



Abbott RealTime HCV Genotype II

REF 08L21-90
51-608500/R1
Rx Only

Key to symbols used

GTIN

Global Trade Item Number



Manufacturer

REF

Reference Number

LOT

Lot Number

IVD

In Vitro Diagnostic Medical Device

INTERNAL CONTROL

Internal Control

AMPLIFICATION REAGENT PACK A

Amplification Reagent Pack A

AMPLIFICATION REAGENT PACK B

Amplification Reagent Pack B

AMPLIFICATION REAGENT PACK C

Amplification Reagent Pack C

CONTROL +

Positive Control

CONTROL -

Negative Control



Temperature Limitation



Use By



Consult instructions for use



Caution



Warning

See REAGENTS section for a full explanation of symbols used in reagent component naming.

Customer Service: 1-800-553-7042

Customer Service International: Call your Abbott Representative

This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

NAME

Abbott RealTime HCV Genotype II

INTENDED USE

The Abbott RealTime HCV Genotype II is an in vitro reverse transcription-polymerase chain reaction (RT-PCR) assay for use with the Abbott *mSample* Preparation System reagents and with the Abbott *m2000sp* and *m2000rt* instruments for the qualitative identification of hepatitis C virus (HCV) genotypes 1, 1a, 1b, and 2 - 5 in plasma or serum from individuals chronically infected with HCV.

The Abbott RealTime HCV Genotype II is intended for use as an aid in the management of HCV-infected individuals and in guiding the selection of therapeutic treatment indicated for the above listed genotypes. The assay is intended for use on patients who are chronically infected with HCV, are being considered for antiviral treatment, and are positive for HCV RNA.

The Abbott RealTime HCV Genotype II assay is not for screening blood, plasma, serum or tissue donors for HCV.

SUMMARY AND EXPLANATION OF THE TEST

The Hepatitis C virus (HCV), a significant cause of blood-borne hepatitis, is an enveloped virus containing a single-stranded positive sense RNA genome of approximately 9,500 nucleotides.¹ It has been identified as the major etiological agent for post-transfusion non-A and non-B hepatitis worldwide. Based on genetic similarity, HCV has been classified into six major genotypes (1 – 6) and numerous subtypes (1a, 1b, etc.).² HCV genotype impacts the response of HCV-infected patients to peg-interferon/ribavirin combination therapy.³ Before starting combination therapy, it is recommended that the genotype of the infecting HCV isolate be determined so that the patient can receive the most appropriate therapy regimen.⁴

HCV genotype 1 is the predominant HCV genotype in the United States (73.32%) followed by HCV genotype 2 (13.10%) and HCV genotype 3 (12.13%) and HCV genotype 4 (1.32%). HCV genotypes 5 and 6 each represent less than or equal to 1% of HCV genotype determinations in the US.⁵

The Abbott RealTime HCV Genotype II Positive Control is standardized against the Second WHO International Standard for Hepatitis C Virus RNA (NIBSC Code 96/798).⁶

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The Abbott RealTime HCV Genotype II assay consists of two reagent kits:

- Abbott RealTime HCV Genotype II Amplification Reagent Kit
- Abbott RealTime HCV Genotype II Control Kit

The Abbott RealTime HCV Genotype II is an in vitro reverse transcription-polymerase chain reaction (RT-PCR) assay for the identification of the genotype(s) of hepatitis C virus (HCV) in plasma or serum from individuals chronically infected with HCV. The Abbott RealTime HCV Genotype II assay detects genotypes 1, 1a, 1b and 2 – 5 through the use of genotype-specific fluorescent-labeled oligonucleotide probes.

Sample Preparation

The Abbott *m2000sp* provides automated sample preparation using a magnetic microparticle-based protocol (Abbott *mSample* Preparation System) to process 0.5 mL samples (ACD-A, CPD, potassium EDTA, or sodium EDTA plasma or serum). During the sample preparation protocol, HCV virions are disrupted by guanidine isothiocyanate, RNA is captured on the magnetic microparticles, inhibitors are removed by washing steps, and RNA is eluted off the microparticles. The bound nucleic acids are eluted and transferred to a 96 deep-well plate. The nucleic acids are then ready for amplification. The Internal Control (IC) is introduced into each specimen at

the beginning of the sample preparation process to demonstrate that the process was completed correctly for each specimen and control.

Amplification Master Mix

The Abbott *m2000sp* instrument automates the assembly of three amplification master mixes (A, B, and C) by combining the respective Abbott RealTime HCV Genotype II Oligonucleotide Reagent (A, B, or C) with thermostable rTth DNA polymerase enzyme and Activation Reagent. The Abbott *m2000sp* dispenses the resulting master mixes into the Abbott 96-Well Optical Reaction Plate along with aliquots of the nucleic acid samples prepared by the Abbott *m2000sp*. Each processed sample is added to one well containing Master Mix A, one well containing Master Mix B, and one well containing Master Mix C.

Amplification

The Abbott RealTime HCV Genotype II assay uses four sets of PCR primers. One set of primers targets a sequence within the 5' untranslated region (*UTR*) of the HCV genome. This primer set is designed to amplify all HCV isolates. The second primer set is designed to amplify the non structural 5b (*NS5b*) region of genotype 1a. The third HCV primer set is designed to amplify the *NS5b* region of genotype 1b. By contrast, the IC primer set is designed to amplify a portion of the hydroxypyruvate reductase gene of the pumpkin plant, *Cucurbita pepo* and is delivered in an Armored RNA[®] particle that has been diluted in negative human plasma.

During the amplification reaction, the target RNA is converted to complementary DNA (cDNA) by the reverse transcriptase activity of the thermostable rTth DNA polymerase.⁷ 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.

Detection

The assay requires three separate reactions to detect genotypes 1, 1a, 1b and 2 – 5:

- Reaction A is designed to detect all HCV isolates, type 3 isolates, and subtype 1a isolates.
- Reaction B is designed to detect type 1 isolates, type 2 isolates, and subtype 1b isolates.
- Reaction C is designed to detect type 4 isolates and type 5 isolates.

Reaction	Probe	Fluorescent Dye
A	All HCV Isolates	FAM
	Genotype 3	NED
	Subtype 1a	VIC
B	Genotype 1	NED
	Genotype 2	FAM
	Subtype 1b	VIC
C	Genotype 4	VIC
	Genotype 5	FAM

A - C	Internal Control (IC)	Quasar 670 ^a
	Reference Dye	ROX ^b

^a Quasar 670 dye contains the same spectral properties as Cy 5.

^b ROX is a Passive Reference Dye in Oligonucleotide Reagents A, B, and C.

Amplification of both HCV and IC targets take place simultaneously in the same reaction. The HCV genotype-specific probes and IC-specific probe within each reaction are all labeled with different fluorophores, thus allowing for simultaneous detection of HCV genotype-specific and IC-specific amplified products.

During the annealing portion of each amplification cycle, the probes hybridize to their respective amplification target, if present. The 5' end of each HCV-specific probe is labeled with a fluorescent moiety while the 3' terminus is labeled with a quenching moiety and a Minor Groove Binder (MGB™) group. In the absence of the HCV target sequences, the probe fluorescence is quenched. In the presence of the HCV target sequences, the probe hybridizes to its complementary sequence. During PCR extension, the 5' to 3' exonuclease (or Taqman) activity of the rTth polymerase degrades the hybridized probe into constituent nucleotides thus separating the quencher and the fluorophore allowing fluorescent emission and detection.⁸

The Abbott *m2000rt* instrument detects the resultant fluorescence of the different fluorophores in each reaction well after each cycle.

PREVENTION OF NUCLEIC ACID CONTAMINATION

The possibility of nucleic acid contamination is minimized because:

- Reverse transcription, PCR amplification, and oligonucleotide hybridization occur in a sealed 96-Well Optical Reaction Plate.
- Detection is carried out automatically without the need to open the 96-Well Optical Reaction Plate.
- Aerosol barrier pipette tips are used for all pipetting. The pipette tips are discarded after use.
- Separate dedicated areas are used to perform the Abbott RealTime HCV Genotype II assay. Refer to the SPECIAL PRECAUTIONS section of this package insert.

REAGENTS

Abbott RealTime HCV Genotype II Amplification Reagent Kit (List No. 08L21-90)

1. **INTERNAL CONTROL** Abbott RealTime HCV Genotype II Internal Control (List No. 8L21Y)

(2 vials, 1.2 mL per vial)

- Less than 0.01% noninfectious Armored RNA with internal control sequences in negative human plasma. Negative human plasma 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. Preservatives: 0.1% ProClin® 300 and 0.15% ProClin 950.

2. Abbott RealTime HCV Genotype II Amplification Reagent Packs (List No. 08L21) (Reagent Packs A, B, C / 24 Tests)

(1) **AMPLIFICATION REAGENT PACK A** Reagent Pack A.

- 1 bottle (0.9 mL) HCV Genotype II Oligonucleotide Reagent A. Less than 0.1% synthetic oligonucleotides (6 primers, 5 probes), less than 0.1% dNTPs, and 10.4% dimethylsulfoxide in a buffered solution with a reference dye. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.
- 1 bottle (0.141 mL) Thermostable rTth Polymerase Enzyme (2.9 to 3.5 Units/μL) in buffered solution.
- 1 bottle (0.400 mL) Activation Reagent, 30 mM manganese chloride solution. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

(2) **AMPLIFICATION REAGENT PACK B** Reagent Pack B.

- 1 bottle (0.9 mL) HCV Genotype II Oligonucleotide Reagent B. Less than 0.1% synthetic oligonucleotides (6 primers, 5 probes), less than 0.1% dNTPs, and 10.4% dimethylsulfoxide in a buffered solution with a reference dye. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.
- 1 bottle (0.141 mL) Thermostable rTth Polymerase Enzyme (2.9 to 3.5 Units/ μ L) in buffered solution.
- 1 bottle (0.400 mL) Activation Reagent, 30 mM manganese chloride solution. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

(3) **AMPLIFICATION REAGENT PACK C** Reagent Pack C.

- 1 bottle (0.9 mL) HCV Genotype II Oligonucleotide Reagent C. Less than 0.1% synthetic oligonucleotides (4 primers, 4 probes), less than 0.1% dNTPs, and 10.4% dimethylsulfoxide in a buffered solution with a reference dye. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.
- 1 bottle (0.141 mL) Thermostable rTth Polymerase Enzyme (2.9 to 3.5 Units/ μ L) in buffered solution.
- 1 bottle (0.400 mL) Activation Reagent, 30 mM manganese chloride solution. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

NOTE: Amplification reagents are intended for single-use only. Residual reagent will remain in the reagent pack bottles after use. Unused reagents should be discarded.

Abbott RealTime HCV Genotype II Control Kit (List No. 08L21-80)

1. **CONTROL -** Abbott RealTime HCV Genotype II Negative Control (List No. 8L21Z)

(4 vials, 1.3 mL per vial)

- Negative human plasma 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. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

2. **CONTROL +** Abbott RealTime HCV Genotype II Positive Control (List No. 8L21W)

(4 vials, 1.3 mL per vial)

- Noninfectious Armored RNA representing the 5' untranslated region (5' UTR) sequences of HCV genotype 1a and HCV genotype 4 in negative human plasma. Negative human plasma 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. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

NOTE: Control lots can be used interchangeably with amplification reagent kit lots.

WARNINGS AND PRECAUTIONS

IVD In Vitro Diagnostic Medical Device

FOR IN VITRO DIAGNOSTIC USE

The Abbott RealTime HCV Genotype II assay is indicated only for individuals who have been determined to be chronically infected with HCV.

The Abbott RealTime HCV Genotype II 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 in donated blood, plasma, serum, or tissue.

The Abbott RealTime HCV Genotype II reagents are intended to be used only on the Abbott m2000 System consisting of the Abbott m2000sp for sample processing and the Abbott m2000rt for amplification and detection.

Only use Uracil-N-Glycosylase (UNG) List No. 08L21-66 when performing the Uracil-N-Glycosylase protocol.

Do not use kits or reagents beyond expiration date.

If the Abbott *m2000sp* master mix addition protocol is aborted, seal the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott *m2000sp* Operations Manual, Hazards section, along with the gloves used to handle the plate. Do not import the test order onto the Abbott *m2000rt*.

The appropriate PCR plate must be selected when samples are loaded into the Abbott *m2000rt* instrument.

NOTE: The Abbott *m2000rt* protocol must be started within 90 minutes of the initiation of the Master Mix Addition protocol (Assay Protocol Step 12).

If the Abbott *m2000rt* instrument run is not initiated within 90 minutes, or is interrupted or aborted, seal the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott *m2000rt* Operations Manual along with the gloves used to handle the plate.

Safety Precautions

Refer to the Abbott *m2000sp* Operations Manual, Hazards Section and the Abbott *m2000rt* Operations Manual, Hazards Section for instructions on safety precautions.



CAUTION: The Control Kit and Internal Control contain human sourced and/or potentially infectious components. Components sourced from human blood have been 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. No known test method can offer complete assurance that products derived from human sources will not transmit infection. Therefore, all human sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled as if infectious, using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories,⁹ OSHA Standard on Bloodborne Pathogens,¹⁰ CLSI Document M29-A3,¹¹ and other appropriate biosafety practices.^{11,12} Therefore, all human sourced materials should be considered potentially infectious.

The Abbott RealTime HCV Genotype II Control Kit, Internal Control, HCV Oligonucleotide Reagents A, B, and C, and Activation Reagent contain a 3:1 mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one which are components of ProClin.



H319	Causes serious eye irritation.
H317	May cause an allergic skin reaction.
P261	Avoid breathing mist / vapors / spray.
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves / protective clothing / eye protection.
P272	Contaminated work clothing should not be allowed out of the workplace.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P337+P313	If eye irritation persists: get medical advice / attention.
P363	Wash contaminated clothing before reuse.
P501	This material and its container must be disposed of in a safe way.

These precautions include, but are not limited to, the following:

- Wear gloves when handling specimens or reagents.
- Do not pipette by mouth.

- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where these materials are handled.
- Clean and disinfect spills of specimens by using a tuberculocidal disinfectant such as 1.0% sodium hypochlorite or other suitable disinfectant.^{13,14}
- Decontaminate and dispose of all specimens, reagents, and other potentially contaminated materials in accordance with local, state, and federal regulations.^{15,16}

Special Precautions

Handling Precautions

The Abbott RealTime HCV Genotype II assay is only for use with human serum or plasma (ACD-A, CPD, potassium EDTA, or sodium EDTA) specimens that have been handled and stored in capped tubes as described in the **SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE** section.

During preparation of samples, compliance with good laboratory practices is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of ribonucleases (RNases) into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with RNA.

Amplification reactions such as PCR are sensitive to accidental introduction of product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the RealTime reagents used in the amplification step become contaminated by accidental introduction of even a few molecules of amplification product. Measures to reduce the risk of contamination in the laboratory include physically separating the activities involved in performing PCR and complying with good laboratory practices.

Work Areas

The *m2000sp* and the *m2000rt* instruments may be operated in the same location. Use two dedicated areas within the laboratory for performing the Abbott RealTime HCV Genotype II assay.

- The **Sample Preparation Area** is dedicated to processing samples (specimens and Abbott RealTime HCV Genotype II Controls), and to adding processed samples and controls to the Abbott 96-Well Optical Reaction Plate. **All reagents used in the Sample Preparation Area should remain in this dedicated area at all times. Laboratory coats, pipettes, pipette tips, and vortexers used in the Sample Preparation Area must remain in this area and not be moved to the Amplification Area. Do not bring amplification product into the Sample Preparation Area.**
- The **Amplification Area** is dedicated to the amplification and detection of amplified product. Laboratory coats and equipment used in the **Amplification Area** must remain in this area and not be moved to the **Sample Preparation Area**.

Components contained within a kit are intended to be used together. Do not mix components from different kit lots. For example, do not use the negative control from control kit lot X with the positive controls from control kit lot Y. Additionally, do not mix and match Amplification Reagent Packs A, B, and C from different amplification kit lots.

Do not use kits or reagents beyond expiration date.

Work areas and instrument platforms must be considered potential sources of contamination. Change gloves after contact with potential contaminants (such as specimens, eluates, and/or amplified product) before handling unopened reagents, negative control, positive controls, or specimens. Refer to the Abbott *m2000sp* and *m2000rt* Operations Manuals for instructions on instrument cleaning procedures.

If the Abbott *m2000sp* instrument run is aborted, dispose of all commodities and reagents according to the Abbott *m2000sp* Operations Manual. If the Abbott *m2000sp* master mix addition protocol is aborted, seal the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott *m2000sp* Operations Manual, Hazards Section, along with the gloves used to handle the plate.

If the Abbott *m2000rt* instrument run is interrupted or aborted, seal the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott *m2000rt* Operations Manual along with the gloves used to handle the plate.

Decontaminate and dispose of all specimens, reagents, and other potentially biohazardous materials in accordance with local, state, and federal regulations.^{15,16} All materials should be handled in a manner that minimizes the chance of potential contamination of the work area. **NOTE: Autoclaving the sealed Abbott 96-Well Optical Reaction Plate will not degrade the amplified product and may contribute to the release of the amplified product by opening the sealed plate. The laboratory area can become contaminated with amplified product if the waste materials are not carefully handled and contained before and after processing.**

Aerosol Containment

To reduce the risk of nucleic acid contamination due to aerosols formed during manual pipetting, aerosol barrier pipette tips must be used for all manual pipetting. The pipette tips must be used only one time. Clean and disinfect spills of specimens and reagents as stated in the Abbott *m2000sp* and Abbott *m2000rt* Operations Manuals.

Contamination and Inhibition

The following precautions should be observed to minimize the risks of RNase contamination, cross-contamination between samples, and inhibition:

- Wear appropriate personal protective equipment at all times.
- Use powder-free gloves.
- Change gloves after having contact with potential contaminants (such as specimens, eluates, and/or amplified product).
- To reduce the risk of nucleic acid contamination due to aerosols formed during pipetting, pipettes with aerosol barrier tips must be used for all sample and IC reagent pipetting. The length of the tip should be sufficient to prevent contamination of the pipette barrel. While pipetting, care should be taken to avoid touching the pipette barrel to the inside surface of the sample tube or container. The use of extended aerosol barrier pipette tips is recommended.
- Change aerosol barrier pipette tips between ALL manual liquid transfers.
- Clean and disinfect spills of specimens and reagents as stated in the Abbott *m2000sp* and the Abbott *m2000rt* Operations Manuals, Hazards section.
- Replace any empty or partially used 200 μ L and 1000 μ L disposable tip trays with full trays before every run.
- The Abbott *mSample* Preparation System (4 x 24 Preps) reagents are single use only. Use new reagent vessels, reaction vessels, and newly opened reagents for every new Abbott RealTime HCV Genotype II assay run. At the end of each run, discard all remaining reagents from the Abbott *m2000sp* worktable as stated in the Abbott *m2000sp* Operations Manual and the Abbott *mSample* Preparation System (4 x 24 Preps) product information sheet.

Contamination From External dU-Containing Amplified Product

Laboratories that use or have used HCV amplification assays that include post-PCR processing of the amplified product may be contaminated by dU-containing amplified product. Such contamination may cause inaccurate results in the Abbott RealTime HCV Genotype II assay. Refer to the Monitoring the Laboratory for the Presence of Amplification Product section of this package insert.

When negative controls persistently generate a HCV genotype result, are invalid or where contamination with dU containing HCV amplified product is likely to have occurred, it is recommended that the laboratory use the Uracil-N-Glycosylase (UNG) (List No. 08L21-66) contamination control procedure if decontamination of the laboratory is unsuccessful.

STORAGE INSTRUCTIONS

Abbott RealTime HCV Genotype II Amplification Reagent Kit (List No. 08L21-90)



The Abbott RealTime HCV Genotype II Amplification Reagent Packs and Internal Control vials must be stored at -25 to -15°C when not in use. Care must be taken to separate the Abbott RealTime HCV Genotype II Amplification Reagent Packs that are in use from direct contact with samples and controls.

Abbott RealTime HCV Genotype II Control Kit (List No. 08L21-80)



The Abbott RealTime HCV Genotype II Negative and Positive Controls must be stored at -25 to -15°C .

INDICATION OF INSTABILITY OR DETERIORATION OF REAGENTS

When a positive or negative control value is out of the expected range, it may indicate deterioration of the reagents. Associated test results are invalid and samples must be retested.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE

Specimen Collection and Storage

Human serum or plasma (ACD-A, CPD, potassium EDTA, or sodium EDTA) specimens may be used with the Abbott RealTime HCV Genotype II assay. Follow the manufacturer's instructions for processing collection tubes.

Freshly drawn specimens (whole blood) may be held at 2 to 30°C for up to 6 hours priorto centrifugation.

After centrifugation, remove serum or plasma from cells. Serum or plasma specimens may be stored:

- At 15 to 30°C for up to 24 hours
- At 2 to 8°C for up to 3 days
- At -25 to -15°C for up to 60 days
- At -70°C for up to 60 days

Multiple freeze/thaw cycles should be avoided. If frozen, thaw specimens at 15 to 30°C or at 2 to 8°C . Once thawed, if specimens are not being processed immediately, they can be stored at 2 to 8°C for up to 6 hours.

Specimens showing particulate matter or turbidity should be clarified by centrifugation at 2000 g for 5 minutes prior to testing.

Specimen Transport

Ship specimens frozen on dry ice. Specimens should be packaged and labeled in compliance with applicable state and federal regulations covering the transport of clinical specimens and etiologic agents/infectious substances.

INSTRUMENT PROCEDURE

The Abbott RealTime HCV Genotype II application file must be installed on the Abbott *m2000sp* and Abbott *m2000rt* instruments from the Abbott RealTime HCV Genotype II *m2000* System Combined Application CD-ROM prior to performing the assay. For detailed information on application file installation, refer to the Abbott *m2000sp* and *m2000rt* Operations Manuals, Operating Instructions section.

ABBOTT REALTIME HCV GENOTYPE II ASSAY PROCEDURE

Materials Provided

- Abbott RealTime HCV Genotype II Amplification Reagent Kit (List No. 08L21-90)

Materials Required But Not Provided

- Abbott RealTime HCV Genotype II Control Kit (List No. 08L21-80)

Sample Preparation Area

- Abbott *m2000sp* Instrument
- Abbott *mSample* Preparation System (4 x 24 Preps) (List No. 04J70-24)
- Abbott RealTime HCV Genotype II *m2000* System Combined Application CD-ROM
- Uracil-N-Glycosylase (UNG) (List No. 08L21-66) protocol[†]
- 5 mL Reaction Vessels
- 200 mL Reagent Vessels
- Transport Tubes (Master Mix Vials)
- Abbott 96-Well Optical Reaction Plate
- Abbott 96-Deep-Well Plate
- Abbott Splash-Free Support Base
- Abbott Optical Adhesive Cover
- Abbott Adhesive Cover Applicator
- Sample Racks
- Round-bottom 11.6 to 16 mm Sample Tubes
- Vortex Mixer
- Centrifuge capable of 2000 *g*
- Calibrated Precision Pipettes capable of delivering 20 μ L-1000 μ L
- 20 μ L-1000 μ L Aerosol Barrier Pipette Tips for precision pipettes
- Molecular Biology Grade Water (RNase Free)^{††}
- 1.7 mL Molecular Biology Grade Microcentrifuge Tubes (Dot Scientific, Inc. or equivalent)^{††}
- Cotton Tip Applicators (Puritan or Equivalent)^{††}

[†] **NOTE: If required per the Contamination From External dU-Containing Amplified Product section of this package insert.**

^{††} **NOTE: These items are used in the procedure for Monitoring the Laboratory for the Presence of Amplification Product. Refer to the QUALITY CONTROL PROCEDURES section of this package insert.**

Amplification Area

- Abbott *m2000rt* Instrument
- Abbott RealTime HCV Genotype II *m2000* System Combined Application CD-ROM
- Abbott *m2000rt* Optical Calibration Kit (List No. 04J71-93)

Other Materials

- Biological safety cabinet approved for working with infectious materials.
- Sealable plastic bags

Procedural Precautions

Read the instructions in this package insert carefully before processing samples.

The Abbott RealTime HCV Genotype II Internal Control, Negative Control, and Positive Control vials are intended for single-use only and should be discarded after use.

Sample tubes should be inspected for air bubbles. If found, remove them with a sterile pipette tip. Reagent bubbles may interfere with proper detection of reagent levels in the reagent vessel, causing insufficient reagent aspiration, which could impact results. **Caution should be taken to avoid cross-contamination between samples by using a new sterile pipette tip for each tube.**

Use aerosol barrier pipette tips or disposable pipettes only one time when pipetting specimens or Internal Control. To prevent contamination to the pipette barrel while pipetting, care should be taken to avoid touching

the pipette barrel to the inside of the sample tube or container. The use of extended aerosol barrier pipette tips is recommended.

If the Abbott *m2000sp* master mix addition protocol is not initiated, re-cap the Amplification Reagent vials and return the Amplification Reagent Packs to –25 to –15°C storage. Once thawed, the Abbott RealTime HCV Genotype II Amplification Reagent Packs can be frozen and thawed a maximum of three additional times. If the Abbott *m2000sp* master mix addition protocol is aborted, then discard the amplification reagents.

NOTE: The *m2000rt* protocol must be started within 90 minutes of the initiation of the Master Mix Addition protocol.

If the Abbott *m2000rt* instrument run is not initiated within 90 minutes, or is interrupted or aborted, seal the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott *m2000rt* Operations Manual along with the gloves used to handle the plate.

Monitoring procedures for the presence of amplification product can be found in the **QUALITY CONTROL PROCEDURES** section in this package insert.

To reduce the risk of nucleic acid contamination, clean and disinfect spills of specimens by including the use of a tuberculocidal disinfectant such as 1.0% sodium hypochlorite or other suitable disinfectant.

The Abbott RealTime HCV Genotype II Controls must be processed in conjunction with specimens to be tested. The use of the Abbott RealTime HCV Genotype II Controls is integral to the performance of the Abbott RealTime HCV Genotype II assay. Refer to the **QUALITY CONTROL PROCEDURES** section of this package insert for details.

ASSAY PROTOCOL

For a detailed description of how to operate the Abbott *m2000sp* instrument and Abbott *m2000rt* instrument, refer to the Abbott *m2000sp* and *m2000rt* Operations Manuals, Operating Instructions section.

Laboratory personnel must be trained to operate the Abbott *m2000sp* and *m2000rt* instruments. The operator must have a thorough knowledge of the applications run on the instruments and must follow good laboratory practices.

Sample Preparation Area

All specimen preparation must take place in the dedicated Sample Preparation Area. Refer to the Handling Precautions section of this package insert before preparing samples.

- 1. A maximum of 24 samples or 72 reactions (3 reactions per sample) can be performed per run. A negative control and a positive control are included in each run, therefore allowing a maximum of 22 specimens to be processed per run.**

Check sample volume. The Abbott RealTime HCV Genotype II assay minimum sample volume and associated rack requirements on the Abbott *m2000sp* are:

Rack	Tube Diameter ^a	Minimum Sample Volume
13 mm	11.6 to 14.0 mm	1.0 mL
16 mm	15.0 to 16.0 mm	1.3 mL
13mm	Reaction Vessel	0.8 mL

^a Refers to sample tube outer diameter

- If frozen, thaw specimens at 15 to 30°C or at 2 to 8°C. Once thawed, specimens can be stored at 2 to 8°C for up to 6 hours before processing.
- Before use, vortex specimens three times for 2 to 3 seconds. **Ensure that bubbles or foam are not created.** If found, remove them with a new sterile pipette tip for each tube. **Specimens showing particulate matter or turbidity should be clarified by centrifugation at 2000 g for 5 minutes prior**

to testing. Aliquot each specimen into clean tubes or vials if necessary. Refer to the Abbott *m2000sp* Operations Manual for tube sizes. Avoid touching the inside of the cap when opening tubes.

2. Thaw assay controls and IC at 15 to 30°C or at 2 to 8°C; see **QUALITY CONTROL PROCEDURES** section of this package insert.
 - Once thawed, assay controls and IC can be stored at 2 to 8°C for up to 24 hours before use.
 - Vortex each control three times for 2 to 3 seconds before use. **Ensure that bubbles or foam are not created.** If found, remove them with a new sterile pipette tip for each tube. Ensure that the contents of each vial are at the bottom after vortexing by tapping the vials on the bench to bring liquid to the bottom of the vial.
3. Thaw amplification reagents at 15 to 30°C or at 2 to 8°C and store at 2 to 8°C until required for the amplification master mix procedure. This step can be initiated before completion of the sample preparation procedure.

Note: Do not vortex the Amplification Reagent Pack.

- Once thawed, store at 2 to 8°C for up to 24 hours if amplification reagents are not being processed immediately.

NOTE: Use one bottle of *m*Lysis Buffer and two vials of Internal Control to support processing up to 24 samples. Each sample is tested with each of the three RealTime HCV Genotype II Amplification Reagent Packs. Use one each of RealTime HCV Genotype II Amplification Reagent Packs A, B, and C to support processing up to 24 samples.

Abbott *m2000sp* Procedure

4. Gently invert the Abbott *m*Sample Preparation bottles to ensure a homogeneous solution. If crystals are observed in any of the reagent bottles upon opening, allow the reagent to equilibrate at room temperature until the crystals disappear. Do not use the reagents until the crystals have dissolved. Ensure bubbles or foam is not generated; if present, remove with a sterile pipette tip, using a new tip for each bottle.
5. Vortex each IC three times for 2 to 3 seconds before use. Ensure bubbles or foam is not generated; if present, remove with a sterile pipette tip, using a new tip for each vial.
6. Using a calibrated precision pipette **DEDICATED FOR INTERNAL CONTROL USE ONLY**, add **2000 µL of IC to one bottle of *m*Lysis Buffer**. Mix by gently inverting the container 5 to 10 times to minimize foaming.
7. Place the negative control, the positive control, and the patient specimens into the Abbott *m2000sp* sample rack.
8. Place the 5 mL Reaction Vessels into the *m2000sp* 1 mL subsystem carrier.
9. Load the carrier racks containing the Abbott *m*Sample Preparation System reagents and the Abbott 96-Deep-Well Plate on the Abbott *m2000sp* worktable as described in the Abbott *m2000sp* Operations Manual, Operating Instructions.
10. From the Run Sample Extraction screen, select and initiate the sample extraction protocol as described in the *m2000sp* Operations Manual, Operating Instruction.
Following completion of the Sample Extraction protocol, proceed to Step 11 for master mix preparation or processed samples may be stored in the Abbott 96-Deep-Well Plate prior to initiating the Abbott *m2000sp* Master Mix Addition protocol:
 - At 2 to 30°C for up to 4 hours
 - At –20°C or colder for up to 7 days
 - Thaw processed samples prior to initiating the Master Mix Addition Protocol

NOTE: Change gloves before handling the amplification reagents.

11. Load the three Amplification Reagent Packs (A, B, and C) and three master mix vials on the *m2000sp* worktable after sample preparation is completed. Load the three Amplification Reagent Packs (A, B, and C) into the reagent positions (1, 2, and 3), respectively.
 - The three RealTime HCV Genotype II Amplification Reagent Packs support up to 24 reactions.
 - Ensure the Amplification reagents are thoroughly thawed before use.
 - Label each vial cap within each amplification reagent pack (i.e. A, B, C) prior to opening the amplification reagents.
 - Prior to opening the amplification reagents, ensure that the contents are at the bottom of the vials by tapping the vials in an upright position on the bench.
 - Remove the amplification vial caps.
12. Select the appropriate deep well plate from the Run Master Mix Addition screen that matches the corresponding sample preparation extraction. Initiate the Abbott *m2000sp* Master Mix Addition protocol. Follow the instructions as described in the Abbott *m2000sp* Operations Manual, Operating Instructions section.

NOTE: The *m2000rt* protocol (step 17) must be started within 90 minutes of the initiation of the Master Mix Addition protocol (step 12).

If the Abbott *m2000sp* master mix addition protocol is not initiated, re-cap the Amplification Reagent vials and return the Amplification Reagent Packs to -25 to -15°C storage. Once thawed, the Abbott RealTime HCV Genotype II Amplification Reagent Packs can be frozen and thawed a maximum of three additional times. If the Abbott *m2000sp* master mix addition protocol is aborted, then discard the amplification reagents.

Amplification Area

13. Switch on and initialize the Abbott *m2000rt* in the amplification area.
 - **The Abbott *m2000rt* requires 15 minutes to warm up.**

NOTE: Change laboratory coats and gloves before returning to the sample preparation area.
14. Place the Abbott 96-Well Optical Reaction Plate into the Abbott Splash-Free Support Base after the Abbott *m2000sp* instrument has completed addition of samples and master mix.
15. Seal the Abbott 96-Well Optical Reaction Plate according to the Abbott *m2000sp* Operations Manual, Operating Instructions section. Export the completed PCR plate results via a network connection directly to a mapped *m2000rt* (or indirectly via a CD).

Abbott *m2000rt* Procedures

For a detailed description of how to perform an Abbott *m2000rt* protocol, refer to the Operating Instructions section in the Abbott *m2000rt* Operations Manual.

16. Place the Abbott 96-Well Optical Reaction Plate in the Abbott *m2000rt* instrument. Import the *m2000sp* test order via a network connection directly to a mapped *m2000rt* (or indirectly via a CD) per the Import Order instructions in the Abbott *m2000rt* Operations Manual, Operating Instructions section.
17. Initiate the Abbott RealTime HCV Genotype II protocol as described in the Abbott *m2000rt* Operations Manual. The Abbott *m2000rt* completes the run in approximately 2 hours and forty-five minutes.

If the Abbott *m2000rt* instrument run is not initiated within 90 minutes from initiation of Master Mix Addition (Step 12), or is interrupted or aborted, seal the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott *m2000rt* Operations Manual along with the gloves used to handle the plate.
18. After the Abbott *m2000rt* instrument has completed the amplification and detection protocol, remove the 96-Well Optical Reaction Plate and dispose according to the instructions in the **Handling Precautions** section of this package insert.

POST PROCESSING PROCEDURES

1. Remove the Abbott 96-Deep-Well Plate, Reaction Vessels, Reagent Vessels, Amplification Reagent Packs, and Master Mix Vials from the worktable and dispose of according to the Abbott *m2000sp* Operations Manual.
2. Place the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott *m2000rt* Operations Manual along with the gloves used to handle the plate.
3. Clean the Splash-Free Support Base before next use, according to the Abbott *m2000rt* Operations Manual.

QUALITY CONTROL PROCEDURES

Abbott *m2000rt* Optical Calibration

Refer to the Calibration Procedures section in the Abbott *m2000rt* Operations Manual for a detailed description of how to perform an Abbott *m2000rt* Optical Calibration. Optical calibration of the Abbott *m2000rt* instrument is required for the accurate measurement and discrimination of dye fluorescence during the Abbott RealTime HCV Genotype II assay.

The following Abbott *m2000rt* Optical Calibration Plates are used to calibrate the Abbott *m2000rt* instrument for the Abbott RealTime HCV Genotype II assay:

- FAM™ Plate (Carboxyfluorescein)
- ROX™ Plate (Carboxy-X-rhodamine)
- VIC® Plate (Proprietary dye)
- NED™ Plate (Proprietary dye)
- Cy5® Plate (Cyanine)

Detection of Inhibition

A defined, consistent quantity of IC nucleic acid is introduced into each specimen and control at the beginning of sample preparation and measured on the Abbott *m2000rt* to demonstrate proper specimen processing and assay validity. The IC is comprised of a RNA sequence unrelated to the HCV target sequences.

The median amplification cycle at which the IC target sequence fluorescent signal is detected in the negative and positive control samples establishes an IC validity range to be met by all subsequent processed specimens on that run.

An error is displayed when a specimen or control fails to meet their respective IC specification. Specimens whose IC CN value exceeds the established range must be retested starting with sample preparation. Refer to **RESULTS** section of this package insert and the Abbott *m2000rt* System Operations Manual for a list of error codes and flags.

Negative and Positive Controls

A negative control and a positive control (are included in each test order to evaluate run validity. An error is displayed when a control result is invalid. Refer to the Abbott *m2000rt* Operations Manual for an explanation of the corrective actions for the error. If the negative or positive controls are invalid, 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, clean the Abbott *m2000sp* instrument and the Abbott *m2000rt* instrument according to the Operations Manuals and repeat the sample processing for controls and specimens following the **Procedural Precautions** section of this package insert. If negative controls are persistently reactive, contact Abbott Molecular Customer Service.

Monitoring the Laboratory for the Presence of Amplification Product

It is recommended that this test be done at least once a month to monitor laboratory surfaces and equipment for contamination by amplification product. It is very important to test all areas that may have been exposed to processed specimens, controls, and/or amplification product. This includes routinely handled objects such as pipettes, the Abbott *m2000sp* and Abbott *m2000rt* function keys, laboratory bench surfaces, and microcentrifuges.

1. Add 0.8 mL RNase-free water to a separate 1.7 mL RNase-free microcentrifuge tube for each laboratory surface to be monitored.
2. Saturate the cotton tip of an applicator (Puritan or equivalent) in the RNase-free water from the microcentrifuge tube.
3. Using the saturated cotton tip of the applicator, wipe the area to be monitored using a sweeping motion. Place the applicator back into the microcentrifuge tube from step 1.
4. Swirl the cotton tip in the RNase-free water 10 times, and then press the applicator along the inside of the microcentrifuge tube so that the liquid drains back into the solution at the bottom of the tube. Discard the applicator.
5. For each additional area to be monitored repeat steps 2 through 4.

Note: A small amount of *mWash 1* buffer is added to each monitor sample in order to ensure that the ionic strength of the sample is sufficient for liquid level detection during processing on the *m2000sp*.

6. Pipette 0.5 mL of the *mWash 1* buffer to a clean tube using the pipette dedicated for Internal Control use.
7. Add 20 μ L of the *mWash 1* buffer from step 6 to each microcentrifuge tube from step 4.
8. Cap the microcentrifuge tubes.
9. Transfer liquid from each microcentrifuge tube to unique 5 mL Reaction Vessels.
10. Bring the volume of each 5 mL Reaction Vessel to 1.5 mL with RNase-free water.
11. Place the 5 mL Reaction Vessels into the Abbott *m2000sp* sample rack and complete the assay following the ASSAY PROTOCOL section of this package insert.

The Uracil-N-Glycosylase (UNG) (List No. 08L21-66) protocol should not be used to monitor the laboratory for presence of amplification product.

12. The presence of contamination is indicated by the detection of HCV nucleic acid in the swab samples.
13. If HCV nucleic acid is detected on equipment, follow the cleaning and decontaminating guidelines given in that equipment's operations manual. If HCV nucleic acid is detected on surfaces, clean the contaminated areas with 1.0% (v/v) sodium hypochlorite solution, followed by 70% ethanol or water.

Note: Chlorine solutions may pit equipment and metal. Use sufficient amounts or repeated applications of 70% ethanol or water until chlorine residue is no longer visible.

14. Repeat testing of the contaminated area by following Steps 1 through 13.

RESULTS

Abbott RealTime HCV Genotype II is a qualitative assay. The Abbott RealTime HCV Genotype II Controls are used to establish run validity for the HCV Genotype II assay.

The Abbott RealTime HCV Genotype II assay employs two determinations on each assay response to accurately designate HCV genotypes:

- Cycle Number (CN) and
- CN number difference, as compared to the HCV-All-probe cycle number, for each of the genotype specific probes (1, 1a, 1b, 2, 3, 4, and 5).

A sample is reported to contain a HCV genotype when the CN threshold is exceeded, and when the genotype-specific CN value is within a predetermined number of cycles of the HCV-All CN value for the same specimen. Multiple genotypes can be detected simultaneously (see Interpretation of Results below).

INTERPRETATION OF RESULTS

If the controls are valid, then proceed to results and interpretations. The Abbott *m2000rt* instrument automatically reports the genotype result on the Abbott *m2000rt* workstation. Assay results and interpretations will look similar to the following examples:

Location	Sample ID	Sample Type	Result	Interpretation	Flags	Error Code
A1	HCV-GT_NEG	Control	Passed			
B1	HCV-GT_POS	Control	Passed ^a			
C1	Patient1		1 1a ^b			
D1	Patient2		1 1b ^b			
E1	Patient3		HCV not detected ^c			
F1	Patient4		2			
G1	Patient5		2, 3 ^d			
H1	Patient6		HCVDetected ^e	No Genotype Result ^f		

^a The results log will indicate amplification curves for GT 1 and GT 4 based on the use of 5' UTR RNA sequences. If the negative or positive controls are invalid, refer to **Quality Control Procedures, Negative and Positive Control** section of this package insert.

^b Genotype 1a and genotype 1b results may be reported without genotype 1.

^c The assay did not detect HCV. Ensure HCV concentration is at least 500 IU/mL and repeat RealTime HCV Genotype II assay.

^d When reporting multiple genotypes, consider inclusion of this statement: Multiple genotype assay results may be caused by a mixed genotype infection, recombination of HCV genotypes, or assay probe cross-reactivity.

^{e,f} The assay detected HCV but did not produce a genotype result. Ensure HCV concentration is at least 500 IU/mL and repeat RealTime HCV Genotype II assay. If repeat testing generates the same result, consider an alternative HCV genotype method.

For more information about error codes and flags, refer to the Abbott *m2000rt* Operations Manual.

LIMITATIONS OF THE PROCEDURE

- **FOR IN VITRO DIAGNOSTIC USE.**
- Optimal performance of this test requires appropriate specimen collection, handling, preparation, storage, and transport to the test site (refer to the **SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE** section of this package insert).
- Human serum or plasma (ACD-A, CPD, potassium EDTA, or sodium EDTA) specimens may be used with the Abbott RealTime HCV Genotype II assay. The use of other anticoagulants has not been validated with the Abbott RealTime HCV Genotype II assay.
- Use of the Abbott RealTime HCV Genotype II assay is limited to personnel who have been trained in the procedures of a molecular diagnostic assay and the Abbott *m2000sp* and *m2000rt* instruments.
- The instruments and assay procedures reduce the risk of contamination by amplification product. However, nucleic acid contamination from the positive control or specimens must be controlled by good laboratory practice and careful adherence to the procedures specified in this package insert.

- A specimen with an interpretation of “No Genotype Result” can not be presumed to be negative for the tested genotypes.
- Multiple genotype assay results may be caused by a mixed genotype infection, recombination of HCV genotypes, or assay probe cross-reactivity.¹⁷
- The Abbott RealTime HCV Genotype II assay is capable of detecting both genotypes in a genotype mixture when the concentrations of both genotypes are near equal; however, the assay may not detect the lower concentration genotype.
- Performance has not been established with the Abbott RealTime HCV Genotype II assay for HCV genotype 6 specimens.
- HCV genotype 6 specimens may generate a HCV genotype 1 result with the Abbott RealTime HCV Genotype II assay based on probe cross-reactivity of the HCV genotype 1 probe.
- As with any diagnostic test, results from the Abbott RealTime HCV Genotype II assay should be interpreted in conjunction with other clinical and laboratory findings. A specimen with a result of “HCV not detected” cannot be presumed to be negative for HCV RNA.
- Contamination from HCV positive controls and clinical specimens can be avoided only by good laboratory practices and careful adherence to the procedures specified in this package insert.

SPECIFIC PERFORMANCE CHARACTERISTICS

REPRODUCIBILITY / PRECISION

The Reproducibility / Precision of Abbott RealTime HCV Genotype II was evaluated by testing a 36-member panel (2 vials of 18 unique members) representing HCV genotypes 1a, 1b, 2, 3, 4, and 5, each at three concentration levels (500 to 1000, 5000 to 10000, > 50000 IU/mL). All panel members were composed of HCV positive donor units diluted in defibrinated human plasma.

A total of 3 Abbott RealTime HCV Genotype II Amplification reagent lots were used. Each of the 3 clinical sites tested 2 of the 3 Amplification reagent lots for 5 nonconsecutive days each, resulting in a total of 10 runs at each site.

The percent correctly identified rate for the Abbott RealTime HCV Genotype II assay was 99.8% (1070/1072) overall for genotypes 1 – 5. The overall No Result (“HCV detected, No Genotype Result” or “HCV not detected”) rate was 0.2% (2/1072) for genotypes 1 – 5.

Within-run, between-run, between-lot, between-site, and total standard deviations and %CV for cycle number (CN) were determined. The total SD ranged from 0.25 to 1.55, the within-run component SD ranged from 0.15 to 1.37, the between-run component SD ranged from 0.00 to 0.14, the between-lot component SD ranged from 0.00 to 0.73, and the between-site component SD ranged from 0.00 to 0.71.

The results, representative of the reproducibility / precision of the Abbott RealTime HCV Genotype II assay, are summarized in Tables 1 and 2.

Table 1. Abbott RealTime HCV Genotype II Reproducibility Study
Overall Analyses by HCV Genotype for all Sites and Lots Combined

HCV Genotype Panel	Total Number of Eligible Results ^a (T)	Number of Correctly Identified Results (D)	Number “No Result” ^b Determinations NR (NR%)	Percent Correct Detection Rate Excludes “No Result” ^b		
				Percent Detected % [D/(T – NR)]	95% CI ^c	
				LL	UL	
1a	180	180	0 (0.0)	100 (180/180)	97.9	100
1b	179	179	0 (0.0)	100 (179/179)	97.9	100
2	178	178	0 (0.0)	100 (178/178)	97.9	100
3	179	179	0 (0.0)	100 (179/179)	97.9	100
4	178	177	1 (0.6)	100 (177/177)	97.9	100
5	178	177	1 (0.6)	100 (177/177)	97.9	100
Overall ^d	1072	1070	2 (0.2)	100 (1070/1070)	99.6	100

^a This number includes all Abbott RealTime HCV Genotype II valid assay results.

^b For HCV genotypes 1 through 5, Abbott RealTime HCV Genotype II assay results “HCV Detected, No Genotype Result” or “HCV not detected” are considered “No Result.”

^c 95% Lower and Upper Confidence Interval limits.

^d Denotes analysis based on assay results from all genotyped panels 1a, 1b, 2, 3, 4, and 5 combined.

Table 2. Abbott RealTime HCV Genotype II Precision Study – Overall Analysis all Sites and Lots Combined

HCV Genotype Panel	Panel Concentration Level	n	Mean CN ^a	Within-Run Component		Between-Run Component		Between-Lot Component		Between-Site Component		Total ^d	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1a	High	60	22.95	0.24	1.1	0.14	0.6	0.08	0.3	0.14	0.6	0.32	1.4
1b	High	59 ^b	21.65	0.18	0.9	0.01	0.1	0.13	0.6	0.09	0.4	0.25	1.1
2	High	58 ^b	21.81	0.25	1.2	0.00	0.0	0.16	0.7	0.12	0.5	0.32	1.5
3	High	60	20.87	0.47	2.2	0.00	0.0	0.34	1.6	0.00	0.0	0.58	2.8
4	High	59 ^b	22.76	0.57	2.5	0.11	0.5	0.09	0.4	0.17	0.7	0.61	2.7
5	High	58 ^b	21.40	0.41	1.9	0.00	0.0	0.43	2.0	0.71	3.3	0.92	4.3
1a	Medium	60	25.29	0.27	1.1	0.00	0.0	0.11	0.4	0.11	0.4	0.31	1.2
1b	Medium	60	24.45	0.15	0.6	0.00	0.0	0.10	0.4	0.17	0.7	0.25	1.0
2	Medium	60	23.77	0.26	1.1	0.00	0.0	0.18	0.8	0.16	0.7	0.36	1.5
3	Medium	60	24.15	0.39	1.6	0.00	0.0	0.30	1.3	0.00	0.0	0.49	2.0
4	Medium	58 ^{b,c}	24.51	0.25	1.0	0.00	0.0	0.27	1.1	0.36	1.5	0.52	2.1
5	Medium	60	25.84	0.63	2.4	0.00	0.0	0.56	2.2	0.00	0.0	0.84	3.3
1a	Low	60	29.11	0.27	0.9	0.12	0.4	0.13	0.4	0.08	0.3	0.33	1.1
1b	Low	60	27.59	0.27	1.0	0.00	0.0	0.15	0.5	0.21	0.7	0.37	1.3
2	Low	60	25.94	1.37	5.3	0.00	0.0	0.73	2.8	0.00	0.0	1.55	6.0
3	Low	59 ^b	27.67	1.00	3.6	0.00	0.0	0.00	0.0	0.36	1.3	1.06	3.8
4	Low	60	28.30	0.39	1.4	0.00	0.0	0.17	0.6	0.42	1.5	0.60	2.1
5	Low	59 ^c	28.76	0.83	2.9	0.00	0.0	0.45	1.6	0.15	0.5	0.96	3.3

^a HCV genotype probe specific cycle number.

^b Invalid replicates (8 total) were not included in the analysis.

^c Replicates without a HCV genotype identification (2 total) were not included in the analysis.

^d The total variability contains Within-Run, Between-Run, Between-Lot and Between-Site variability.

Limit of Detection (LoD) by Genotype

The assay limits of detection (LoD) were estimated for each HCV genotype (1a, 1b, 2, 3, 4, and 5). For each genotype, a single HCV specimen was diluted in HCV negative human plasma as well as in human serum to make panels containing the following HCV concentrations: 1000, 500, 250, 100, and 25 IU/mL.

Each panel member was tested with a minimum of 2 replicates per run, with 2 runs per day, for 4 or 5 days and with 2 Abbott RealTime HCV Genotype II Amplification Reagent lots, for a total of 40 measurements.

The results for each HCV genotype in plasma and serum are summarized in Table 3.

Table 3. Abbott RealTime HCV Genotype II - Limit of Detection Summary
Limit of Detection Estimates (IU/mL)

Sample Type	HCV Genotype					
	1a	1b	2	3	4	5
Plasma	100	500	500	100	500	100
Serum	100	500	250	25	500	100

The limit of detection of the Abbott RealTime HCV Genotype II assay is 500 IU/mL.

Accuracy

The accuracy of the Abbott RealTime HCV Genotype II assay was evaluated by testing 266 HCV genotype 1 (144 of genotype 1a, 122 of genotype 1b), 116 HCV genotype 2, 87 HCV genotype 3, 79 HCV genotype 4, and 27 HCV genotype 5 specimens. Nucleotide sequencing was used to determine the reference genotype of each specimen tested in this study.

The percent correctly identified (Accuracy) rate for the Abbott RealTime HCV Genotype II assay while excluding “No Result” determinations was 99.6% (265/266) for HCV genotype 1, 99.1% (110/111) for HCV genotype 2, 100.0% (86/86) for HCV genotype 3, 98.7% (76/77) for HCV genotype 4, and 100.0% (24/24) for HCV genotype 5, and 99.3% (139/140) for subtype 1a and 99.1% (114/115) for subtype 1b.

The percent correctly identified (Accuracy) rate for the Abbott RealTime HCV Genotype II assay while excluding “No Result” determinations was 99.5% (561/564) overall for genotypes 1 – 5.

The results, representative of the Abbott RealTime HCV Genotype II assay, are summarized in Tables 4 and 5.

Table 4. Abbott RealTime HCV Genotype II– Accuracy Analysis for HCV Genotypes 1 Through 5

HCV Genotype By Sequencing	Total Number of Eligible Results ^a	Number of Eligible Results Excluding "No Result" ^b	Number of RealTime Results in Agreement with Sequencing	Percent Correctly Identified (Accuracy)	95% CI ^c	
					LL	UL
1 ^d	266	266	265	99.6 (265/266)	97.9	99.9
2 ^e	116	111	110	99.1 (110/111)	95.1	99.8
3 ^f	87	86	86	100 (86/86)	95.7	100
4 ^g	79	77	76	98.7 (76/77)	93.0	99.8
5 ^h	27	24	24	100 (24/24)	86.2	100
Overall 1 through 5 ⁱ	575	564	561	99.5 (561/564)	98.4	99.8

^a This number includes all valid Abbott RealTime HCV Genotype II assay and Sequencing results and excludes 11 mixed infection results by the Abbott RealTime HCV Genotype II assay.

^b For HCV genotypes 1 through 5, Abbott RealTime HCV Genotype II assay results “HCV Detected, No Genotype Result” or “HCV not detected” are considered “No Result.”

^c 95% Lower and Upper Confidence Interval limits.

^d Includes HCV genotype 1 and all subtypes of 1 (1a, 1b) results.

^e Four out of 116 HCV genotype 2 samples were identified as “HCV Detected, No Genotype Result” and one sample was identified as “HCV not detected.”

^f One out of 87 HCV genotype 3 samples was identified as “HCV Detected, No Genotype Result.”

^g Two out of 79 HCV genotype 4 samples were identified as “HCV Detected, No Genotype Result.”

^h Two out of 27 HCV genotype 5 samples were identified as “HCV Detected, No Genotype Result” and one sample was identified as “HCV not detected.”

ⁱ Denotes analysis based on results from HCV genotypes 1 through 5 combined.

Table 5. Abbott RealTime HCV Genotype II– Accuracy Analysis for HCV Genotypes 1a and 1b

HCV Genotype By Sequencing	Total Number of Eligible Results ^a	Number of Eligible Results Excluding "No Result" ^b	Number of RealTime Results in Agreement with Sequencing	Percent Correctly Identified (Accuracy)	95% CI ^c	
					LL	UL
1a ^d	144	140	139	99.3 (139/140)	96.1	99.9
1b ^e	122	115	114	99.1 (114/115)	95.2	99.8

^a This number includes all valid Abbott RealTime HCV Genotype II assay and Sequencing results and excludes 4 mixed infection results by the Abbott RealTime HCV Genotype II assay.

^b For HCV subtypes 1a and 1b, Abbott RealTime HCV Genotype II Assay results “HCV Detected, No Genotype Result,” “HCV not detected,” or HCV genotype “1” without being subtyped are considered “No Result.”

^c 95% Lower and Upper Confidence Interval limits.

^d Four out of 144 HCV genotype 1a samples were identified as genotype 1 only but not as subtype 1a.

^e Seven out of 122 HCV genotype 1b samples were identified as genotype 1 but not as subtype 1b.

The overall Abbott RealTime HCV Genotype II “No Result” rate for all HCV genotypes combined was 1.9% (11/575). Overall, HCV genotype 1 samples were not subtyped in 4.1% (11/266) of specimens: 2.8% (4/144) for subtype 1a and 5.7% (7/122) for subtype 1b.

Analytical Specificity

Potentially Interfering Substance

The susceptibility of the Abbott RealTime HCV Genotype II assay to interference by elevated levels of potentially interfering substances was evaluated in two studies.

In the first study, HCV negative plasma samples and plasma samples containing 10,000 IU/mL of HCV genotype 2 armored RNA were spiked with high levels of hemoglobin (500 mg/dL), bilirubin (20 mg/dL), protein (9 g/dL), or triglycerides (3000 mg/dL) and tested. In the second study, HCV negative plasma samples and plasma samples containing 1000 IU/mL of HCV genotype 1 virus were spiked with high levels of hemoglobin (2 g/L), bilirubin (342 uM), protein (120 g/L), or triglycerides (37.34 mM) and tested.

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

Two studies were conducted to assess the susceptibility of the assay to potential interference from high levels of drugs commonly prescribed for the treatment of hepatitis C virus (HCV) and other related diseases. Antivirals and antibiotics at concentrations in excess of peak plasma or serum levels were tested. The drugs listed below were tested in five pools in which each drug was present in excess of reported peak plasma or serum levels. Each drug pool was spiked into an HCV serologically negative plasma aliquot and HCV positive plasma aliquot (HCV genotype 2 armored RNA at 10,000 IU/mL in Study 1 and HCV genotype 1 virion at 1000 IU/mL in Study 2) for testing. Non-spiked aliquots were used as controls. No interference in the performance of the Abbott RealTime HCV Genotype II assay was observed in the presence of the following drugs for all HCV positive and negative samples tested:

Drug Category	Drug Name
Anti-HIV-1 Non-Nucleoside Reverse Transcriptase Inhibitors	Nevirapine, NVP
Anti-HIV-1 Nucleoside/Nucleotide Reverse Transcriptase Inhibitors	Zidovudine, AZT Lamivudine, 3TC Didanosine Efavirenz ^a Stavudine, d4T Abacavir sulfate Tenofovir disoproxil fumarate, TNV
Anti-HIV-1 Protease Inhibitors	Amprenavir Indinavir sulfate Saquinavir, SQV Kaletra ^b (Lopinavir and Ritonavir) Nelfinavir
Anti-HIV-1 Fusion Inhibitors	Enfuvirtide, T-20
Anti-HBV Polymerase Inhibitors	Lamivudine, 3TC (see above) Adefovir Entecavir Tenofovir disoproxil fumarate, TNV (see above) Abacavir sulfate (see above)
Anti-HCV Drugs	Interferon alfa-2a ^c Interferon alfa-2b Peginterferon alfa-2a Peginterferon alfa-2b Ribavirin
Anti-HSV-1/HSV-2/VZV	Acyclovir Valacyclovir
Anti-CMV	Ganciclovir Valganciclovir hydrochloride
Macrolide Antibiotic	Azithromycin Clarithromycin ^a
DNA Gyrase Inhibitor	Ciprofloxacin

^a Clarithromycin and Efavirenz were only tested in Study 2.

^b Kaletra is a combination of Lopinavir and Ritonavir

^c Interferon alfa-2a only tested in Study 1.

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 each of the following DNA virus markers, RNA viruses, non-viral hepatitis, or autoimmune disease states. Two specimens for each condition were tested. In addition, HCV negative specimens positive for the virus markers and conditions listed below were spiked with genotype 1a virions.

DNA and RNA Viruses

Hepatitis A Virus
Hepatitis B Virus
Human Immunodeficiency Virus

Autoimmune States and Non-viral Hepatitis

Anti-nuclear antibodies (ANA)
Rheumatoid factor (RF)
Hepatocellular carcinoma
Alcoholic hepatitis
Non-alcoholic steatohepatitis (NASH)
Cirrhosis
Autoimmune hepatitis

The disease states tested, including autoimmune disorders, viral infections, and non-viral liver disease, have been shown not to yield false-negative or false-positive HCV genotype results or to interfere with the identification of HCV genotypes by Abbott RealTime HCV Genotype II.

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 Genotype II 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 4, 1, and 0.5 µg/mL to HCV RNA negative samples and samples that contained HCV genotype 1 targeted to 1000 and 10,000 IU/mL.

Human immunodeficiency virus 1 (HIV-1) ^a	BK human polyomavirus
Human immunodeficiency virus 2 (HIV-2) ^a	Human papilloma virus 16 (HPV-16)
Human T-lymphotropic virus I (HTLV-I) ^a	Human papilloma virus 18 (HPV-18)
Hepatitis B virus (HBV)	Flavivirus ^a
Epstein-Barr virus (EBV)	<i>Neisseria gonorrhoeae</i>
Herpes simplex virus 1 (HSV-1)	<i>Chlamydia trachomatis</i>
Herpes simplex virus 2 (HSV-2)	<i>Candida albicans</i>
Cytomegalovirus (CMV)	<i>Staphylococcus aureus</i>
Human herpesvirus 6B (HHV-6B)	<i>Staphylococcus epidermidis</i>
Human herpesvirus 8 (HHV-8)	<i>Mycobacterium smegmatis</i>
Varicella-zoster virus (VZV)	Human genomic DNA
Vaccinia virus (VACV)	

^a Viral lysate

No interference in the performance of Abbott RealTime HCV Genotype II 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 4 µg/mL for all the HCV positive and negative samples tested.

Performance of the Assay with HCV-Negative Specimens

The performance of Abbott RealTime HCV Genotype II with HCV negative specimens was evaluated by analyzing 370 unique HCV negative specimens; 135 HCV serologically-negative serum and 235 HCV

serologically-negative plasma specimens. The observed percent of results “HCV not detected” for this study was 100% (370/370), with 95% CI: 99.0 to 100%.

Analytical Carryover

Potential sample carryover within the Abbott RealTime HCV Genotype II assay was evaluated by testing a high positive HCV genotype 2 sample (targeted to a concentration of 1×10^7 IU/mL) interspersed with replicates of a negative sample (Abbott RealTime HCV Genotype II Negative Control).

A combined total of 327 valid measurements for the negative sample and 330 valid measurements for the HCV genotype 2 positive sample were generated in two studies. The Abbott RealTime HCV Genotype II assay did not exhibit detectable carryover from high positive samples to negative samples; percent of results “HCV not detected” for negative samples was 100% (327/327), with 95% CI: 98.2% to 100%.

Mixed Infections

A panel consisting of mixed HCV genotype specimens, representing all possible combinations of HCV genotypes 1a, 1b, and 2 through 5, were prepared for testing. Each panel member consisted of a mixture of two distinct genotypes at 500:500 IU/mL, 1×10^7 : 1×10^7 IU/mL, 1×10^7 :500 IU/mL, and 500: 1×10^7 IU/mL concentration ratios. Each combination was tested in replicates of three.

Native HCV virions were utilized for low level 500 IU/mL target concentrations and Armored RNA stocks were utilized for high level target concentrations. HCV genotypes 2 through 5 armored RNA stocks were composed of representative 5' *UTR* sequences. HCV genotypes 1a and 1b armored RNA stocks were composed of one armored RNA containing a 5' *UTR* representative sequence and a second armored RNA containing a *NS5b* representative sequence.

The Abbott RealTime HCV Genotype II assay detected both genotypes in a genotype mixture when the concentrations of both genotypes were near equal (96.7 % [87/90]). In mixed infections of unequal concentration, the assay detected only the genotype at the higher concentration (100 % [90/90]).

Specimen Stability

Specimen stability testing for both the 5' *UTR* and the *NS5b* regions of HCV in whole blood, serum, and plasma was performed. For each test condition, samples from ten unique donors were spiked with HCV genotype 1a virions at a target concentration of 2000 IU/mL, divided into aliquots and stored at the test conditions.

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, $-20 \pm 5^\circ\text{C}$ for up to 60 days, or -70°C for up to 60 days.

Multiple freeze/thaw cycles should be avoided and should not exceed five freeze/thaw cycles. Frozen specimens may be thawed at 15 to 30°C or 2 to 8°C. Once thawed, if specimens are not processed immediately, store specimens at 2 to 8°C for up to 6 hours.

CLINICAL STUDIES

Study Population

The clinical specimens included in the clinical studies consisted of retrospectively collected serum or plasma specimens from chronic HCV-infected (CHC) subjects, treated with pegylated Interferon alfa 2a or 2b and Ribavirin combination therapy, with treatment outcomes. Duration of treatment of the subjects was defined based on the pretreatment HCV genotype assignment determined by the clinical test of record used at each particular site. Of a total of 447 CHC subjects enrolled from 8 different health care facilities, 260 subjects which had treatment outcome data were used for the clinical usefulness analysis.

Pretreatment HCV genotype assignment was based upon clinical site test of record; subsequently, determinations of HCV genotype at pretreatment (screen or baseline) were also performed using the Abbott RealTime HCV Genotype II assay.

Subject demographics of the study population with clinical outcome are presented in Table 6.

Table 6. Subject Demographics

Characteristics	Category	Number of subjects Included (n)	Percentage of Total
Total Number of Subjects		260	100
Age	< 40 years	70	26.9
	≥ 40 years	190	73.1
Gender	Female	88	33.8
	Male	172	66.2
Race/Ethnicity	Asian	1	0.4
	Caucasian	66	25.4
	Black	0	0.0
	Hispanic / Latino	0	0.0
	American Indian/ Alaska Native	0	0.0
	Other	26	10.0
	Not Available	167	64.2
Pretreatment ALT Liver Enzyme Quotient^a	≤ 3	158	60.8
	> 3	75	28.8
	Not Available	27	10.4
Pretreatment HCV Antibody Test Result	Detected	224	86.2
	Not Available	36	13.8
HIV and/or HBV Co-Infection	HBV	2	0.8
	HIV	29	11.2
	No	229	88.1
Biopsy Result	Cirrhotic	23	8.8
	Non-Cirrhotic	237	91.2
Treatment Assignment	24 weeks	77	29.6
	48 weeks	146	56.2
	Other	37	14.2
Sustained Virological Response Status	SVR	174	66.9
	Non-SVR	86	33.1

^a Quotient is calculated as ALT level divided by Upper Limit of Normal (ULN) specific to the local laboratory.

Clinical Study Results and Statistical Analysis

Based on genetic similarity, HCV has been classified into 6 major genotypes (1 – 6) and numerous subtypes (1a, 1b, etc.). According to literature “HCV genotype impacts the response of HCV-infected patients to peg-Interferon/Ribavirin combination therapy. Current guidelines for the management and treatment of HCV recommend that before starting treatment the genotype of the infecting HCV isolate be determined so that the patient can receive the most appropriate therapy regimen. Patients infected with HCV genotype 1 have a 40% to 50% likelihood of achieving SVR with a low dose of combination therapy and 48 weeks of treatment. Patients infected with HCV genotypes 2 or 3 have an 80% or more likelihood of achieving SVR with a low dose of combination therapy and only 24 weeks of treatment.”³

The clinical usefulness of the Abbott RealTime HCV Genotype II assay was assessed by evaluating the association between HCV genotype (as determined by Abbott RealTime HCV Genotype II Assay) and the probability of achieving sustained virological response (SVR) in subjects included in the clinical study population.

The performance of the Abbott RealTime HCV GT II assay is presented in Table 7.

Table 7. Results of Abbott RealTime HCV GT II assay vs. SVR

		SVR		Total
		Yes	No	
HCV Genotype	1	56	43	99
	2	35	6	41
	3	34	12	46
	4	36	21	57
	5	13	4 ^a	17
	Total	174	86	260

^a One patient with no SVR had 24 weeks of treatment.

The likelihood ratios by the Abbott RealTime HCV Genotype II Assay result are shown in Table 8.

Table 8. Rate of SVR and Likelihood Ratio

HCV Genotype	Rate of SVR		Likelihood Ratio		Genotype Percent
	Estimate	95% CI ^a	Estimate	95% CI ^a and p-value	
1	56.6% (56/99)	(49.0%, 64.0%)	0.64 (56/174) / (43/86)	(0.475, 0.879) p = 0.0055	38.1% (99/260)
2	85.4% (35/41)	(72.7%, 93.0%)	2.88 (35/174) / (6/86)	(1.314, 6.521) p = 0.0062	15.8% (41/260)
3	73.9% (34/46)	(61.0%, 83.9%)	1.40 (34/174) / (12/86)	(0.774, 2.579) p = 0.2726	17.7% (46/260)
4	63.2% (36/57)	(51.8%, 73.5%)	0.85 (36/174) / (21/86)	(0.532, 1.370) p = 0.5059	21.9% (57/260)
5	76.5% (13/17)	(53.4%, 90.5%)	1.61 (13/174) / (4/86)	(0.566, 4.715) p = 0.4186	6.5% (17/260)

^a 95% Lower and Upper Confidence Interval limits.

Without knowledge of HCV genotype, the probability of SVR is 66.9% (174/260). The resulting SVR rate for genotype 1 was statistically significantly lower (56.6%) than the average SVR rate (without knowledge of HCV genotype). SVR rate for genotype 2 was statistically significantly higher (85.4%) than the average SVR rate. Observed SVR rate for genotype 3 was higher (73.9%) than the average SVR rate, with borderline statistical significance. The observed rates of SVR for genotypes 4 and 5 were within the expected values.

The odds ratio (the association between genotype and achieving SVR) and the relative risk (ratio of SVR rates for two different groups defined by genotype) from the Abbott RealTime HCV Genotype II Clinical Study are presented in the Table 9.

Table 9. Odds Ratio and Ratio of SVR Rates (Relative Risk)

Genotype Comparison	SVR Rate Comparison	Ratio of SVR Rates (Relative Risk)	Odds Ratio	95% CI^a for Odds Ratio
1 vs 2	56.6% vs 85.4%	0.663	0.223	(0.071, 0.608)
1 vs 3	56.6% vs 73.9%	0.765	0.460	(0.194, 1.046)
1 vs 2 + 3	56.6% vs 79.3%	0.713	0.340	(0.166, 0.682)
3 vs 2	73.9% vs 85.4%	0.866	0.486	(0.135, 1.606)
1 vs 4	56.6% vs 63.2%	0.896	0.760	(0.366, 1.588)
4 vs 2	63.2% vs 85.4%	0.740	0.294	(0.087, 0.879)
4 vs 3	63.2% vs 73.9%	0.854	0.605	(0.234, 1.527)
4 vs 2 + 3	63.2% vs 79.3%	0.796	0.447	(0.198, 1.011)
1 vs 5	56.6% vs 76.5%	0.740	0.401	(0.090, 1.428)
5 vs 2	76.5% vs 85.4%	0.896	0.557	(0.111, 3.164)
5 vs 2 + 3	76.5% vs 79.3%	0.964	0.848	(0.225, 4.006)
3 vs 5	73.9% vs 76.5%	0.967	0.872	(0.174, 3.621)
4 vs 5	63.2% vs 76.5%	0.826	0.527	(0.112, 2.025)

^a 95% Lower and Upper Confidence Interval limits

From the Abbott RealTime HCV Genotype II clinical study, subjects with HCV genotype 1 had a statistically lower SVR rate relative to those with HCV genotype 2; subjects with HCV genotype 1 had also a statistically lower SVR rate relative to those with genotypes 2+3, and HCV genotype 2 infected patients achieved SVR at a statistically higher rate than patients infected with HCV genotype 4. The study also showed that subjects with HCV genotype 1 achieved a lower SVR rate than subjects with HCV genotype 3 (statistical significance was borderline) and subjects with HCV genotype 4 achieved a lower SVR rate than subjects with HCV genotypes 2+3 (statistical significance was borderline).

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