

SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: In vitro polymerase chain reaction (PCR) assay for genotyping hepatitis C virus

Device Trade Name: Abbott RealTime HCV Genotype II
Abbott RealTime HCV Genotype II Control Kit
Uracil-N-Glycosylase (UNG)

Device Procode: OBF

Applicant's Name and Address: Abbott Molecular Inc.
1300 E. Touhy Ave
Des Plaines IL 60018

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P120012

Date of FDA Notice of Approval: June 20, 2013

Expedited: Not applicable

II. INDICATIONS FOR USE

Abbott RealTime HCV Genotype II

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.

Abbott RealTime HCV Genotype II Control Kit

The Abbott RealTime HCV Genotype II Controls are used to establish run validity of the Abbott RealTime HCV Genotype II assay when used for determining the genotype(s) of hepatitis C virus (HCV) in plasma or serum from individuals chronically infected with HCV.

Uracil-N-Glycosylase (UNG)

The Uracil-N-Glycosylase (UNG) procedure is to be used in conjunction with Abbott RealTime HCV Genotype II 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.

III. CONTRAINDICATIONS

None

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the labeling for the Abbott RealTime HCV Genotype II, the Abbott RealTime HCV Genotype II Control Kit, and the Uracil-N-Glycosylase (UNG).

V. DEVICE DESCRIPTION

The Abbott RealTime HCV Genotype II is an *in vitro* reverse transcription-polymerase chain reaction (RT-PCR) assay for determining the genotype(s) of hepatitis C virus (HCV) in plasma and 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.

The Abbott RealTime HCV Genotype II assay is performed on the Abbott *m2000* System consisting of a sample preparation unit, the Abbott *m2000sp*, and an amplification and detection unit, the Abbott *m2000rt*. Application parameters specific to the Abbott RealTime HCV Genotype II assay are contained in an assay specific application file, housed on a CD-ROM and loaded onto the Abbott *m2000sp* and Abbott *m2000rt* instruments.

The Abbott RealTime HCV Genotype II assay is intended for use with serum, potassium EDTA plasma, sodium EDTA plasma, citrate phosphate dextrose (CPD) plasma, and acid citrate dextrose-A (ACD-A) plasma.

The Abbott RealTime HCV Genotype II assay consists of several kits and ancillary reagents:

1. Abbott RealTime HCV Genotype II Amplification Reagent Kit (List No. 8L21-90), which includes:
 - a. Two vials of Abbott RealTime HCV Genotype II Internal Control (8K24Y) consisting of non infectious armored RNA sequence unrelated to HCV in negative human plasma, and
 - b. Three Abbott RealTime HCV Genotype II Amplification Reagent Packs (A, B, and C) which contain one vial of each of the following:
 - i. rTth Polymerase Enzyme Reagent (566850099) and
 - ii. Activation Reagent (935910099) and

- iii. One of three HCV Genotype II Oligonucleotide Reagents
 - HCV Genotype II Oligonucleotide Reagent A (8K24A0099) for detection of all HCV genotypes, and genotypes 1a and 3.
 - HCV Genotype II Oligonucleotide Reagent B (8K24B0099) for detection of genotypes 1, 1b, and 2.
 - HCV Genotype II Oligonucleotide Reagent C (8K24C0099) for detection of genotypes 4, 5, and 6.

The Abbott RealTime HCV Genotype II Amplification Reagent Kit contains sufficient reagents to process 24 tests.

2. Abbott RealTime HCV Genotype II Control Kit (List No. 8L21-80), which includes:
 - a. Abbott RealTime HCV Genotype II Negative Control (List No. 8K24Z), consisting of negative human plasma, and
 - b. Abbott RealTime HCV Genotype II Positive Control (List No. 8K24W), consisting of noninfectious armored RNA with HCV sequences (Genotypes 1a and 4) in negative human plasma. The amount of HCV RNA in the Abbott RealTime HCV Genotype II Positive Control is standardized against the Second WHO International Standard for HCV RNA, NIBSC code: 96/798.³

Three reactions with each clinical specimen are carried out with Oligonucleotide Reagents A, B, and C to determine the HCV genotype present in the specimen. The Negative Control and Positive Control of the Control Kit are used to establish the run validity of the Abbott RealTime HCV Genotype II assay.

3. There is also an ancillary reagent, Uracil-N-glycosylase (UNG) for use in conjunction with Abbott RealTime HCV Genotype II (List No. 08L21-68), which is offered as an optional procedure to mitigate the effects of pre-existing laboratory contamination from other assays, either commercial or lab-developed, which utilize dUTP and whose PCR products are templates for further amplification by Abbott RealTime HCV Genotype II. Laboratories which do not have pre-existing contamination by dU containing products will not benefit from the UNG procedure. When the Abbott RealTime HCV (P100017) viral load assay was approved, the UNG was also approved as an IVD.

Sample Preparation

The Abbott *m2000sp* provides automated sample preparation using a magnetic microparticle-based protocol (Abbott *m* Sample 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.

Reagent Preparation and Reaction Plate Assembly

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.

Customers using the optional UNG procedure add 27 units of UNG to each thermostable rTth DNA Polymerase Enzyme bottle prior to the automated assembly of the three amplification master mixes

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*) region 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.¹ 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.

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.²

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

VI. ALTERNATIVE PRACTICES AND PROCEDURES

Currently, there are no other FDA approved medical devices for determining the hepatitis C virus (HCV) genotype(s) in plasma or serum. The only currently available conventional alternative methodology for this indication is nucleic acid sequencing. Each alternative has its own advantages and disadvantages. A patient should fully discuss the alternatives with his/her physician to select the method that best meets expectations and lifestyle.

VII. MARKETING HISTORY

The Abbott RealTime HCV Genotype II assay received CE certification and was launched in July 2008 outside of the United States, under the list number of 8K24. The following countries receive the Abbott RealTime HCV Genotype II assay:

Algeria, Armenia, Australia, Austria, Azerbaijan, Bahrain, Belgium, Bosnia, Brazil, Bulgaria, Cameroon, Canada, Colombia, Costa Rica, Croatia, Ethiopia, France, Germany, Herzegovina, Ireland, Israel, Italy, Mexico, Moldova, Montenegro, Netherlands, New Zealand, Oman, Pakistan, Poland, Portugal, Romania, Russia, Saudi Arabia, Singapore, Slovenia, South Africa, Spain, Sweden, Switzerland, Taiwan, Tunisia, Turkey, United Arab Emirates, United Kingdom, United States, and Vietnam.

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

When used according to the instructions in the package insert, there are no known direct adverse effects of this device on the health of the user.

Failure of the test to perform as indicated, or human error during performance of the test may lead to a false result.

In general, the risks associated with inaccurate test results can be: error in identification of the HCV genotype which can result in error or delay in treatment when genotype-specific treatment is instituted; or delay in determining the patient's true genotype. A misidentification test result may lead a clinician to assign an incorrect treatment duration and dosage.

Although there are several potential miscalls that could occur if the device was to malfunction, the device-related adverse event that has the potential to be more serious is the reporting of inaccurate genotype results involving genotypes 1, 4 and 5 (48 weeks of treatment) versus genotypes 2 and 3 (24 weeks of treatment). If the device erroneously calls a genotype 1 specimen as genotype 2 or 3, the physician may not have as many treatment options as the physician should, and the patient will likely be treated for 24 weeks instead of 48 weeks. This would decrease the patient's chance of obtaining a successful outcome from treatment (sustained virological response). Genotype 1 patients who receive a genotype 4 or 5 result may be deprived of treatment choices available only to genotype 1 HCV patients. This is likely to be a non-serious event, because patients would be still treated with a proven valid therapy, even if not the most current or optimal.

If the true genotype is 2 or 3 and the device miscalls the test result as genotype 1, the patient may receive drugs that are not indicated for genotypes other than genotype 1 and may receive treatment for 48 weeks instead of 24 weeks. Unnecessary longer treatment

places the patient at risk for adverse effects for an additional 24 weeks. If genotypes 4 or 5 are miscalled genotype 1, the patients may receive drugs that are not indicated for this genotype and be at risk for more adverse events than they would if receiving treatment for genotypes 4 or 5.

The possibility of genotype miscalls will be even more important in the future, when more direct-acting viral agents with different degrees of toxicity become available since these drugs are expected to be more genotype specific.

A test that fails to produce a HCV genotype determination, "No Result" or "HCV detected but did not produce a genotype result" may lead a clinician to repeat the test or use an alternative genotype method. Since HCV treatment decisions are not required "STAT" there would be no long term health risk to the patient.

The likelihood of erroneous results from the use of this device is minimal if the appropriate instructions are followed as stated in the package insert.

No specific adverse events occurred during the development of the clinical studies. For related information, please see Section X below.

IX. SUMMARY OF NON CLINICAL LABORATORY STUDIES

Nonclinical studies were performed at multiple sites to evaluate the performance characteristics of the Abbott RealTime HCV Genotype II assay. The studies are described below.

Reproducibility/Precision

The purpose of this study was to evaluate the precision and reproducibility of the Abbott RealTime HCV Genotype II assay according to the Clinical and Laboratory Standards Institute protocol EP5-A2. 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 1,000; 5000 to 10,000; and > 50,000 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 reproducibility 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 (SD) 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, met the acceptance criteria by detecting greater than or equal to 95.0% of each genotype/subtype (low, medium, high reproducibility panel members combined) as the expected genotype/subtype by the Abbott RealTime HCV Genotype II assay. Results 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/Reproducibility 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

This study was conducted to determine the limit of detection (LOD) of the Abbott RealTime HCV Genotype II assay for HCV genotypes 1, 1a, 1b and 2 – 5 in plasma and serum samples using the Abbott *m2000* System. 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: 1,000, 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 met the acceptance criterion that the concentration of HCV at which the Abbott RealTime HCV Genotype II assay detects with 95% probability for each specimen type (serum and plasma), genotype, and lot shall be at or below 500 IU/ml. 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

This study was conducted to evaluate the ability of the Abbott RealTime HCV Genotype II assay to accurately identify HCV genotypes 1, 2, 3, 4, and 5, and subtypes 1a and 1b as determined by nucleotide sequencing.

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, 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 met the criteria of accuracy close to 100% with a lower bound of the two-sided 95% confidence interval greater than 90% for HCV genotypes 1 through 4 and close to 100% accuracy with a lower bound greater than 80% for HCV genotype 5 (based on the particular design of this study). 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 (3 of those 11 results corresponded to mixed infection results as defined by the sequencing 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. These results met the acceptance criteria of a rate of “No Result” not exceeding 10%.

Analytical Specificity

Potentially Interfering Substances

Two separate studies were conducted with the intent to evaluate the Abbott RealTime HCV Genotype II assay’s susceptibility to interference by elevated levels of potentially interfering substances.

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 1,000 IU/mL of HCV genotype 1a virion were spiked with high levels of hemoglobin (2 g/L), bilirubin (342 µM), protein (120 g/L), or triglycerides (37.34 mM) and tested.

The results met the acceptance criteria that, for the HCV negative samples spiked with the various interfering substances, the percent with the correct assay result (HCV RNA not detected) must be greater than or equal to 95.0%, and that, for the HCV positive samples spiked with various interfering substances, the percent with the correct result must be greater than or equal 95.0%. No interference with 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 additional 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 1,000 IU/mL in Study 2) for testing. Non-spiked aliquots were used as controls. The results met the acceptance criteria that HCV RNA must not be detected greater than or equal to 95.0% of the time in the HCV negative samples spiked with various analytical therapeutic drugs, and that the appropriate HCV genotype must be detected greater than or equal to 95.0% of the time in the HCV positive samples spiked with various analytical therapeutic drugs. No interference with 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 disporoxil 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 disporoxil 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 tested only 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 Abbott RealTime HCV Genotype II 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.

<u>DNA and RNA Viruses</u>	<u>Autoimmune States and Non-viral Hepatitis</u>
Hepatitis A Virus	Anti-nuclear antibodies (ANA)
Hepatitis B Virus	Rheumatoid factor (RF)
Human Immunodeficiency Virus	Hepatocellular carcinoma
	Alcoholic hepatitis
	Non-alcoholic steatohepatitis (NASH)
	Cirrhosis
	Autoimmune hepatitis

The acceptance criteria were defined as the assay shall produce the correct results when tested with clinical specimens positive for the listed DNA and RNA virus markers and non-viral disease states: HAV, HBV, HIV-1, Rheumatoid Arthritis (RA), Anti-nuclear antibody (ANA), Hepatocellular carcinoma, Alcoholic hepatitis, Autoimmune hepatitis, Non-alcoholic steatohepatitis (NASH), and Cirrhosis. The disease states tested, including autoimmune disorders, viral infections, and non-viral liver disease, have been shown not to yield any 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 purpose of these studies was to assess the potential of nucleic acids from viruses and microorganisms to cross-react and interfere with the HCV genotype determination by the Abbott RealTime HCV Genotype II assay. 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 1,000 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

The acceptance criteria were defined as HCV RNA must not be detected greater than or equal to 95.0% of the time in the assay results for HCV negative samples spiked with viral or microorganism nucleic acids; and HCV genotype 1 must be detected greater than or equal to 95.0% of the time in the assay results for HCV positive samples spiked with viral or microorganism nucleic acids.

The results show that no interference with the performance of the Abbott RealTime HCV Genotype II assay was observed for all of the HCV positive and negative samples tested 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 concentrations up to 4 µg/mL.

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%. These results met the acceptance criteria for this study, defined as the lower bound of the two-sided 95% confidence interval greater than or equal to 99.0% and the point estimate of the percent specificity greater than or equal to 99.5%.

Mixed Infections

This study was conducted to evaluate the ability of the Abbott RealTime HCV Genotype II assay to detect mixed HCV genotype infected specimens using the Abbott *m2000* System.

A panel consisting of mixed HCV genotype specimens, representing all possible combinations of HCV genotypes 1a, 1b, and 2 through 5, was prepared for testing. Each panel member consisted of a mixture of two distinct genotypes at 500:500 IU/mL, 1 x 10⁷:1 x 10⁷ IU/mL, 1 x 10⁷:500 IU/mL, and 500:1 x 10⁷ 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 an NS5b representative sequence.

The acceptance criterion was the simultaneous detection of two different genotypes greater than or equal to 95.0% of the time when both genotypes are present in the sample

at equal concentrations. The Abbott RealTime HCV Genotype II assay met the acceptance criterion, detecting both genotypes in a genotype mixture when the concentrations of both genotypes were near equal (96.7 % [87/90]). For samples containing two genotypes of unequal concentration, the assay detected only the genotype at the higher concentration (100.0 % [90/90]).

Analytical Carryover

These studies were conducted to evaluate the potential of carryover from high positive samples to subsequent samples when using the Abbott RealTime HCV Genotype II assay. Potential sample carryover 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%.

Specimen Stability

The purpose of this study was to evaluate the specimen stability for both the 5' UTR and the NS5b regions of HCV in whole blood, serum, and plasma. For each test condition, samples from ten unique donors were spiked with HCV genotype 1a virions at a target concentration of 2,000 IU/mL, divided into aliquots and stored at the test conditions. The results of the studies support the following conclusions: 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, they can be stored at 2 to 8°C for up to 6 hours.

Reagent Stability

The purpose of this stability study was to determine the expiration dating of the Abbott RealTime HCV Genotype II Amplification Reagent Kit and the Abbott RealTime HCV Genotype II Control Kit.

Abbott Molecular submitted a stability protocol for review and comment that included three lots of negative and positive control in the study design, and included modified acceptance criteria and action limits, which are the same as the final specifications. The action limit for each panel member probe is the baseline median value plus 3.44 Cycle Numbers (+3.44 CN). This was chosen since:

(1) Variance studies showed that the most conservative between-run cycle number standard deviation (SD) for an HCV genotype probe response for a 2,000 IU/mL panel member and the 5,000 IU/mL Positive Control (PC) was 0.86. Calculating a C_p of 1.33, where process capability is shown to be within ± 4 SD, predicts that results will be in a

range of 6.88 CN (0.86 SD x 8 = 6.88). Since reagent degradation will only result in later CN values, this value is changed to a one-sided value of +3.44 CN.

(2) Sample size calculations demonstrate that a minimum of three panel member replicates with an upper action limit range of 3.44 CN is sufficient to measure the stability response.

To demonstrate the shelf life integrity at the intended storage condition (ISC), three lots of each of the Abbott RealTime HCV Genotype II Amplification Reagent Packs (A, B and C) components and three lots of Negative Control and Internal Control, and one lot of Positive Control were stored at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ for the duration of the stability study. To evaluate temperature and transport extremes, one lot of each of the Abbott RealTime HCV Genotype II Amplification Reagent Packs (A, B and C) components and one lot of Negative Control, Positive Control, and Internal Control were stored at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ for 46 to 50 hours, followed by three cycles of 6 to 18 hours in a container with dry ice, then at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ for 6 to 18 hours, followed by storage at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$. The Abbott RealTime HCV Genotype II Amplification Reagent Pack Oligonucleotide Reagents (A, B, and C) were tested with a panel member (PM) set, Abbott RealTime HCV Genotype II Internal Control (IC), and Abbott RealTime HCV Genotype II Controls that were stored at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ for the duration of the study.

The Abbott RealTime HCV Genotype II Amplification Reagent Kits and Control Kits stored at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ were tested at baseline, and thereafter at months 7 and 10. Additional time points will be performed at months 13, 18, and 19 and data will be supplied in the annual reports.

The on-going stability studies for the Abbott RealTime HCV Genotype II Amplification Reagent Kit and the Abbott RealTime HCV Genotype II Control Kit components have been completed for 10 months with kits stored at the $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ stability conditions. Expiration dating is assigned at 9 months to allow for a safety margin. All stability studies using three different lots of Abbott RealTime HCV Genotype II Amplification Reagent Kits and the Abbott RealTime HCV Genotype II Control Kits meet the stability criteria over the time period tested.

Conclusions Drawn from the Non Clinical Studies

The Abbott RealTime HCV Genotype II assay was evaluated to demonstrate performance claims for cross-reactivity, interference, carryover, precision/reproducibility, mixed infections, and stability. The results of the non-clinical studies, in conjunction with results of the clinical trial studies, support the intended use statement of the Abbott RealTime HCV Genotype II assay.

X. SUMMARY OF CLINICAL STUDIES

The clinical usefulness of the Abbott RealTime HCV Genotype II assay for the identification of hepatitis C virus (HCV) genotypes 1, 1a, 1b, and 2 - 5 in plasma or serum from individuals chronically infected with HCV to aid in guiding the selection of therapeutic treatment was determined by the clinical study described below. Data from this clinical study were the basis for the PMA approval decision. A summary of the clinical study is presented below.

A. Study Design

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.

Testing of clinical specimens was performed at three independent sites. The study was a multicenter retrospective clinical study that examined the association between HCV genotype (as determined by the Abbott Real Time HCV Genotype II Assay) and the probability of achieving sustained virological response (SVR) in patients treated with interferon alfa-2a or 2b and ribavirin combination therapy. SVR status was defined as the absence of HCV RNA at the end of treatment (EOT) and six months later (end of follow up, EOF). The primary endpoint evaluated was SVR rate for each genotype. The benefit of this study is to show that there is an association between HCV genotype and SVR rate when the genotype is determined by the device. This information helps the physician to predict the patient's response to treatment based on the Abbott RealTime HCV Genotype II assay result.

The data for this study was obtained with specimens from patients receiving the standard dual combination therapy. Pretreatment HCV genotype assignment was based upon the 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.

1. Clinical Inclusion and Exclusion Criteria

Enrollment in the study was limited to subjects and specimens meeting the following inclusion criteria

Subject Inclusion Criteria:

- Signed and dated an IRB/IEC approved consent form
- Both genders
- 18 years of age or older
- Subject has documented chronic HCV infection (i.e. positive HCV antibody and/or HCV RNA positive in two specimens six months apart, alanine aminotransferase (ALT) results if available, liver biopsy consistent with chronic HCV infection if available, or physician diagnosis of chronic HCV infection)
- Subject has completed HCV anti-viral treatment with pegylated interferon alfa 2a or 2b and ribavirin
- Subject has a serum specimen or a specimen collected in ACD-A, CPD, potassium EDTA, or sodium EDTA anticoagulant available at pretreatment with a minimum volume of 1.3 mL
- Subject had an HCV RNA level > 500 IU/mL at pretreatment

- Subject infected with HCV genotype 1a, 1b, 2, 3, 4, or 5
- A historical HCV genotype result with test method at pretreatment (Test of Record)
- A historical HCV RNA assay result by an FDA approved assay at pretreatment
- A historical HCV RNA assay result by an FDA approved assay at End of Treatment (EOT) and End of Follow-up (EOF) or a physician documented treatment outcome

Specimen Inclusion Criteria:

- Subject or his/her legal representative has signed and dated an IRB/IEC Committee approved consent form or has a consent waiver, if required
- Archived plasma or serum specimen obtained from subjects meeting study inclusion/exclusion criteria

Subjects and specimens were not permitted to enroll in the study if they met any of the following exclusion criteria:

Subject Exclusion Criteria:

- Subjects who received investigational HCV anti-viral therapy
- Subjects previously enrolled in this study

Specimen Exclusion Criteria:

- Specimen volume is less than 1.3 mL plasma or serum
- Specimen is not handled or stored in accordance with the Abbott RealTime HCV Genotype II Clinical Brochure

2. Follow-up Schedule

Due to the nature of the study, there was no follow up schedule programmed for the subjects.

3. Clinical Endpoints

The primary endpoint evaluated was anticipation of SVR rate, the analysis was based on the Abbott RealTime HCV Genotype II assay results and the subject's Sustained Virologic Response (SVR) status.

B. Accountability of PMA Cohort

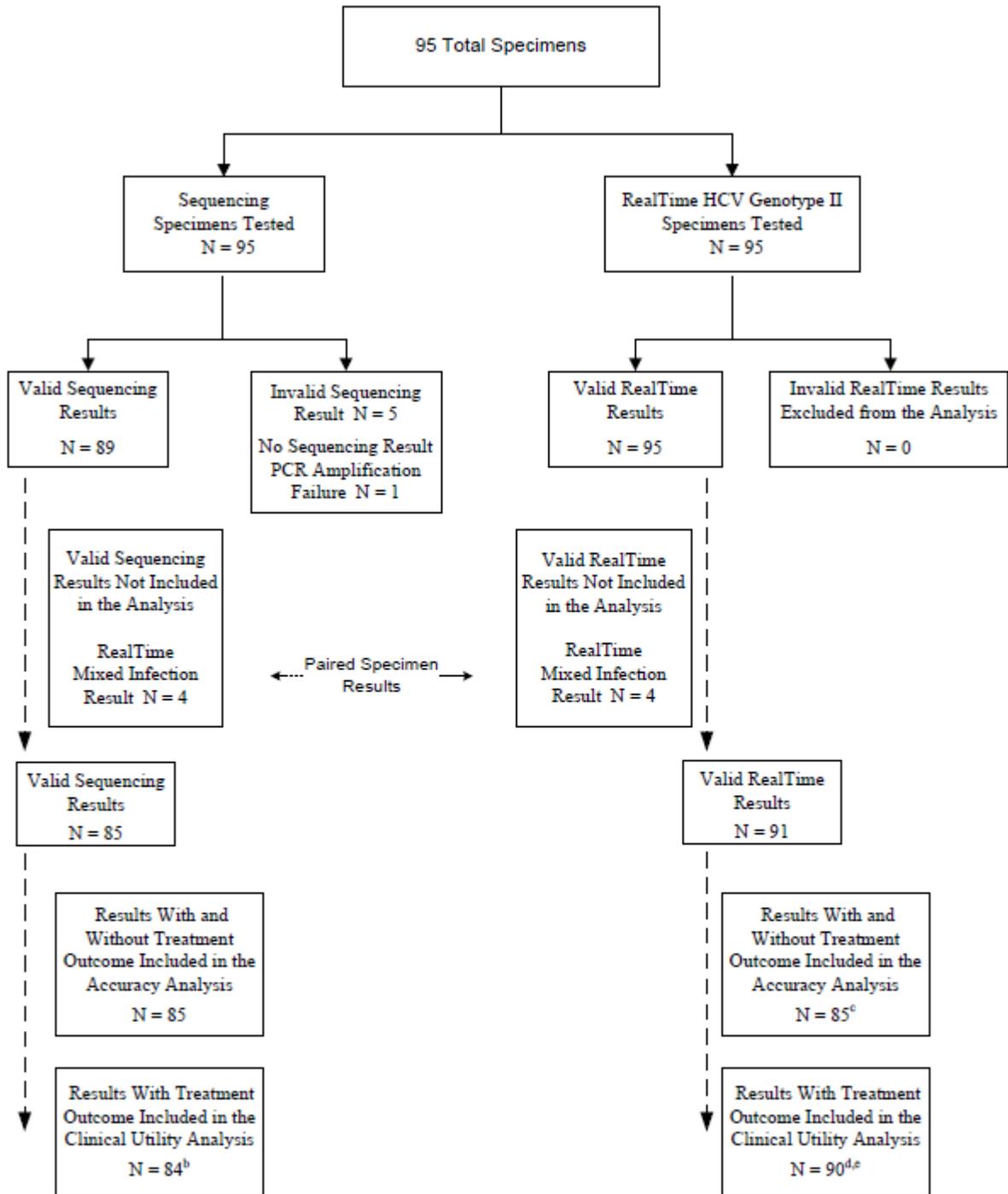
Retrospective specimen samples were sourced from 8 clinical collection sites and commercial vendors (with appropriate Certificates of Analysis) under Institutional Review Board (IRB) or Institutional Ethic Committee (IEC)-approved Abbott RealTime HCV Specimen Collection Protocols.

Specimen results from subjects with treatment outcome data were used for the clinical usefulness analysis and accuracy analysis. Specimen results from subjects who did not have treatment outcomes were used in the analysis for accuracy only.

The study population consisted of 447 CHC subjects enrolled from eight different health care facilities. These 447 subjects were distributed among the three testing sites as follows: 95 subjects in testing site 1; 168 subjects in testing site 2; and 155 subjects in testing site 3. Twenty-nine subjects were excluded from the study due to insufficient sample volume, leaving a total of 418 evaluable subjects. In addition, a set of 302 additional specimens used for internal verification accuracy studies was used to supplement the accuracy studies. At the testing sites, subjects were excluded from the study due to a number of possible reasons, including protocol deviations and withdrawals, testing errors with the Abbott RealTime HCV Genotype II assay or Sequencing method, and mixed HCV genotype infection determinations in the Abbott RealTime HCV Genotype II assay. Refer to the following flow charts for detailed description of the included and excluded results for the accountability of the PMA cohort per testing site, and the additional internal verification accuracy supplementary specimens.

After exclusion of samples as specified above and reflected in the following charts, a total of 260 final results were used in the clinical usefulness analysis (including 90 specimens from site 1, 143 specimens from site 2, and 27 from site 3). A total of 575 results were used in the accuracy analyses (including 85 specimens from site 1, 139 specimens from site 2, 139 specimens from site 3; and 220 specimens from an internal additional accuracy analysis, submitted for sequencing evaluation to supplement the accuracy studies). The mentioned 575 results exclude 11 mixed infection results produced by the Abbott RealTime HCV Genotype II assay (3 of which corresponded to mixed infection results as defined by the sequencing assay).

Specimen Disposition for Clinical Testing Site 1^a



^a Sequencing performed at the University of Washington Molecular Virology Laboratory.

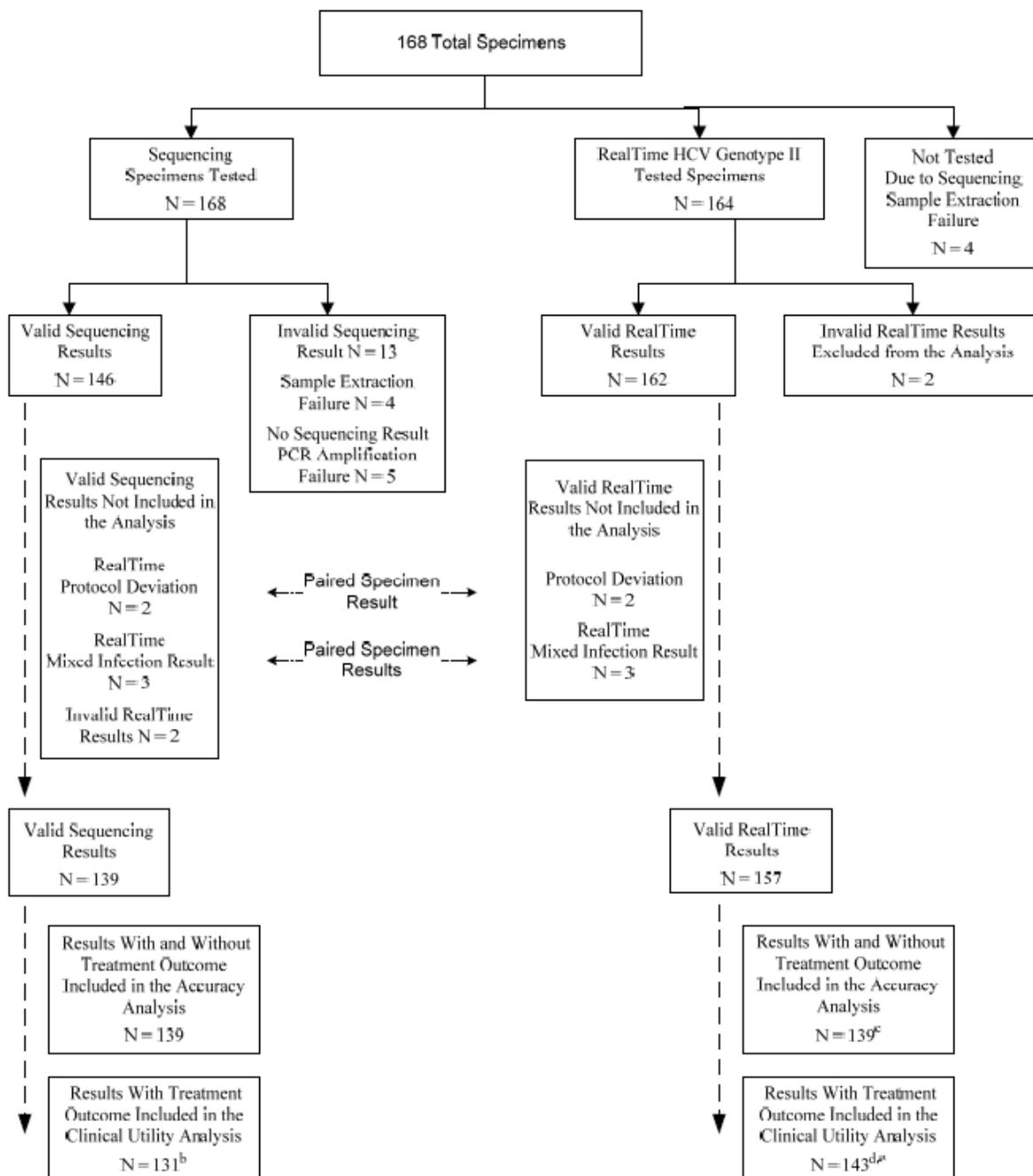
^b Excludes 1 “No Result” from total.

^c Excludes 5 invalid sequencing results and 1 sequencing PCR amplification failure from total.

^d Excludes 1 “No Result” from total.

^e Six RealTime results included in the Clinical Utility Analysis do not have a sequencing result.

Specimen Disposition for Clinical Testing Site 2^a



^a Sequencing performed at the University of Washington Molecular Virology Laboratory.

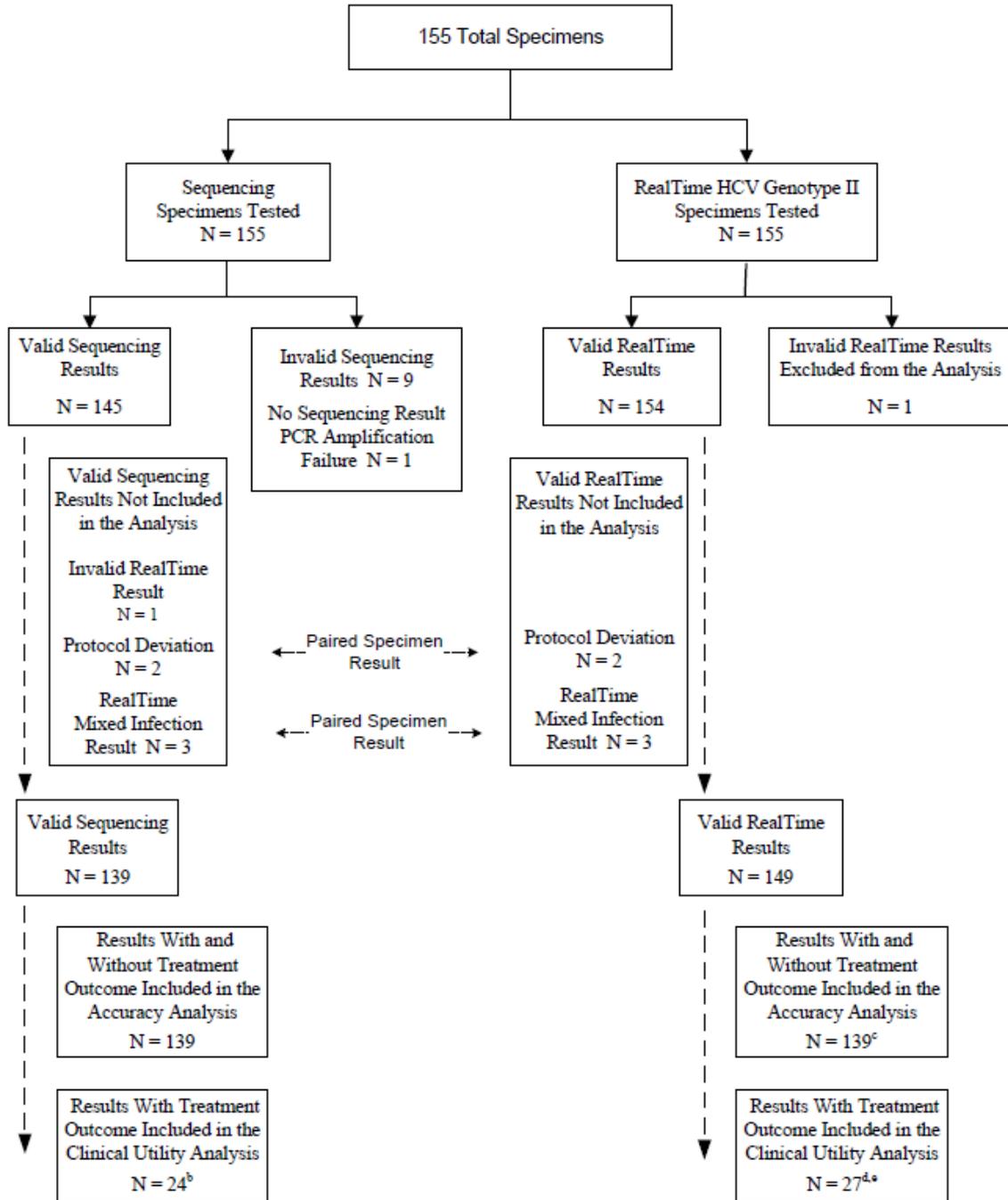
^b Excludes 7 “No Results” and 1 specimen result without outcome from total.

^c Excludes 13 invalid sequencing results (<250) and 5 sequencing PCR amplification failures from total.

^d Excludes 13 “No Results” and 1 specimen result without outcome from total.

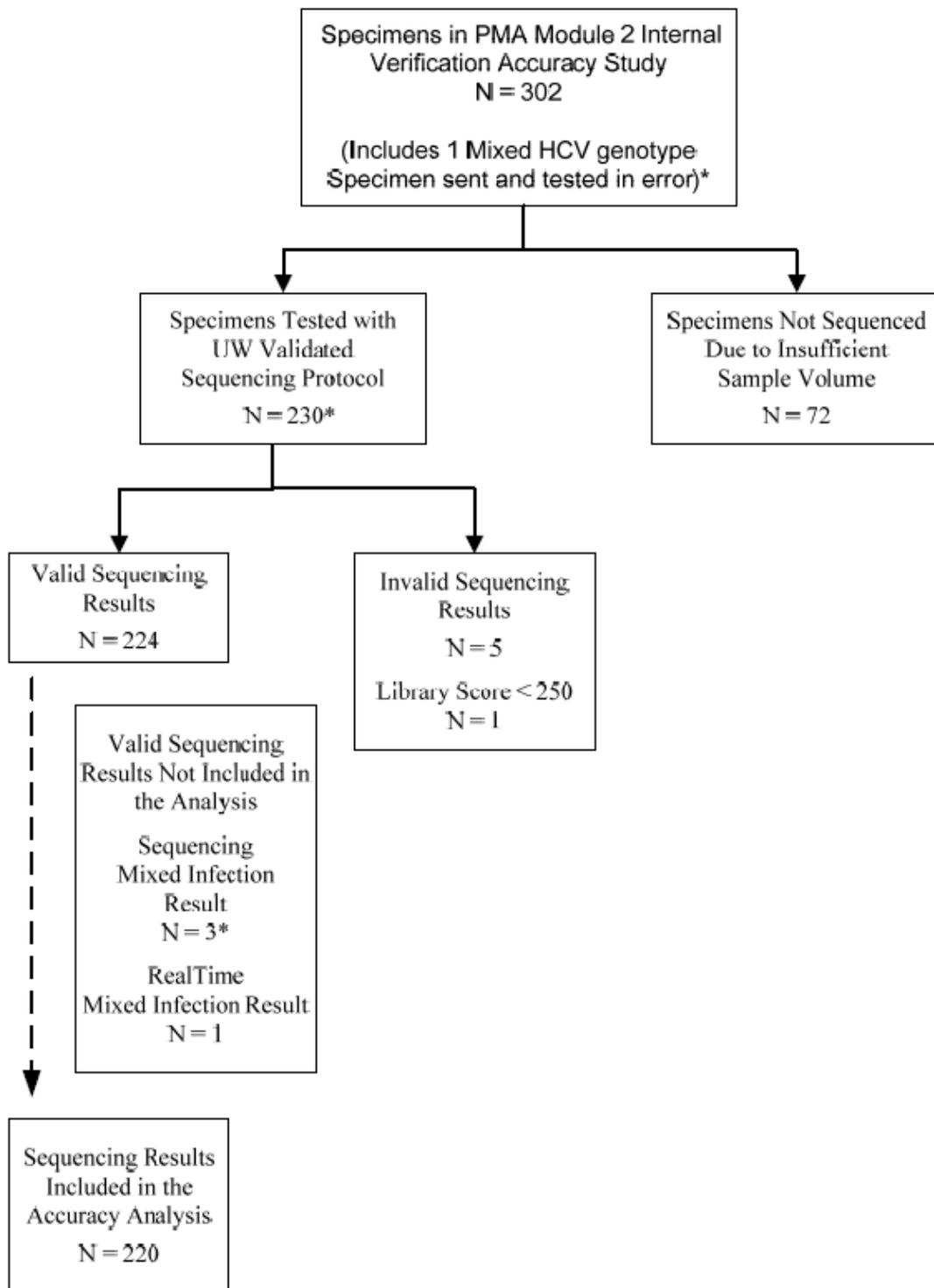
^e Twelve RealTime results included in the Clinical Utility Analysis do not have a sequencing result.

Specimen Disposition for Clinical Testing Site 3^a



- ^a Sequencing performed at the University of Washington Molecular Virology Laboratory.
- ^b Excludes 4 “No Results” and 111 specimen results without outcome from total.
- ^c Excludes 9 invalid sequencing results and 1 sequencing PCR amplification failure from total.
- ^d Excludes 8 “No Results” and 114 specimen results without outcome from total.
- ^e Three RealTime results included in the Clinical Utility Analysis do not have a sequencing result.

Specimen Disposition for Internal Verification Accuracy Specimens



* One mixed HCV genotype specimen sent and tested in error

C. Study Population Demographics and Baseline Parameters

The demographics of the study population are typical for an HCV genotype study performed in the US. Subject demographic data, pretreatment HCV characteristics (antibodies, HCV RNA viral load, genotype, alanine transaminase (ALT), and liver disease stage, if available), treatment assignment and outcome (SVR) were collected. In addition, HIV and HBV infection status were collected for each subject. Subject demographics of the study population with clinical outcome are presented in Table 6 below.

Table 6. Subject Demographics

Characteristics	Category	Number of subjects Included (n)	Percentage of Total
Total Number of Subjects		260	100.0
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
Pretreatment HCV Genotype Assignment^b	1	2	0.8
	1a	45	17.3
	1b	49	18.8
	2	48	18.5
	3	44	16.9
	4	59	22.7
	5	12	4.6
	6	1	0.4
	Mixed	0	0.0

Characteristics	Category	Number of subjects Included (n)	Percentage of Total
Pretreatment HCV RNA Concentration			
Genotype 1	$\leq 8.0 \times 10^5$ IU/mL	0	0.0
	$> 8.0 \times 10^5$ IU/mL	2	100.0
Subtype 1a	$\leq 8.0 \times 10^5$ IU/mL	10	22.2
	$> 8.0 \times 10^5$ IU/mL	34	75.6
	Not Available	1	2.2
Subtype 1b	$\leq 8.0 \times 10^5$ IU/mL	24	49.0
	$> 8.0 \times 10^5$ IU/mL	25	51.0
Genotype 2	$\leq 8.0 \times 10^5$ IU/mL	10	20.8
	$> 8.0 \times 10^5$ IU/mL	34	70.8
	Not Available	4	8.3
Genotype 3	$\leq 8.0 \times 10^5$ IU/mL	16	36.4
	$> 8.0 \times 10^5$ IU/mL	28	63.6
Genotype 4	$\leq 8.0 \times 10^5$ IU/mL	15	25.4
	$> 8.0 \times 10^5$ IU/mL	44	74.6
Genotype 5	$\leq 8.0 \times 10^5$ IU/mL	1	8.3
	$> 8.0 \times 10^5$ IU/mL	8	66.7
	Not Available	3	25.0
Genotype 6	$\leq 8.0 \times 10^5$ IU/mL	1	100.0
	$> 8.0 \times 10^5$ IU/mL	0	0.0
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.

^b Pretreatment HCV genotype assignment based upon clinical site test of record.

D. Safety and Effectiveness Results

1. Safety Results

The Abbott RealTime HCV Genotype II assay is an *in-vitro* diagnostic test that requires specimens derived from a subject's blood sample; therefore it may involve removal of blood from an individual for testing purposes. The test presents safety hazards similar to those for any other test where blood is drawn.

False positive and false negative results are discussed in Section VIII. Briefly, inaccurate genotype results involving genotypes 1, 4 and 5 (48 weeks of treatment) versus genotypes 2 and 3 (24 weeks of treatment) are the most potentially serious false positive events. If the device erroneously calls a genotype 1 specimen as genotype 2 or 3, the physician may not have as many treatment options as the physician should, and the patient will likely be treated for 24 weeks instead of 48 weeks. This would decrease the patient's chance of obtaining a successful outcome from treatment (sustained virological response). On the other

hand, Genotype 1 patients who receive a genotype 4 or 5 result may be deprived of treatment choices available only to genotype 1 HCV patients. This is likely to be a non-serious event, because patients would be still treated with a proven valid therapy, even if not the most current or optimal.

A potential false negative, such as a test result that fails to produce a HCV genotype determination, "No Result" or "HCV detected but did not produce a genotype result", may lead a clinician to repeat the test or use an alternative genotype method. Since HCV treatment decisions are not required "STAT" there would be no long term health risk to the patient.

Based on the data from the accuracy study, the probability of the device to generate a miscall of the genotype is very low. In the accuracy study, only one miscall was observed for each of genotypes 1 (265/266), 2 (110/111), and 4 (76/77). There were no miscalls for genotype 3 (86/86) and genotype 5 (24/24). Therefore, the percent of patients that might experience a harmful event due to a device miscall of the genotype is less than 1% based on 3 miscalls in a total of 564 eligible results.

There were no significant adverse effects of the device reported while the study was conducted.

2. Effectiveness Results

Clinical Study Results and Statistical Analyses

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

Based on genetic similarity, HCV has been classified into 6 major genotypes (1 – 6) and numerous subtypes (1a, 1b, etc.). According to the 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."⁴

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	
1	56	43	99
2	35	6	41
3	34	12	46
4	36	21	57
5	13	4*	17
Total	174	86	260

* One (1) 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.

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).

Clinical assessment of the results:

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. The 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, and statistical significance, which depends on the sample size, was not anticipated in this clinical study for these genotypes.

3. Subgroup Analyses

Apart from the parameters described above, there were no additional characteristics evaluated for potential association with outcomes.

E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included three investigators. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

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 FDA Microbiology Devices 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. Effectiveness Conclusions

The results of the precision and reproducibility studies demonstrated acceptable performance of the Abbott RealTime HCV Genotype II assay across the assay's measurement range for the studied sources of variability of different sites, lots, runs, days and replicates.

The Abbott RealTime HCV Genotype II assay demonstrated acceptable analytical sensitivity as determined by limit of detection studies for the different studied genotypes.

As part of the analytical studies, Abbott Molecular conducted an accuracy study obtaining the percent agreement between the results of the Abbott RealTime HCV Genotype II assay and the results from a validated bidirectional sequencing assay using archived clinical specimens. The results confirmed satisfactory accuracy values for all genotypes, namely, accuracy close to 100% for genotypes 1, 1a, 1b, and 2-5, and lower bound limits of the two-sided 95% confidence interval above 90% for genotypes 1, 1a, 1b, and 2-4, and above 80% for genotype 5 (based on the specific design of these studies). All accuracy results are sufficient and acceptable.

Studies to evaluate potential susceptibility of the Abbott RealTime HCV Genotype II assay to interference from endogenous substances present in blood showed no evidence of interference of the Abbott RealTime HCV Genotype II assay when testing samples containing elevated levels of hemoglobin, bilirubin, protein,

triglycerides.

Likewise, studies to evaluate susceptibility of the assay to potential interference from high levels of drugs commonly prescribed for the treatment of HCV and other related diseases indicated no interference of the Abbott RealTime HCV Genotype II assay when testing samples containing Anti-HIV-1 Reverse Transcriptase Inhibitors, Protease Inhibitors, and Fusion Inhibitors; Anti-HBV Polymerase Inhibitors; current Anti-HCV Drugs; and other drugs used for treatment of HSV and CMV.

No cross-reactivity was observed when testing specimens that were positive for a number of DNA and RNA virus markers (such as HAV, HBV, and HIV), non-viral hepatitis, or autoimmune disease states. No cross-reactivity was observed when nucleic acid or viral lysate from different microorganisms were added in high levels to genotype 1a positive and negative HCV specimens.

Mixed infections studies (studies with samples containing mixtures of two HCV genotypes), showed that the Abbott RealTime HCV Genotype II assay is able to detect both genotypes in a genotype mixture when the concentrations of both genotypes were approximately equal (96.7 % (87/90)). However, when the concentrations of the genotypes were widely disparate, the assay detected only the genotype at the higher concentration. Multiple genotype results from the Abbott RealTime HCV Genotype II assay may also be caused by recombination of HCV genotypes or assay probe cross-reactivity

Matrix comparison studies demonstrated equivalent performance of the Abbott RealTime HCV Genotype II assay with serum samples and plasma specimens collected in potassium ethylenediaminetetra-acetic acid (EDTA), sodium EDTA, citrate phosphate dextrose (CPD), and acid citrate dextrose-A (ACD-A) collection tubes.

Specimen stability studies were able to supported the following conclusions: 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 ± 5°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, they can be stored specimens at 2 to 8°C for up to 6 hours.

The clinical usefulness analysis that examined the association between HCV genotype as determined by the Abbott Real Time HCV Genotype II Assay and the probability of achieving SVR showed informative results with statistical significance and provided the following conclusions: 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. The 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, and statistical significance, which depends on the sample size, was not anticipated in this clinical study for these genotypes.

B. Safety Conclusions

The risks of the Abbott RealTime HCV Genotype II assay are based on nonclinical laboratory data as well as data collected in the clinical studies conducted to support PMA approval as described above.

Due to the nature of the specimen type, the test presents no more safety hazard to an individual being tested than other tests where blood is drawn. As such, subjects may experience pain, potential infection and possibly excessive bleeding at the venipuncture specimen collection site.

Adverse events derived from potential false positive and false negative results are discussed in Section VIII, and briefly summarized here. Potential false positive results involving genotypes 1, 4 and 5 (48 weeks of treatment) versus genotypes 2 and 3 (24 weeks of treatment) are the most serious false positive events. If the device erroneously calls a genotype 1 specimen as genotype 2 or 3, the physician may not have as many treatment options as the physician should, and the patient will likely be treated for 24 weeks instead of 48 weeks. This would decrease the patient's chance of obtaining a successful outcome from treatment (sustained virological response). On the other hand, Genotype 1 patients who receive a genotype 4 or 5 result may be deprived of treatment choices available only to genotype 1 HCV patients. This is likely to be a non-serious event, because patients would be still treated with a proven valid therapy, even if not the most current or optimal. Potential false negative results, such as a test result that fails to produce a HCV genotype determination, "No Result" or "HCV detected but did not produce a genotype result", may lead a clinician to repeat the test or use an alternative genotype method. Since HCV treatment decisions are not required "STAT" there would be no long term health risk to the patient.

Based on the data from the accuracy study, the probability of the device to generate a miscall of the genotype is very low. In the accuracy study, only one miscall was observed for genotype 1 (265/266), genotype 2 (110/111), and genotype 4 (76/77). There were no miscalls for genotype 3 (86/86) and genotype 5 (24/24). Therefore, the percent of patients that might experience a harmful event due to a miscall of the genotype is less than 1% based on a total of three miscalls in a total of 564 eligible results.

There were no significant adverse effects of the Abbott RealTime HCV Genotype II assay reported while the study was conducted.

C. Benefit-Risk Conclusions

The probable benefits of the device are also based on data collected in the clinical studies conducted to support the PMA approval as described above.

When used for the proposed intended use, the benefits of the Abbott RealTime HCV Genotype II assay to the clinician and patient include an established device performance in a manner that demonstrates consistently accurate test results and the ability to predict the patient's response to treatment based on the assay results, as indicated by the demonstrated association between HCV genotype (determined by the device) and SVR rate.

The risks associated with the Abbott RealTime HCV Genotype II assay, when used as intended, are those related to failure of the device to perform as indicated or to an error in the interpretation of the result. These risks include a possible error in identification of the HCV genotype, which can result in error or delay in patient treatment when genotype-specific treatment is instituted or in delay in determining the patient's true genotype. A misidentification test result may lead a clinician to assign an incorrect treatment duration and dosage.

The risks associated with the use of the Abbott RealTime HCV Genotype II assay are mitigated by the product labeling, which provides the device intended use, directions for use, information on quality control, interpretation of results, conditions for storage of reagents, precautions, and by the results of the studies conducted by the sponsor when evaluating the performance and clinical usefulness of the device.

In conclusion, given the available information mentioned above, the presented data from the non-clinical and clinical studies support that, 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 probable benefits of the Abbott RealTime HCV Genotype II assay outweigh the probable risks associated with its use. There are no substantial clinical concerns with the approval of this device.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the instructions for use. The data from the preclinical studies demonstrated acceptable analytical sensitivity, precision/reproducibility, and analytical specificity of the Abbott RealTime HCV Genotype II assay when used according to the instructions for use, the warnings and precautions, and limitations sections as stated in the labeling. The clinical usefulness studies and the statistical analysis of clinical data in this application have shown that specific HCV genotype (1, 1a, 1b, 2, 3, 4, or 5) identification as defined by the Abbott RealTime HCV Genotype II assay is informative for assessing the probability of achieving sustained virological response (SVR) in chronically infected HCV patients treated with interferon alfa-2a or 2b and ribavirin combination therapy, and that the test is safe and effective when used according to the directions for use in the labeling. Therefore, this device should benefit the physician in the management of chronically infected HCV patients.

XIII. CDRH DECISION

CDRH issued an approval order on June 20, 2013. The final conditions of approval can be found in the approval order.

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

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

1. Myers TW, Gelfand DH. Reverse Transcription and DNA Amplification by a *Thermus thermophilus* DNA Polymerase. *Biochem* 1991;30:7661–6.
2. Higuchi R., Dollinger G., Walsh, PS, et al. Simultaneous amplification and detection of specific DNA sequences. *Biotechnology* 1992;10:413–417.
3. Saldanha J, Heath A, Aberham A, et al. World Health Organization collaborative study to establish a replacement WHO international standard for hepatitis C virus RNA nucleic acid amplification technology assays. *Vox Sang* 2005;88:202-4.
4. Ghany MG, Nelson DDR, Strader DB, et al. AASLD Practice Guideline *Hepatology* 2011;54(4):1433-44.