	3a	270	4.51	0.06	0.03	0.01	0.04	0.08
8	3a	270	2.61	0.07	0.02	0.01	0.03	0.08
9	3a	270 ^g	1.34	0.17	0.00	0.04	0.07	0.19
10	3a	252 ^{c,h,i}	0.73 ^j	0.29	0.05	0.08	0.00	0.31

^a Standard deviations are in log IU/mL.

^b The total variability contains within-run, between-run, between-lot, and between-site variability.

^c One invalid replicate not included.

^d Target not detected for one sample of panel 4.

^e Target not detected for twenty-eight samples of panel 5.

^f Four invalid replicates not included.

^g One replicate was an outlier. Without this replicate, the Panel 9 mean concentration was 1.34 log IU/mL, within-run component SD was 0.15, the between-run component SD was 0.00, the between-lot component SD was 0.04, the between-site SD was 0.06, and the total SD was 0.17.

^h Target not detected for seventeen samples of panel 10.

ⁱ Two replicates were outliers. Without these replicates, the Panel 10 mean concentration was 0.71 log IU/mL, within-run component SD was 0.26, the between-run component SD was 0.05, the between-lot component SD was 0.06, the between-site SD was 0.00, and the total SD was 0.27.

^j Concentration is below the assay LoD (1.08 log IU/mL).

**Abbott RealTime HCV:
Reproducibility
(IU/mL)**

Panel Member	Genotype	n	Mean Concentration (IU/mL)	Within-Run Component %CV	Between-Run Component %CV	Between-Lot Component %CV	Between-Site Component %CV	Total %CV ^a
1	1a	270	96,867,941	9.9	0.0	4.3	18.1	21.1
2	1a	269 ^b	141,936	10.6	7.2	0.0	11.9	17.5
3	1a	270	1,501	14.5	5.3	0.0	3.6	15.9
4	1a	269 ^{c,d}	13	54.4	0.0	3.9	8.7	55.2
5	1a	242 ^e	5 ^j	56.5	0.0	10.2	18.1	60.2
6	3	266 ^f	9,315,103	12.9	3.5	4.9	13.7	19.8
7	3a	270	32,732	12.5	5.8	1.9	10.1	17.2
8	3a	270	413	14.4	4.7	2.2	6.1	16.5
9	3a	270 ^g	25	119.8	14.7	8.1	19.3	122.5
10	3a	252 ^{b,h,i}	8 ^j	268.3	0.0	49.7	0.0	272.9

^a The total variability contains within-run, between-run, between-lot, and between-site variability.

^b One invalid replicate not included.

^c Two replicates were outliers. Without these replicates, the Panel 4 mean concentration was 13 IU/mL, within-run component %CV was 41.5, the between-run component %CV was 2.7, the between-lot component %CV was 4.6, the between-site %CV was 11.6, and the total %CV was 43.5.

^d Target not detected for one sample of panel 4.

^e Target not detected for twenty-eight samples of panel 5.

^f Four invalid replicates not included.

^g One replicate was an outlier. Without this replicate, the Panel 9 mean concentration was 23 IU/mL, within-run component %CV was 36.1, the between-run component %CV was 0.0, the between-lot component %CV was 7.1, the between-site %CV was 12.5, and the total %CV was 38.9.

^h Target not detected for seventeen samples of panel 10.

ⁱ Two replicates were outliers. Without these replicates, the Panel 10 mean concentration was 6 IU/mL, within-run component %CV was 96.2, the between-run component %CV was 0.0, the between-lot component %CV was 28.0, the between-site %CV was 0.0, and the total %CV was 100.2.

^j Concentration is below the assay LoD (12 IU/mL).

Analytical Specificity

Potentially Interfering Substances

The susceptibility of the Abbott RealTime HCV assay to interference by elevated levels of potentially interfering substances was evaluated. HCV negative plasma samples and plasma samples containing 50 IU/mL and 10,000 IU/mL of HCV RNA were spiked with high levels of hemoglobin, bilirubin, protein, or triglycerides and tested.

No interference in the performance of the Abbott RealTime HCV assay was observed in the presence of the following endogenous substances for all HCV positive and negative samples tested:

Hemoglobin 2 g/L
 Triglycerides 37 mM
 Bilirubin 342 µM
 Protein 120 g/L

Antivirals and antibiotics at concentrations in excess of peak plasma or serum levels were tested in five pools. No interference in the performance of the Abbott RealTime HCV assay was observed in the presence of the following drug pools for all HCV positive and negative samples tested:

Drug Pool Drugs Tested

- 1 Zidovudine, Saquinavir, Ritonavir, Clarithromycin, Interferon 2b
- 2 Abacavir sulfate, Amprenavir, Peginterferon 2a, Peginterferon 2b, Ribavirin
- 3 Tenofovir disoproxil fumarate, Lamivudine, Indinavir sulfate, Ganciclovir, Valganciclovir hydrochloride, Acyclovir
- 4 Stavudine, Efavirenz, Lopinavir, Enfuvirtide, Ciprofloxacin
- 5 Nevirapine, Nelfinavir, Azithromycin, Valacyclovir

Cross-reactivity studies with clinical specimens

The specificity of the assay was evaluated by testing patient specimens that were positive for at least one of the following DNA virus markers, RNA viruses, non-viral hepatitis, or autoimmune disease states.

DNA and RNA Viruses	n	Autoimmune States and Non-viral Hepatitis	n
Hepatitis A Virus	2	Systemic lupus erythematosus (SLE)	10
Hepatitis B Virus	12	Anti-nuclear antibodies (ANA)	12
Human T-Cell Leukemia Virus-I	5	Rheumatoid factor (RF)	12
Human T-Cell Leukemia Virus-II	5	Hepatocellular carcinoma	2
Human Immunodeficiency Virus-1	12	Alcoholic hepatitis	2
Human Immunodeficiency Virus-2	10	Non-alcoholic steatohepatitis (NASH)	2
Flavivirus		Cirrhosis	2
(West Nile virus and GB virus-C)	10	Autoimmune hepatitis	2

HCV RNA was detected but not quantifiable (less than LoD) in four specimens (two RF, one SLE, and one anti-HIV-1). The specimens were retested in duplicate. HCV RNA was not detected in either retest of one RF specimen and the results were considered negative. HCV RNA was detected below the LoD in one or both replicates of the remaining three specimens (one RF, one SLE, and one anti-HIV-1). Insufficient specimen volume did not allow for resolution. The disease states tested including autoimmune disorders, viral infections, and non-viral liver disease have been shown not to interfere with the quantitation of HCV RNA by the Abbott RealTime HCV assay.

Performance of the assay with HCV-Negative Specimens

The specificity of the Abbott RealTime HCV assay was evaluated by analyzing 760 unique HCV negative specimens; 380 plasma specimens and 380 serum specimens. HCV RNA was detected in two of the specimens tested. The observed specificity for this study was 99.74% (758/760) (95% CI 99.05 to 99.97%).

Cross-Reactivity studies using nucleic acid or viral lysate

The following viruses and microorganisms were evaluated for potential cross-reactivity in the Abbott RealTime HCV assay. Purified nucleic acid or viral lysate from each microorganism or virus was added at a targeted concentration of 100,000 copies/mL or human genomic DNA was added at 1 µg/mL to HCV RNA negative samples and samples that contained HCV RNA targeted to 50 IU/mL and 10,000 IU/mL.

Human immunodeficiency virus 1	Vaccinia virus
Human immunodeficiency virus 2	BK human polyomavirus
Human T-lymphotropic virus 1	Human papilloma virus 16
Hepatitis B virus	Human papilloma virus 18
Epstein-Barr virus	<i>Neisseria gonorrhoeae</i>
Herpes simplex virus 1	<i>Chlamydia trachomatis</i>
Herpes simplex virus 2	<i>Candida albicans</i>
Cytomegalovirus	<i>Staphylococcus aureus</i>
Human herpesvirus 6B	<i>Staphylococcus epidermidis</i>
Human herpesvirus 8	<i>Mycobacterium gordonae</i>
Varicella-zoster virus	<i>Mycobacterium smegmatis</i>
Dengue virus 1	Human genomic DNA

No interference in the performance of the Abbott RealTime HCV assay was observed in the presence of viral or microorganism DNA/RNA at a concentration of 100,000 copies/mL or in the presence of human genomic DNA at less than or equal to 1 µg/mL for all the HCV positive and negative samples tested.

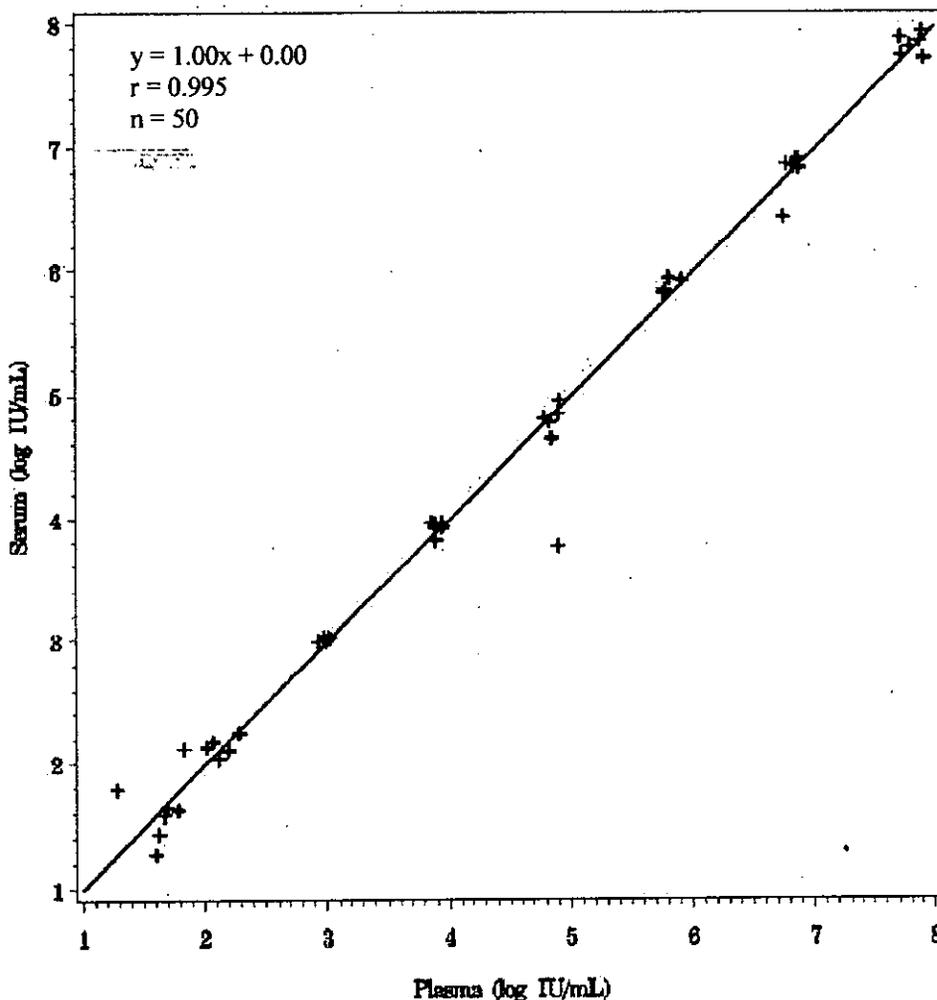
Analytical Carryover

Potential sample carryover within the Abbott RealTime HCV assay was evaluated by testing 372 high titer HCV positive samples (Abbott RealTime HCV Calibrator B with a target concentration of 7.00 log IU/mL) interspersed with 372 negative samples (Abbott RealTime HCV Negative Control). The Abbott RealTime HCV assay did not exhibit detectable carryover from high positive samples to negative samples. The upper 95% CI for percent carryover was 0.99%.

Serum vs. Plasma Across the Linear Range

HCV serologically negative specimens from donors were collected as serum and as plasma in tubes and spiked with an HCV viral stock or with an Armored HCV RNA stock to HCV RNA concentrations across the linear range for a total of 50 matched pairs. The HCV RNA concentrations across the linear range for a total of 50 matched pairs. The HCV RNA concentration from the plasma and the serum specimens were compared. The mean difference between serum and plasma specimens was -0.02 log IU/mL (95% CI -0.08 to 0.04%). The results are presented in the following figure.

Abbott RealTime HCV: Serum vs Plasma Across the Linear Range



Recommended storage stability

The stability study data support 18 month dating for the Abbott RealTime HCV Amplification Reagent Kits when stored at -10°C or colder.

The stability study data support 18 month dating for the Abbott RealTime HCV Control and Calibrator Kit components and the Internal Control from the Amplification Reagent Kit when stored at -10°C or colder.

The stability study data support 30 month dating for the Uracil-N-Glycosylase (UNG) for use in conjunction with Abbott RealTime HCV kit when stored at -15°C to -25°C.

Specimen stability

Specimen stability testing for HCV in whole blood, serum, and plasma was performed. For each test condition, samples from ten unique donors were spiked with HCV virions at a target concentration of 1,000 IU/mL. The samples were divided into aliquots and stored at the test conditions listed in the table below. Freshly drawn specimens (whole blood) may be held at 2 to 30°C for up to 6 hours prior to centrifugation. Serum or plasma specimens may be stored at 15 to 30°C for up to 24 hours, 2 to 8°C for up to 3 days, -10 to -30°C for 60 days, or -70°C or colder for 60 days. Multiple freeze/thaw cycles should be avoided and should not exceed three freeze/thaw cycles. Frozen specimens may be thawed at 15 to 30°C or 2 to 8°C. Thawed specimens may be stored at 2 to 8°C for up to 6 hours, if not processed immediately. Serum and plasma specimens may be stored at -10°C or colder for 60 days. Stability test results are summarized in the table below.

Abbott RealTime HCV: Specimen Stability (log IU/mL)

Sample Type	Test Condition	Test Condition Mean	Baseline Condition Mean	Mean Difference
Whole Blood (Plasma)	6 hours at 28 to 32°C	3.132	3.129	0.003
	6 hours at 2 to 8°C	3.077	3.129	-0.052
Whole Blood (Serum)	6 hours at 28 to 32°C	3.042	3.082	-0.040
	6 hours at 2 to 8°C	3.058	3.082	-0.024
Plasma	24 hours at 28 to 32°C ^b	2.662	2.939	-0.277
	72 hours at 2 to 8°C	2.872	2.939	-0.067
	24 hours at 28 to 32°C, ^b 48 hours at 2 to 8°C	2.601	2.939	-0.338
	60 days at -10 to -30°C, thaw at 2 to 8°C	2.597	2.750	-0.153
	60 days at ≤70°C, thaw at 2 to 8°C	2.603	2.750	-0.147
	5 freeze/thaw cycles ^a , 6 hours at 2 to 8°C	2.728	2.733	-0.005

Sample Type	Test Condition	Test Condition Mean	Baseline Condition Mean	Mean Difference
Serum	24 hours at 28 to 32°C ^b	2.572	2.902	-0.330
	72 hours at 2 to 8°C	2.869	2.902	-0.033
	24 hours at 28 to 32°C, ^b 48 hours at 2 to 8°C	2.478	2.902	-0.424
	60 days at -10 to -30°C, thaw at 2 to 8°C	2.743	2.903	-0.160
	60 days at ≤70°C, thaw at 2 to 8°C	2.853	2.903	-0.050
	5 freeze/thaw cycles ^a , 6 hours at 2 to 8°C	2.774	2.705	0.069

^a freeze at ≤-70°C / thaw at 28 to 32°C

^b 28 to 32°C represents the upper range of room temperature exposure

B. Animal Studies

Not applicable

C. Additional Studies

Not applicable

X. SUMMARY OF PRIMARY CLINICAL STUDIES

The applicant performed a clinical study to establish a reasonable assurance of safety and effectiveness of the Abbott RealTime HCV for quantitation of HCV viral loads in the US, Germany, and Taiwan. Data from this clinical study were the basis for the PMA approval decision. A summary of the clinical study is presented below.

A. Study Population

Retrospectively collected specimens from subjects enrolled in fourteen different multi-center clinical trials were studied. The study population consisted of 356 evaluable chronic hepatitis C (CHC) infected subjects, treated with pegylated interferon alfa 2a or 2b and ribavirin combination therapy.

For site 1, a total of 160 treatment naive, HCV genotype 1, 2, and 3 subjects were enrolled from two European, Phase IV, treatment, safety and efficacy studies.

For site 2, a total of 200 treatment naive, HCV genotype 1 and 2 subjects were enrolled from ten investigator-initiated trials from two hospitals in Asia. Forty three subjects did not meet inclusion/exclusion criteria leaving 157 evaluable subjects.

For site 3, a total of 46 CHC subjects, HCV genotype 1, 2, and 3, treated by standard of care at Liver Clinics at two US Medical Centers, were enrolled from two genetic studies. Seven subjects did not meet inclusion/exclusion criteria leaving 39 evaluable subjects.

Determination of HCV RNA viral levels at Screening, Baseline, Week 4, and Week 12 were performed using the Abbott RealTime HCV Assay. Two predictive analyses were established from the study population based on the availability of specimens at clinically relevant time points as follows: Week 4/RVR Analysis was performed for the subset of subjects with viral load results available for Week 4, EOT, and EOF time points, and Week 12/EVR Analysis was performed for the subset of subjects with viral load results available for Baseline, Week 12, EOT, and EOF time points. Baseline demographics of the overall study population and each site are presented in the table below.

There were no samples from subjects with HCV genotypes 4, 5, and 6 tested in clinical studies with the Abbott RealTime HCV Assay. Therefore, the non-1 genotype label used in the table below and subsequent tables refers to genotypes 2 and 3.

Subject Demographics

Characteristics	Category	Number of Subjects (n)	Percentage of Total
Total Number of Subjects		356	100.0
Age	< 40 years	52	14.6
	≥ 40 years	304	85.4
Gender	Female	172	48.3
	Male	184	51.7
Race/Ethnicity	Asian	163 ^b	45.8
	Black	3	0.8
	Caucasian	180	50.6
	Hispanic / Latino	5	1.4
	Not available	5	1.4
Genotype	1	231 ^c	64.9
	2	96	27.0
	3	29 ^d	8.1
Baseline HCV RNA Genotype 1 ^a	≤ 8.0 x 10 ⁵ IU/mL	138	59.7
	> 8.0 x 10 ⁵ IU/mL	90	39.0
	Missing	3	1.3
Baseline HCV RNA Genotype Non-1 ^a	≤ 8.0 x 10 ⁵ IU/mL	104	83.2
	> 8.0 x 10 ⁵ IU/mL	20	16.0
	Missing	1	0.8

Baseline Biopsy Result	Cirrhotic	30	8.4
	Non-Cirrhotic	326	91.6

^a 8.0×10^5 IU/mL = 5.90 log IU/mL based upon Test of Record

^b One subject's race/ethnicity was Asian and Caucasian mixed and was categorized as Asian

^c One subject's HCV genotype from Site 2 was 1b/2b and was treated as a genotype 1.

^d One subject from Site 1 was co-infected with HCV genotypes 1a and 3a and was treated as a non-1 genotype.

^e Genotype Non-1 in this table and subsequent tables refers to genotypes 2 and 3 and does not include genotypes 4, 5, and 6.

Within-Subject Variability in Absence of Treatment

The objective of this analysis was to estimate the relative contributions of biological variability and assay variability to within subject HCV RNA variability in the absence of treatment.

One-hundred and fifty subjects had both screening and baseline results. These two results were used to estimate within subject variability, which includes biological variability as well as total assay variability. The total assay variability, biological variability, and within subject variability from these results were estimated and are shown in the following table.

Within Subject Variability

Genotype	n	Total Assay Variability SD ^a	Biological Variability SD ^a	Within Subject Variability SD ^a
1	142	0.06	0.33	0.34
Non-1	8	0.06	0.50	0.50

^a Standard deviations are in log IU/mL.

B. Safety and Effectiveness Results

Clinical Study Results and Statistical Analyses

The use of HCV RNA quantitation 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 Weeks 4 and 12.

The primary objective of this study was to evaluate the clinical utility of the Abbott RealTime HCV Assay for the clinical management of patients infected with HCV by estimating the Negative Predictive Value (NPV) and Positive Predictive Value (PPV) for achieving sustained virologic response (SVR) at established clinically relevant time points during antiviral treatment (Week 4/RVR and Week 12/EVR).

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

- Rapid Virologic Response (RVR) Analysis = HCV RNA <LoD (12 IU/mL or 1.08 log IU/mL) at Week 4 antiviral therapy.

- Early Virologic Response (EVR) = achievement of a 2-log drop or greater of HCV RNA or HCV RNA negative at Week 12 of antiviral therapy.
- Sustained Virologic Response (SVR) = HCV RNA negative 24 weeks after cessation of treatment.
 - Undetermined SVR status = Shortened treatment duration or missing EOT / EOF time points with recommended treatment duration.
- Positive Predictive Value (PPV) = the probability of SVR given an on-treatment virologic response at Week 4 or 12.
- Negative Predictive Value (NPV) = the probability of not achieving SVR given no on-treatment virologic response at Week 4 or 12.

Odds Ratio (OR) describes the measure of association between virologic response and SVR. Factors such as HCV genotype, baseline viral load, cirrhosis, age, and gender are cited in the literature as predictors for SVR. The relationship between SVR and RVR or EVR results was studied after adjusting for baseline covariates. Data from the three study sites were analyzed for both PPV and NPV as pooled data and further stratified by genotype.

Association Between Baseline Covariates and Sustained Virologic Response

Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated for established host- and viral- baseline covariates predictive of SVR with peginterferon/ribavirin combination therapy. The statistical significance of the associations of Age, Gender, Genotype, Baseline HCV RNA level, and Baseline Liver Biopsy result are summarized in the table below.

The data used for this analysis comprises 356 subjects who have baseline characteristics and treatment outcome. For all sites combined, genotype (Non-1) and baseline biopsy result (Non-Cirrhotic) had a significant influence on achieving SVR. HCV genotype (Non-1) odds ratio equals 2.93 (95% CI 1.56, 5.81) and baseline biopsy result odds ratio equals 2.48 (95% CI 1.03, 5.74).

Predictors of Sustained Virologic Response at Baseline

Characteristics	Category	n	Percent with SVR	Odds Ratio (95% CI) Using Univariate Analysis
Age	< 40 years	52	86.54	2.07 (0.87, 5.66)
	≥ 40 years	304	75.66	
Gender	Male	184	77.72	1.06 (0.62, 1.79)
	Female	172	76.74	
Genotype	1	231	71.43	2.93 (1.56, 5.81)
	Non-1 ^b	125	88.00	

Baseline HCV RNA for Genotype 1 ^a	≤ 8.0 x 10 ⁵ IU/mL	138	76.09	1.84 (0.99, 3.43)
	> 8.0 x 10 ⁵ IU/mL	90	63.33	
Baseline HCV RNA for Genotype Non-1 ^a	≤ 8.0 x 10 ⁵ IU/mL	104	89.42	2.11 (0.43, 8.29)
	> 8.0 x 10 ⁵ IU/mL	20	80.00	
Baseline Biopsy Result	Cirrhotic	30	60.00	2.48 (1.03, 5.74)
	Non-Cirrhotic ^b	326	78.83	

^a 352 of the 356 subjects have both baseline HCV RNA and treatment outcome.

^b The significance of genotype (Non-1) and baseline biopsy result (Non-Cirrhotic) predicting SVR is demonstrated by the lower 95% CI limit for the odds ratio exceeding 1.0.

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

The following RVR analysis was performed using HCV RNA < 12 IU/mL (<1.08 log IU/mL) as the prediction rule.

The PPV and NPV and all associated two sided 95% CI, of RVR for SVR (as determined by test of record) were calculated on treatment outcomes in chronic hepatitis C (CHC) subjects and are summarized in the table below.

For all sites combined, the results demonstrate a high PPV for all subjects at 4 weeks independent of genotype. CHC genotype 1 subjects with RVR had a 100.0% (64/64) probability to achieve SVR (odds ratio > 36.6) and hepatitis C genotype non-1 subjects with RVR had a 93.3% (84/90) probability to achieve SVR (odds ratio 6.5, 95% CI 1.6, 26.6). The significance of a rapid virologic response at Week 4 in predicting SVR is demonstrated by the lower 95% CI limit for the odds ratio exceeding 1.0.

CHC genotype 1 subjects who had not achieved RVR had a 36.8% (57/155) probability of not achieving SVR and hepatitis C genotype non-1 subjects who had not achieved RVR had a 31.8% (7/22) probability of not achieving SVR.

In this analysis, RVR has high reliability as a positive predictor for determining if CHC subjects will achieve SVR and low reliability as a negative predictor for determining if CHC subjects will not achieve SVR.

Similarly, RVR analysis based upon < 50 IU/mL as the prediction rule demonstrated high reliability as a positive predictor of SVR. Overall CHC genotype 1 subjects with RVR had a 98.7% (76/77) probability to achieve SVR (unadjusted odds ratio = 49.5) and hepatitis C genotype non-1 subjects with RVR had a 91.9% (91/99) probability to achieve SVR (unadjusted odds ratio = 7.1).

NPV and PPV at Week 4 (RVR) and Corresponding Odds Ratios

Genotype	NPV ^a (%) (95% CI)	PPV ^b (%) (95% CI)	Odds Ratio (95% CI)	
			Unadjusted (95% CI)	Adjusted (95% CI) ^c
1	57/155 ^d (36.8) (34.1, 40.0)	64/64 (100.0) (94.7, 100.0)	> 36.6 ^e (N/A) ^f	71.1 (4.6, > 999.9)
Non-1	7/22 (31.8) (16.7, 46.8)	84/90 ^g (93.3) (90.0, 96.6)	6.5 (1.6, 26.6)	4.9 (1.5, 16.6)

^a NPV: Denominator is the number of subjects with no RVR at 4 weeks; the numerator is the number of subjects who did not achieve SVR among subjects with no RVR at 4 weeks.

^b PPV: Denominator is the number of subjects with RVR at 4 weeks; the numerator is the number of subjects who did achieve SVR among subjects with RVR.

^c Based on the logistic regression model including covariates gender (male vs. female), baseline viral load ($\leq 8.0 \times 10^5$ IU/mL vs. $> 8.0 \times 10^5$ IU/mL), liver disease (cirrhotic vs. non-cirrhotic), and age (< 40 vs. ≥ 40).

^d 8 of 155 subjects who did not achieve RVR and who had undetermined SVR status were assigned "SVR achieved" status for this analysis.

^e The odds ratio calculations are undefined when NPV is 100% or PPV is 100% or missing. Where the denominators for both the NPV and PPV are greater than five, a "minimum" odds ratio was determined by subtracting one specimen from the numerator of the 100% parameter estimate (NPV or PPV)

^f N/A = not applicable.

^g 2 of 90 subjects who did achieve RVR and who had undetermined SVR status were assigned "SVR not achieved" status for this analysis.

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

The PPV and NPV and all associated two sided 95% confidence intervals, for EVR were calculated on the reliability of EVR to predict SVR (as determined by test of record) after completion of therapy in CHC subjects and is summarized in the following table.

CHC genotype 1 subjects who had not achieved EVR had a 91.9% (34/37) probability (NPV) of not achieving SVR and hepatitis C genotype non-1 subjects who had not achieved EVR had a 100.0% (4/4) probability (NPV) of not achieving SVR.

CHC genotype 1 subjects with EVR had a 80.2% (154/192) probability (PPV) to achieve SVR (odds ratio 45.9, 95% CI 13.1, 240.8) and hepatitis C genotype non-1 subjects with EVR had a 91.6% (109/119) probability (PPV) to achieve SVR (odds ratio >32.7). The significance of an early virologic response at Week 12 in predicting SVR is demonstrated by the lower 95% CI limit for the odds ratio exceeding 1.0. In this analysis, EVR has high reliability as a negative predictor for determining if CHC subjects will achieve SVR.

NPV and PPV at Week 12 (EVR) and Corresponding Odds Ratios

Genotype	NPV ^a (%) (95% CI)	PPV ^b (%) (95% CI)	Odds Ratio (95% CI)	
			Unadjusted (95% CI)	Adjusted (95% CI) ^c
1	34/37 ^d (91.9) (79.4, 97.4)	154/192 ^e (80.2) (76.9, 83.8)	45.9 (13.1, 240.8)	53.7 (13.9, 207.7)
Non-1	4/4 (100.0) (51.2, 100.0)	109/119 ^f (91.6) (89.7, 94.8)	> 32.7 ^g (N/A) ^h	118.5 (4.0, > 999.9)

^a NPV: Denominator is the number of subjects with no EVR at 12 weeks; the numerator is the number of subjects who did not achieve SVR among subjects with no EVR at 12 weeks.

^b PPV: Denominator is the number of subjects with EVR at 12 weeks; the numerator is the number of subjects who did achieve SVR among subjects with EVR.

^c Based on the logistic regression model including covariates gender (male vs. female), baseline viral load ($\leq 8.0 \times 10^5$ IU/mL vs. $> 8.0 \times 10^5$ IU/mL), liver disease (cirrhotic vs. non-cirrhotic), and age (<40 vs. ≥ 40)

^d 2 of 37 subjects who did not achieve EVR and who had undetermined SVR status were assigned "SVR achieved" status for this analysis.

^e 8 of 192 subjects who did achieve EVR and who had undetermined SVR status were assigned "SVR not achieved" status for this analysis.

^f 2 of 119 subjects who did achieve EVR and who had undetermined SVR status were assigned "SVR not achieved" status for this analysis.

^g The odds ratio calculations are undefined when NPV is 100% or PPV is 100% or missing. Where the denominators for both the NPV and PPV are greater than five, a "minimum" odds ratio was determined by subtracting one specimen from the numerator of the 100% parameter estimate (NPV or PPV)

^h N/A = not applicable.

XI. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

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 Advisory Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Safety Conclusions

The adverse effects of the device are based on data collected in a clinical study conducted to support PMA approval as described above. Based on the results of the clinical laboratory studies, the Abbott RealTime HCV assay, 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.

B. Effectiveness Conclusions

The effectiveness of the Abbott RealTime HCV assay has been demonstrated for use in quantitation of Hepatitis C Virus (HCV) DNA in human serum or plasma. A reasonable determination of effectiveness of the Abbott RealTime HCV assay 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, to aid in assessing response to treatment in conjunction with other laboratory results and clinical information has been demonstrated.

C. 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 Abbott RealTime HCV assay 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 Abbott RealTime HCV assay 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.

Risk and benefit analysis: As a diagnostic test, the Abbott RealTime HCV assay 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.

XIII. CDRH DECISION

CDRH issued an approval order on May 17, 2011. The final conditions of approval cited in the approval order are described in the approval order.

The applicant's manufacturing facility was inspected and found to be in compliance with the Quality Systems (QS) regulation (21 CFR 820) on December 22, 2010.

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

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

Post-approval Requirements and Restrictions: See approval order.

XV. REFERENCES

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3. Clinical and Laboratory Standards Institute. Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. CLSI Document EP6-A. CLSI: Wayne, PA; 2002.
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S.F. Lovell May 16, 2011

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