SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	In vitro reverse transcription-polymerase chain reaction (PCR)-based assay for detection of HCV RNA.
Device Trade Name:	Alinity m HCV
Device Procode:	MZP
Applicant's Name and Address:	Abbott Molecular, Inc. 1300 E. Touhy Avenue Des Plaines, IL 60018
Date(s) of Panel Recommendation:	None
Premarket Approval Application (PMA) Number:	P190025
Date of FDA Notice of Approval:	March 23, 2020

II. INDICATIONS FOR USE

The Alinity m HCV assay is an in vitro reverse transcription-polymerase chain reaction (RT-PCR) assay for both the detection and quantitation of hepatitis C virus (HCV) RNA, in human plasma (EDTA, Acid Citrate Dextrose) or serum, from HCV antibody positive individuals. The assay is intended for use as an aid in the diagnosis of active HCV infection in individuals with antibody evidence of HCV infection, and to aid in the management of patients with known active HCV infection, including SVR determination. The results from the Alinity m HCV assay must be interpreted within the context of all relevant clinical and laboratory findings.
The Alinity m HCV assay is not intended to be used in screening blood, plasma, serum, tissue or tissue donors for HCV.

III. CONTRAINDICATIONS

The Alinity m HCV assay is not intended to be used in screening blood, plasma, serum, tissue or tissue donors for HCV.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the Alinity m HCV labeling.

V. DEVICE DESCRIPTION

The Alinity m HCV assay utilizes real-time reverse transcriptase polymerase chain reaction (RT-PCR) to amplify and detect HCV RNA genomic sequences that have been extracted from human plasma or serum specimens. The steps of the Alinity m HCV assay consist of sample preparation, reverse transcriptase RT-PCR assembly, amplification/detection, and result calculation and reporting. All steps of the Alinity m HCV assay procedure are executed automatically by the Alinity m System. Manual dilutions may be performed for low-volume specimens to meet the minimum volume requirement, and for high-titer specimens above the upper limit of quantitation (ULOQ).

The Alinity m System is designed to be a random-access analyzer that can perform the Alinity m HCV assay in parallel with other Alinity m assays on the same instrument.

The Alinity m HCV assay requires the following three assay-specific kits in addtion to other, non-assay specific accessory reagents and consumables:

- Alinity m HCV AMP Kit
- Alinity m HCV CTRL Kit
- Alinity m HCV CAL Kit

HCV RNA from human plasma or serum is extracted using the Alinity m Sample Prep Kit 2, Alinity m Lysis Solution, and Alinity m Diluent Solution. The Alinity m System employs magnetic microparticle technology to facilitate nucleic acid capture, wash, and elution. The resulting purified RNA is then combined with liquid unit-dose Alinity m HCV activation reagent and lyophilized unit-dose Alinity m HCV amplification/detection reagents and transferred into a reaction vessel. Alinity m Vapor Barrier Solution is then added to the reaction vessel which is then transferred to an amplification/detection unit for reverse transcription, PCR amplification, and real-time fluorescence detection of HCV.

At the beginning of the Alinity m HCV sample preparation process, a lyophilized unit-dose of internal control on the AMP Tray is rehydrated by the Alinity m System and delivered into each sample preparation reaction. The internal control is then processed through the entire sample preparation and RT-PCR procedure along with the specimens, calibrators, and controls to demonstrate proper sample processing and assay validity.

The Alinity m HCV amplification/detection reagents consist of enzymes, primers, probes, and activation reagents that enable reverse transcription, polymerization, and detection. The Alinity m HCV amplification/detection reagent also contains Uracil-DNA Glycosylase (UDG) as a contamination control for amplicons containing uracil, which may be present in molecular laboratories.

An HCV calibration curve is required for determining the HCV RNA concentration. Two levels of calibrators are processed through sample preparation

and RT-PCR to generate the calibration curve. The concentration of HCV RNA in specimens and controls is then calculated from the stored calibration curve.

Assay controls are tested at or above an established minimum frequency to help ensure that instrument and reagent performance remains satisfactory. During each control event, a negative control, a low-positive control, and a high-positive control are processed through sample preparation and RT-PCR procedures that are identical to those used for specimens.

1. Alinity m HCV AMP Kit

The Alinity m HCV AMP Kit consists of 2 types of multiwell trays:

- Alinity m HCV AMP TRAY 1 (4 trays × 48 tests):
  The Alinity m HCV AMP TRAY 1 contains separate wells of lyophilized, unit-dose RT-PCR amplification/detection reagents and lyophilized, unit-dose internal control.
- Alinity m HCV ACT TRAY 2 (4 trays × 48 tests):
  Alinity m HCV ACT TRAY 2 contains liquid activation reagent.

Each Alinity m HCV AMP TRAY 1 and Alinity m HCV ACT TRAY 2 is provided in a sealed foil pouch (4 pouches of each tray type per Alinity m HCV AMP Kit for up to 192 samples [patient specimens and/or assay controls or calibrators]). Both trays contain 48 unit-dose reagent wells (with reagents as listed above) of which one well of each reagent is used per test (48 tests per tray).

2. Alinity m HCV CAL Kit

The Alinity m HCV CAL Kit is composed of the following reagents:

- Alinity m HCV CAL A (4 tubes × 1.95mL)
- Alinity m HCV CAL B (4 tubes × 1.95mL)

The Alinity m HCV CAL A and Alinity m HCV CAL B tubes are intended for single-use only. The Alinity m System will process 3 replicates from each calibrator tube.

The calibrators are assigned lot-specific HCV RNA concentrations based on the results of testing against the Primary Calibrators.

3. Alinity m HCV CTRL Kit

The Alinity m HCV CTRL Kit is composed of the following reagents:

- Alinity m HCV Negative CTRL (12 tubes × 1.15mL)
- Alinity m HCV Low Positive CTRL (12 tubes × 0.75mL)
- Alinity m HCV High Positive CTRL (12 tubes × 0.75mL)

The Alinity m HCV Negative CTRL, Alinity m HCV Low Positive CTRL, and Alinity m HCV High Positive CTRL tubes are intended for single-use only. Controls are recommended to be tested at or above the minimum frequency of once every 24 hours.

4. Alinity m HCV Application Specification File

The application specification file is a data file that contains a set of parameters in a software-industry-standard JSON (Java Script Object Notation) file format. The parameters determine how the software controls the instrument components to execute the selected assay.

To run an assay on an Alinity m System, an Application Specification File is required. The Alinity m System software interprets the assay information provided in the specific Application Specification File (including definitions for sample extraction, reagent addition, amplification/detection, dilution function, data analysis, and validity evaluation protocols), along with system information, to control the system hardware, run vaidity checks and identify the appropriate algorithms for data generation.

5. Alinity m Sample Prep Kit 2

The Alinity m Sample Prep Kit 2 is provided in a liquid, multi-dose format and is shared with other Alinity m assays. It consists of 2 reagents:

- Alinity m Elution Buffer 2 (4 bottles × 22mL)
- Alinity m Microparticles 2 (4 bottles × 24mL)

The Alinity m Sample Prep Kit 2 is used in conjunction with Alinity m System Solutions as part of the sample preparation protocol to extract and concentrate target molecules from biological samples for subsequent Polymerase Chain Reaction (PCR) amplification, and to remove potential inhibitors from the resulting extract.

The sample preparation procedure consists of lysis/binding, washes, and elution. The sample preparation is performed within a disposable multi-well Integrated Reaction Unit that is loaded onto an APU on the Alinity m System.

6. Alinity m Specimen Dilution Kit I

The Alinity m Specimen Dilution Kit I is intended to allow dilution of specimens for measurement of nucleic acid. It consists of Alinity m Specimen Diluent Tubes with a pierceable cap (24 tubes × 2.45mL). Each Specimen Dilution Kit I supports dilution of up to 24 samples (patient specimens); each tube may only be used once and must not be reused. The Alinity m Specimen Diluent Tubes contain Abbott Molecular Transport Buffer which contains guanidine thiocyanate (GITC) in Tris Buffer.

7. Alinity m Tubes and Caps

- Alinity m LRV Tube: capped Low Residual Volume (LRV) Tubes (12 per kit)
- Alinity m Transport Tube Pierceable Capped: transport tubes closed with pierceable caps (1500 capped tubes per case, 10 boxes of 150 capped tubes)
- Alinity m Transport Tube: 1600 tubes per kit
- Alinity m Pierceable Cap: 2000 caps per kit

- Alinity m Aliquot Tube: 1600 tubes per kit

8. Alinity m System Solutions

The Alinity m System Solutions (below) are used as part of the sample preparation protocol to extract and concentrate target molecules from biological samples for subsequent PCR amplification and to remove potential inhibitors from the resulting extract.

- The Alinity m Lysis Solution: consists of 1 bottle × 975mL.
- The Alinity m Diluent Solution: consists of 4 bottles × 975mL
- The Alinity m Vapor Barrier Solution: consists of 1 bottle × 975 mL

9. Alinity m System

The Alinity m System is a fully integrated and automated molecular diagnostics analyzer which utilizes real-time PCR technology in clinical laboratories. It is an integrated system for performing sample preparation and performing fluorescence-based real-time PCR to provide quantitative and qualitative detection of nucleic acid sequences. It provides sample-to-result uninterrupted processing workflow.

The Alinity m System enables continuous and random-access sample processing by utilizing four (4) independent Assay Processing Units (APUs), each of which has a sample processor and a PCR thermal cycler/reader modules that operate in parallel. The system can process up to 300 tests in approximately 8 hours.

APU sample preparation lanes use multi well disposables and provide functionality for heating wells, mixing the contents of wells, capturing magnetic particles, and moving the magnetic particles from one well to another (extraction unit). Once the sample is purified, the remaining eluate is mixed with amplification reagent and dispensed into a reaction vessel (1 reaction vessel per sample) where it undergoes thermal cycling and amplification/detection (Amp-Detect unit).

The Alinity m System software interprets system and assay specific (provided in the Application Specification File) information, calculates results, and provides the interface for controlling the system hardware.

Using application specifications, customers create orders for calibrators, controls, and specimens. Customers load racks of calibrators, controls, and specimens in the sample input to begin processing. Once the samples are processed, results are reviewed and released through the software user interface.

Additional details can be found in the Alinity m Operator’s Manual.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are several other FDA approved in vitro diagnostic tests for the quantitation of HCV RNA. The patient's medical history and thorough clinical examination, in addition to serology, PCR or nucleic acid testing (NAT), determination of liver enzyme levels, and noninvasive liver elasticity measurements or biopsy of the liver, will provide further information on the status of an HCV infection. Each alternative has its own advantages and disadvantages.

VII. MARKETING HISTORY

The Alinity m HCV AMP Kit (List No. 08N50 095), Alinity m HCV CTRL Kit (List No. 08N50 085), and Alinity m HCV CAL Kit (List No. 08N50 075) are intended to be marketed in the United States.

At this time, the Alinity m HCV assay (List No. 08N50-095) has not been introduced or distributed for sale, and there are no tests sold to date. However, the CE certified Alinity m HCV AMP Kit (List No. 08N50-090/091), Alinity m HCV CTRL Kit (List No. 08N50-080), and Alinity m HCV CAL Kit (List No. 08N50-070) are identical in formulation to the US kits, except for kit labeling, and were introduced to foreign markets outside of the United States as listed in **Table 1.**

Table 1: Alinity m HCV in Foreign Markets Outside of the United States

Australia[a]	Greece	Peru
Austria	Hungary	Poland
Belgium	Iceland	Portugal
Brazil	Ireland	Romania
Bulgaria	Italy	Saudi Arabia
Croatia	Kenya	Slovakia
Cyprus	Latvia	Slovenia
Czech Republic	Liechtenstein	Spain
Denmark	Lithuania	Sweden
Estonia	Luxembourg	Switzerland
Finland	Malta	Turkey
France	Netherlands	UK
Germany	Norway	

[a] 08N-50-091 only

The Alinity m System (List No. 08N53-002) is intended to be marketed in the United States. At this time, the Alinity m System (List No. 08N53-002) has not been introduced for sale into the United States. However. The system was self-certified for commercialization in the European Union, and the European Free Trade Association (EFTA) since December 2017 and is marketed in the countries listed in Table 2 below:

Table 2: Alinity m System - Registered for Sale in Following Countries

Australia	Greece	Netherlands
Austria	Hungary	Norway
Belgium	Iceland	Poland
Brazil	Ireland	Portugal
Bulgaria	Israel	Romania
Colombia	Italy	Saudi Arabia
Croatia	Japan	Slovakia
Cyprus	Kenya	Slovenia
Czech Republic	Latvia	Spain
Denmark	Liechtenstein	Sweden
Estonia	Lithuania	Switzerland
Finland	Luxembourg	Turkey
France	Malta	UK
Germany		

The Alinity m HCV and the Alinity m System have not been withdrawn from marketing for any reason related to its safety or effectiveness.

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 either the device operator or the patient who is tested. No adverse effects occurred during conduct of the clinical studies.

An erroneous "Not Detected" (false negative) result may lead to an HCV infected patient not being identified and not admitted into care. A false negative result is considered a public health concern because of the potential for HCV transmission from an unidentified HCV infected patient; however, this is partially mitigated by current testing guidelines that recommend repeat HCV RNA testing within a certain timeframe for patients with symptoms indicative of an HCV infection, and for patients at high risk of HCV infection. For HCV infected patients undergoing direct acting antiviral (DAA) therapy, a false negative result is not significant because current treatment regimens are all of fixed duration.

An erroneous "HCV Detected" (false positive) result is not considered to be a public health concern because HCV RNA testing is used to confirm serology positive results. In addition, repeat HCV RNA testing and consideration of other clinical evidence of an active HCV infection is recommended prior to initiating HCV DAA therapy. However, a false positive test result could lead to unnecessary exposure to HCV therapy with the potential for adverse effects.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

1. Limit of Detection (LoD)

The limit of detection (LoD) was determined by testing dilutions of the 4th World Health Organization (WHO) International Standard for Hepatitis C Virus for Nucleic Acid Amplification Techniques (NIBSC code: 06/102; genotype 1) prepared in HCV negative human plasma and serum. Testing for each HCV RNA concentration was performed with 4 lots of amplification reagents across multiple days. The results, representative of the analytical sensitivity performance of Alinity m HCV assay in plasma and serum are included in **Table 3**.

Probit analysis of the data determined that the concentration of HCV RNA in plasma detected with 95% probability (LoD by Probit) was 8.50 IU/mL (95% CI: 6.20 to 14.53 IU/mL).

Probit analysis of the data determined that the concentration of HCV RNA in serum detected with 95% probability (LoD by Probit) was 7.96 IU/mL (95% CI:5.06 to 25.52 IU/mL).

The LOD of Alinity m HCV is 12 IU/mL (1.08 Log IU/mL) in plasma and serum.

Table 3: LOD with HCV 4th WHO International Standard (NIBSC 06/102) in EDTA Plasma and Serum Across all Lots

Matrix	Nominal Concentration (IU/mL)	Number of Valid Replicates	Number of detected* Replicates	Hit Rate [%]	LOD by Probit [95% CI]
Plasma	1	95	11	11.6	8.50 IU/mL [6.20 – 14.53 IU/mL]
	2	93	25	26.9	
	3	93	66	71.0	
	6	96	84	87.5	
	9	95	90	94.7	
	12	96	94	97.9	
	15	95	95	100.0	
Serum	1	94	14	14.9	7.96 IU/mL [5.06 – 25.52 IU/mL]
	2	93	24	25.8	
	3	95	67	70.5	
	6	93	84	90.3	
	9	95	91	95.8	
	12	95	92	96.8	
	15	96	96	100.0	

2. Genotype Specific LoD

The LoD of the Alinity m HCV assay was verified for HCV Genotypes 2, 3, 4, 5, and 6 in plasma and serum. For each HCV Genotype, 3 panel members were prepared by diluting a clinical specimen into HCV negative human serum and plasma. Testing was performed across three days. Alinity m HCV detected >95% of HCV replicates at and above 9 IU/mL in plasma and serum. As such, the claimed LOD of the Alinity m HCV was confirmed for all genotypes.

3. Linearity

Linearity of Alinity m HCV was assessed by testing a dilution series of HCV genotype 1 in negative human plasma and serum, each consisting of 9 panel members spanning from 10 to 200,000,000 IU/mL (1.00 to 8.30 Log IU/mL). Two kind of panel members were created: panel members with concentrations from 10 to 10,000 IU/mL (1.00 to 4.00 Log IU/mL) were prepared using an HCV positive clinical specimen, panel members with concentrations 100 to 200,000,000 IU/mL (2.00 to 8.30 Log IU/mL) were prepared using Armored RNA.

There was no significant difference in the linearity between the clinical sample and the armored RNA based panel members. As such they were analyzed in a combined regression (**Figure 1**). Alinity m HCV demonstrated linearity in plasma and serum across the range tested with an upper limit of quantitation (ULoQ) of 8.3 Log IU/mL.

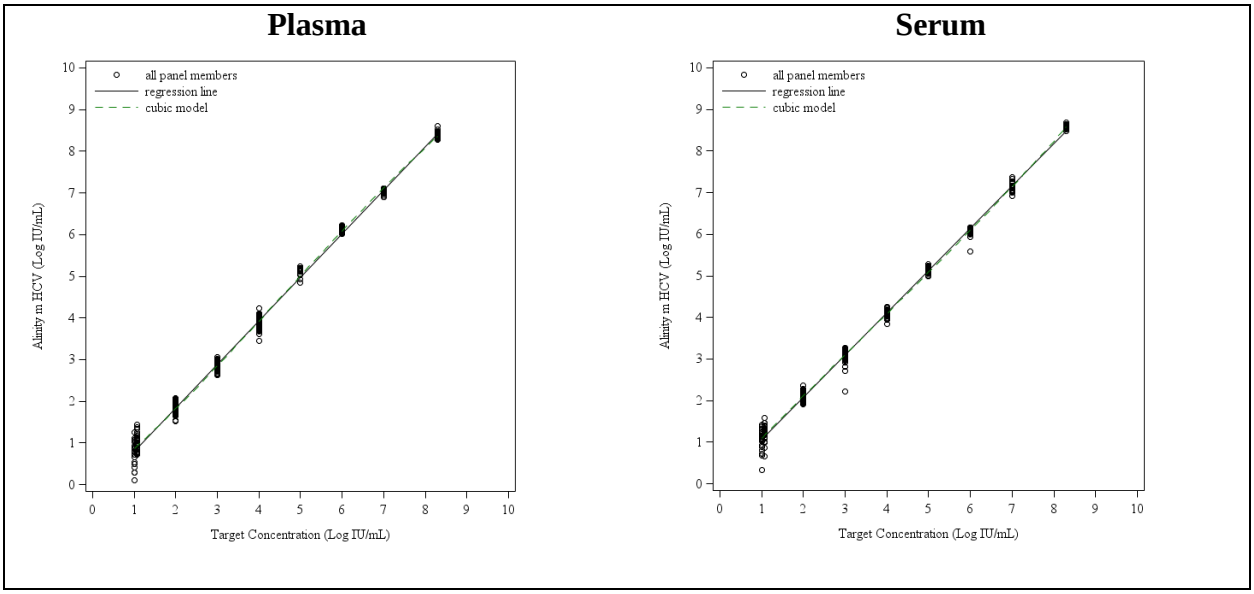

Figure 1: Alinity m HCV Linearity in Serum and Plasma for Genotype 1 - Outliers Included

Linearity was also performed with genotypes 2, 3, 4, 5, and 6. The following equations were obtained for the different genotypes using a least square regression model across the linear range of the Alinity m HCV (outliers were included). The Alinity m HCV is linear across the measuring range for all tested genotypes.

Table 4: Least Square Regression Equations

Genotype	Plasma		Serum	
	Linear Equation	**Maximum Non-linearity (log IU/mL)**	**Linear Equation**	**Maximum Non-linearity (log IU/mL)**
1	y=1.05x - 0.04	0.06	y=1.05x + 0	-0.12
2	y=0.99x + 0.17	0.07	y=1.06x = 0.12	-0.13
3	y=1.02x - 0.02	0.03	y=1.02x + 0.05	0.05
4	y=1.04x - 0.07	0.16	y=0.99x + 0.09	0.15
5	y=1.01x - 0.04	0.15	y=1.00x + 0.04	0.26
6	y=1.03x - 0.02	0.08	y=1.05x – 0.11	-0.08

4. Lower Limit of Quantitation (LLoQ)

The lower limit of quantitation (LLOQ) is defined as the lowest concentration at which HCV RNA is reliably quantitated within a total error. Total error was estimated by two methods: Total Analytical Error (TAE) = |bias| + 2 × SD, and Total Error (TE) =$\sqrt{2}$ × 2 × SD.

TAE and TE of Alinity m HCV for genotypes 1, 2, 3, 4, 5 and 6 in plasma and serum were calculated for panel members with observed concentrations at or near 12 IU/mL (1.08 Log IU/mL) tested in multiple non-clinical studies (LOD/Genotype specific LOD, Linearity, Precision [see below]) as shown in **Table 5**. The panel members were either generated directly from the 4th WHO International Standard for HCV (NIBSC code: 06/102) or their concentrations were traceable to the 4th WHO International Standard for HCV.

The results of these analyses demonstrated that Alinity m HCV can determine the concentration of HCV RNA for genotypes 1, 2, 3, 4, 5, and 6 in plasma and serum at 12 IU/mL (1.08 Log IU/mL) with an acceptable level of accuracy and precision, i.e., TAE and TE less than or equal to 1.00 Log IU/mL. The LLoQ of the Alinity m HCV is 12 IU/mL.

Table 5: LLoQ of the Alinity m HCV (TAE and TE for all Genotypes based on various studies as indicated)

Genotype	Study	Target (Log IU/mL)	Mean (Log IU/mL)	Bias (Log IU/mL)	SD	TAE	TE
PLASMA							
1	Precision	1.08	1.07	-0.01	0.19	0.40	0.55
	LOD	1.08	0.82	-0.26	0.24	0.75	0.69
	Linearity	1.08	1.01	-0.07	0.20	0.47	0.57
2	Genotype LOD	1.08	0.95	-0.13	0.24	0.61	0.68
	Genotype Linearity	1.08	1.04	-0.04	0.18	0.39	0.50
3	Genotype LOD	1.08	1.06	-0.02	0.20	0.41	0.56

Genotype	Study	Target (Log IU/mL)	Mean (Log IU/mL)	Bias (Log IU/mL)	SD	TAE	TE
PLASMA							
	Genotype Linearity	1.08	1.05	-0.03	0.23	0.49	0.65
4	Genotype LOD	1.08	1.10	0.02	0.23	0.48	0.64
	Genotype Linearity	1.08	1.30	0.22	0.20	0.63	0.58
5	Genotype LOD	1.08	1.10	0.02	0.25	0.51	0.70
	Genotype Linearity	1.08	1.15	0.07	0.23	0.53	0.64
6	Genotype LOD	1.08	0.97	-0.11	0.17	0.45	0.49
	Genotype Linearity	1.08	1.11	0.03	0.28	0.59	0.80
SERUM							
1	Precision	1.08	0.96	-0.12	0.23	0.57	0.64
	LOD	1.08	0.85	-0.23	0.22	0.67	0.62
	Linearity	1.08	1.23	0.15	0.20	0.55	0.56
2	Genotype LOD	1.08	1.07	-0.01	0.14	0.30	0.40
	Genotype Linearity	1.08	0.96	-0.12	0.15	0.42	0.41
3	Genotype LOD	1.08	1.10	0.02	0.21	0.44	0.60
	Genotype Linearity	1.08	1.13	0.05	0.26	0.56	0.72
4	Genotype LOD	1.08	1.14	0.05	0.20	0.45	0.57
	Genotype Linearity	1.08	1.27	0.19	0.17	0.54	0.49
5	Genotype LOD	1.08	1.27	0.19	0.15	0.48	0.41
	Genotype Linearity	1.08	1.16	0.08	0.16	0.40	0.46
6	Genotype LOD	1.08	1.06	-0.02	0.15	0.33	0.44
	Genotype Linearity	1.08	1.15	0.07	0.29	0.65	0.82

5. Traceability to the WHO Standard

Primary calibrators and assay calibrators with known concentrations were used throughout product development and product manufacturing to establish traceability to the 4th WHO International Standard for Hepatitis C Virus for Nucleic Acid Amplification Techniques (NIBSC code: 06/102). The concentrations tested for the WHO standard were 3.00 and 2.00 Log IU/mL. The concentrations tested for the primary calibrators ranged from 3.00 to 7.00 Log IU/mL. The Alinity m HCV calibrators and controls were also tested along with the primary calibrators and the WHO standard. All of the panels had observed HCV concentrations similar to the target concentrations, and were linear across the assay's quantitation range, as presented in **Figure 2**.

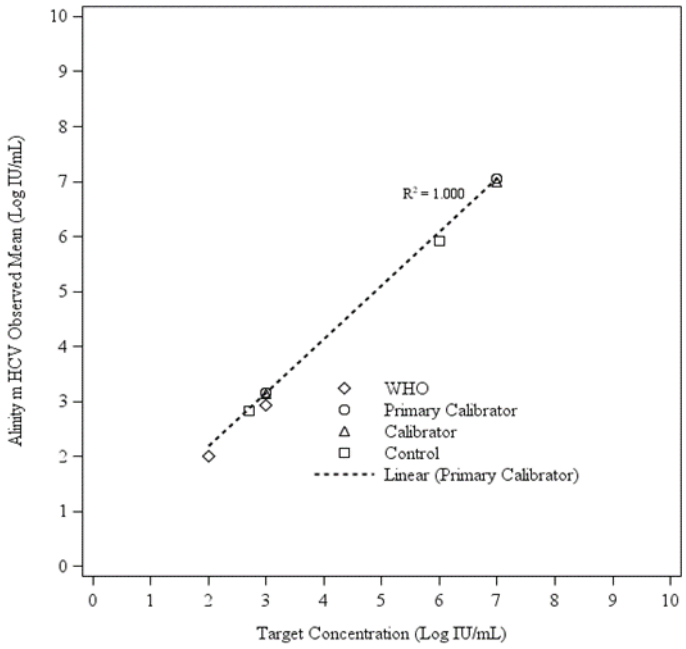

Figure 2: Traceability to the 4th WHO International Standard for HCV (NIBSC code: 06/102)

6. Precision

These studies evaluated the lot-to-lot variability in an internal precision study and the reproducibility of the Alinity m HCV test in plasma on the Alinity m system at 3 external sites with four reagent lots.

a. Internal 12-Day Precision Study

Precision of Alinity m HCV was determined by analyzing a 9-member plasma panel and a 9-member serum panel. Panels span the range of 12 IU/mL (the assay's LLOQ) to 200,000,000 IU/mL. Panel members 1, 2, 3, 5, 7, 8 and 9 were prepared by diluting HCV genotype 1 into HCV negative human plasma and serum, whereas panel members 4 and 6 were prepared by diluting HCV genotype 2 into HCV negative human plasma and serum. Panel members with concentrations from 2 to 5 Log IU/mL (100 to 100,000 IU/mL) were prepared using HCV positive clinical specimens, while panel members with concentrations greater than 5 Log IU/mL (100,00 IU/mL) were prepared using Armored RNA. Each panel member was tested in 4 replicates, twice each day for 12 days, on 3 Alinity m Systems with 1 Alinity m AMP Kit lot by 3 operators (one per instrument) for a total of 288 replicates.

The results, representative of the precision of Alinity m HCV in plasma and serum, (**Table 6**), demonstrated that the within-laboratory standard deviation (SD) is less than or equal to 0.25 Log IU/mL of HCV RNA from 2 to 8 Log IU/mL (100 to 100,000,000 IU/mL), and less than or equal to 0.35 Log IU/mL from 1 to 3 times the LLOQ (1.08 to 1.56 Log IU/mL or 12 to 36 IU/mL).

Table 6: Precision Analysis in Plasma and Serum

Panel	N	Mean Concentration (Log IU/mL)	Within-Run Component		Between-Run Component		Between-Day Component		Within-Laboratory[a]		Between-Instrument/ Operator Component		Total[b]	
			SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
PLASMA														
01	285	1.07	0.18	16.9	0.07	6.4	0.00	0.0	0.19	18.1	0.08	7.9	0.21	19.7
02	286	1.42	0.15	10.8	0.03	2.4	0.00	0.0	0.16	11.0	0.08	6.0	0.18	12.6
03	284	1.91	0.13	6.9	0.02	1.0	0.00	0.0	0.13	6.9	0.10	5.2	0.17	8.7
04	281	2.88	0.12	4.2	0.00	0.0	0.04	1.5	0.13	4.5	0.11	3.7	0.17	5.8
05	285	3.94	0.12	3.0	0.00	0.0	0.02	0.5	0.12	3.1	0.07	1.8	0.14	3.6
06	285	5.15	0.10	2.0	0.01	0.2	0.02	0.5	0.11	2.1	0.07	1.4	0.13	2.5
07	282	6.14	0.07	1.2	0.01	0.1	0.00	0.0	0.07	1.2	0.09	1.4	0.11	1.8
08	285	7.11	0.12	1.7	0.03	0.4	0.00	0.0	0.13	1.8	0.10	1.5	0.16	2.3
09	285	8.55	0.06	0.7	0.03	0.4	0.00	0.0	0.06	0.8	0.15	1.7	0.16	1.9
SERUM														
01	284	0.96	0.22	23.0	0.00	0.0	0.04	4.1	0.23	23.4	0.07	7.0	0.24	24.4
02	283	1.34	0.16	12.1	0.05	3.5	0.03	2.3	0.17	12.8	0.08	6.3	0.19	14.3
03	286	1.81	0.12	6.4	0.05	2.6	0.03	1.8	0.13	7.2	0.06	3.5	0.14	8.0
04	286	2.78	0.09	3.4	0.05	1.9	0.04	1.6	0.12	4.2	0.15	5.2	0.19	6.7
05	286	3.82	0.09	2.5	0.06	1.7	0.01	0.3	0.11	3.0	0.05	1.3	0.12	3.3
06	286	5.10	0.09	1.7	0.00	0.0	0.00	0.0	0.09	1.7	0.08	1.7	0.12	2.4
07	282	6.08	0.08	1.3	0.03	0.5	0.00	0.0	0.09	1.4	0.07	1.1	0.11	1.8
08	286	6.85	0.08	1.2	0.06	0.9	0.05	0.7	0.11	1.7	0.01	0.2	0.12	1.7
09	288	8.60	0.06	0.7	0.02	0.2	0.00	0.0	0.06	0.7	0.13	1.5	0.15	1.7

[a] Within-Laboratory includes Within-Run, Between-Run and Between-Day Components.
[b] Total includes Within-Run, Between-Run, Between-Day and Between-Instrument Components.

b. External 5-Day Reproducibility Study

A multi-site reproducibility study was performed at three external U.S. based sites. Six unique panel members for Genotype 1a and five unique panel members for Genotype 2 covering the measuring range of the assay were formulated in plasma to make an 11 member panel (see **Table 7** below) . Each panel member was tested with 5 replicates per run. Because the Alinity is a random-access analyzer a run is defined as testing a batch of 5 replicates of each of the 11 panel members tested consecutively on the system within 8 hours (i.e., one run per work day) using the same Alinity m HCV reagent lot. Testing was performed on 5 non-consecutive days. Three different Alinity m HCV Amplification Reagent Kit lots were tested (2 per site). Each site used unique lots of the Calibrator Kit, Control Kit, Sample Prep Kit 2 and Systems Solutions. A minimum of three operators at each site were part of the testing. The design (3 sites x 5 replicates in one panel x 1 run/day x 5 days x 2 lots/site) accounts for a total of 150 replicates per panel member. Results are summarized in **Table 8**.

Table 7: Genotypes and Concentration of Reproducibility Panel Members

Panel Member	Genotype	HCV RNA Target Concentration [Log IU/mL]	HCV RNA Target Concentration [IU/mL]
1	1a	8	100,000,000
2	1a	6	1,000,000
3	1a	4	10,000
4	1a	2	100
5	1a	1.18	15
6	2	7	10,000,000
7	2	5	100,000
8	2	3	1,000
9	2	1.7	50
10	2	1.18	15
11	1a	1.0	12

Table 8: Summary of Reproducibility (All Sites)

Genotype	N[a]	Mean Concentration (Log IU/mL)	Within-Run		Between-Run		Within-Laboratory[c]		Between-Lot		Between- Site		Total[d]	
			SD[b]	%CV	SD[b]	% CV	SD[b]	% CV	SD[b]	% CV	SD[b]	% CV	SD[b]	% CV
1	150	8.50	0.08	0.9	0.05	0.6	0.09	1.1	0.12	1.4	0.00	0.0	0.15	1.7
	150	6.15	0.15	2.5	0.03	0.5	0.15	2.5	0.03	0.6	0.01	0.1	0.16	2.6
	150	3.89	0.16	4.2	0.04	1.1	0.17	4.3	0.04	1.0	0.03	0.7	0.18	4.5
	150	1.95	0.21	10.6	0.00	0.0	0.21	10.6	0.06	3.1	0.00	0.0	0.22	11.0
	149	1.06	0.30	28.0	0.09	8.6	0.31	29.3	0.16	15.2	0.00	0.0	0.35	33.0
	128	0.65	0.34	53.0	0.07	10.6	0.35	54.0	0.11	17.5	0.00	0.0	0.37	56.8
2	150	7.30	0.09	1.3	0.01	0.2	0.09	1.3	0.02	0.3	0.05	0.6	0.11	1.5
	150	5.27	0.12	2.3	0.00	0.0	0.12	2.3	0.04	0.8	0.05	0.9	0.14	2.6
	150	3.06	0.20	6.5	0.00	0.0	0.20	6.5	0.06	1.9	0.00	0.0	0.21	6.7
	150	1.44	0.21	14.7	0.08	5.3	0.22	15.6	0.10	6.7	0.00	0.0	0.24	17.0
	149	0.88	0.28	31.4	0.09	10.2	0.29	33.0	0.08	8.8	0.00	0.0	0.30	34.2

[a] Number of valid replicates with detectable viral load
[b] SD = Standard deviations in Log IU/mL
[c] Within-Laboratory includes Within-Run, Between-Run and Between-Day component
[d] Total includes Within-Run, Between-Run, Between-Day, and Between-Instrument components

7. Cross Reactivity of the Alinity m HCV with Other Microorganisms

A comprehensive list of organisms containing viral, fungal and bacterial analytes as indicated in **Table 9** was evaluated for cross-reactivity with the Alinity m HCV test. The study was designed according to CLSI EP7-A2. Organisms were tested by adding either the microorganism or its purified nucleic acid to HCV negative and HCV positive (36 IU/mL and 10,000 IU/mL) plasma at a final concentration of 10^5 U/mL for viruses, protozoans and yeast and 10^6 CFU/mL for bacteria. Cross-reactivity was analyzed from the HCV negative sample, microbial interference was analyzed from the 3x LLoQ (i.e., 36 IU/mL) sample (i.e., detectability) and from the 10,000 IU/mL sample (i.e., quantitation). Three replicates for each cross reactant were tested.

No cross reactivity or microbial interference was observed for any of the tested organsims.

Table 9: Potential Cross-Reactants

Viruses	Bacteria
Adenovirus Type 5	*Chlamydia trachomatis*
BK polyomavirus	*Corynebacterium diphtheriae*
Dengue virus 1	*Mycobacterium gordonae*
Dengue virus 2	*Mycobacterium smegmatis*
Dengue virus 3	*Neisseria gonorrhoeae*
Dengue virus 4	*Propionibacterium acnes*
FSME virus	*Staphylococcus aureus*
GB virus C	*Staphylococcus epidermidis*
Hepatitis A Virus	*Streptococcus pneumoniae*
Hepatitis B Virus	**Yeast/Protozoan**
Hepatitis D Virus	*Candida albicans*
HSV-1	*Trichomonas vaginalis*
HHV-2	
HHV-5 (CMV)	
HHV-4 (EBV)	
HHV-6B	
HHV-8 (Kaposi Sarcoma Virus)	
HIV-2	
HIV-1	
HPV-16	
HPV-18	
HTLV-1	
HTLV-2	
Influenza A	
Japanese encephalitis virus	
Murray Valley encephalitis virus	
Parvovirus B19	
Rubella virus	
St. Louis encephalitis	

Vaccinia virus (VACV) VZV West Nile virus Yellow Fever virus Zika virus	

8. Interfering Substances

a. Interference with Endogenous Substances

The impact of potentially interfering endogenous substances and disease states on the analytical specificity (detection and quantitation) of the Alinity m HCV assay was evaluated by testing spiked samples as well as patient samples with naturally elevated levels of these substances. Hemoglobin (2g/L), triglycerides (37mM), conjugated and unconjugated bilirubin (342µM), albumin (60g/L) and human DNA (2µg/mL) were spiked into HCV negative and HCV positive plasma. Ten donor samples were tested for each interfering substance with one replicate each. As a control, one replicate of each donor sample was also tested without the addition of any potentially interfering endogenous substance. The HCV positive samples were prepared at two HCV levels by adding HCV from a positive patient specimen to HCV negative plasma at a final concentration of 3 × the assay's LLOQ and at 10,000 IU/mL.

No interference was observed in the presence of albumin (60 mg/mL), hemoglobin (2 mg/mL), triglycerides (37 mM), conjugated bilirubin (0.342 mM), unconjugated bilirubin (0.342 mM) or human genomic DNA (2 mg/L) that were introduced in the sample. No interference was observed in specimens collected from individual donors containing the naturally elevated interfering substances, i.e. albumin (>5.1 g/dL), bilirubin (>2 mg/dL), hemoglobin (>2 g/L) or triglycerides (> 325 mg/dL).

b. Interference with Autoimmune Disorders, Serological Markers and non-viral Hepatitis

This study tests the interference of non-viral hepatitis and other conditions (autoimmune) with the detection and quantitation of the Alinity m HCV in the presence of 3x LLOQ HCV. HCV negative clinical specimens from 10 individual donors from each of the following disease states were tested in the presence and absence of HCV at a concentration of 3 x LLOQ: systemic lupus erythematosus (SLE), anti-nuclear antibodies (ANA), rheumatoid factor (RF), alcoholic hepatitis, non-alcoholic steatohepatitis (NASH), cirrhosis, auto-immune hepatitis, and hepatocellular carcinoma.

No interference was observed for specimens collected from patients with the following disease states: systemic lupus erythematosus (SLE), anti-nuclear antibodies (ANA), rheumatoid factor (RF), alcoholic hepatitis, non-alcoholic steatohepatitis (NASH), cirrhosis, auto-immune hepatitis or hepatocellular carcinoma (HCC). Please also refer to the Diagnostic Study in the clinical study section.

c. Interference with Potentially Interfering Drugs

Interference was tested in the presence of drug compounds tested in pools that are listed in Table 12, at a concentration of 3 times the reported Cmax or higher. Not interference was observed.

Table 10: Potentially Interfering Drugs

Pool Tested	Drug Compounds in Pool
1	Abacavir sulfate, Acetaminophen, Acyclovir, Adefovir, Amitriptyline, Amlodipine, Aspirin, Atazanavir, Atenolol, Atorvastatin, Azithromycin, Celecoxib, Cidofovir, Clarithromycin, Clopidogrel
2	Didanosine, Efavirenz, Entecavir, Fluconazole, Fluoxetine, Ibuprofen, Indinavir, Kaletra (Lopinavir and Ritonavir), Lamivudine, Levofloxacin, Maraviroc, Nelfinavir, Nevirapine, Paroxetine
3	Prednisone, Raltegravir, Ribavirin, Rifamate (Rifampin and Isoniazid), Saquinavir, Sertraline, Stavudine, Stribild (Elvitegravir, Cobicistat, Emtricitabine, and Tenofovir), Bactrim (Trimethoprim/Sulfamethoxazole)
4	Darunavir, Ethambutol, Etravirine, Flucytosine, Fluticasone propionate, Furosemide, Hydrochlorothiazide, Levothyroxine, Rifabutin, Rilpivirine, Salmeterol xinafoate, Simeprevir, Sofosbuvir, Telaprevir, Tenofovir alafenamide, Trazodone, Warfarin, Zalcitabine
5	Fosamprenavir, Keflex (Cephalexin), Metformin, Naproxen, Pyrazinamide
6	Tipranavir
7	Ceftriaxone, Ciprofloxacin, Foscarnet, Lisinopril, Peginterferon alfa-2a, Enfuvirtide, Imipramine
8	Cyclosporine, Telbivudine, Valacyclovir, Valganciclovir, Zidovudine, Amphotericin B, Ganciclovir
9	Hydrocodone
10	Biotin
11	Acetaminophen/Hydrocodone)

9. Carryover

The carryover rate for Alinity m HCV was determined by analyzing 362 replicates of HCV negative plasma samples processed from alternating positions with high concentration HCV armored RNA positive plasma samples at 10,000,000 IU/mL, across a total of 16 runs. HCV RNA was not detected in any of the HCV negative samples, resulting in an overall carryover rate of 0.0% (95% CI: 0.0 to 1.1%).

10. Matrix Equivalency Study

a. Serum/Plasma Equivalency Across the Linear Range

Serum/plasma matrix equivalency in Alinity m HCV results for plasma and serum was evaluated by analyzing 27 negative plasma and serum pairs and 58 positive plasma and serum pairs. Ten positive pairs were collected from HCV positive patients. Forty-eight positive pairs were prepared by spiking patient specimens or Armored RNA in paired serum and plasma specimens collected from individuals without a history of HCV. The HCV RNA concentrations for the HCV positive serum/plasma pairs were distributed across the quantitative range of the assay, with the lowest concentration at 1.23 Log IU/mL and the highest concentration at 8.21 LogIU/mL. HCV genotypes 1, 2, 3 ,4, 5 and 6 were represented in the positive samples.

No detection was observed for any of HCV negative plasma and serum samples; all HCV positive plasma and serum samples were detected, resulting in an overall percent agreement between plasma and serum of 100.0 % (95% CI: 95.7 to 100.0%). The least square regression analysis in **Figure 3** between serum and plasma demonstrated that the Alinity m HCV Assay has equivalent performance in reporting quantitative results with serum and plasma since the regression demonstrated a slope of 1.00, intercept of -0.06, r = 0. 994, and mean bias of -0.06 Log IU/mL (plasma minus serum). Accordingly, performance of the test in serum and plasma can be considered equivalent.

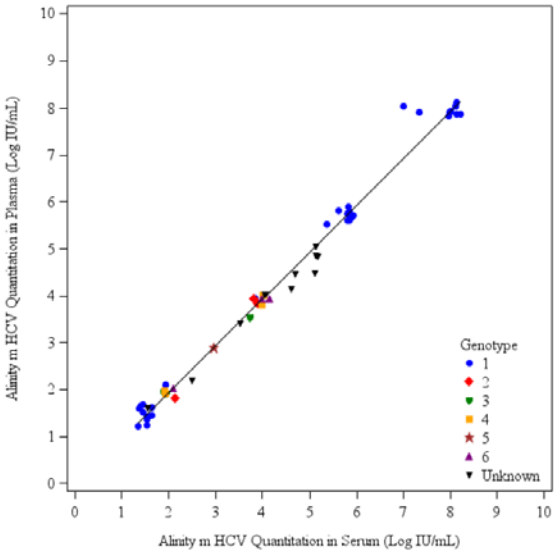

Figure 3: Matrix Equivalence Study (Outliers included) - Least Squares Regression Plot (equation: Y = 1.0 x X + (-0.1); 95% CI Slope: 1.0, 1.0; 95% CI Intercept: -0.2,0.1).

b. Specimen and Collection Tube Type Equivalency

To demonstrate equivalent performance between serum and plasma collection tube types, 25 matched sets of HCV- negative and positive samples were obtained in each of the following sixprimary collection tubes and tested using the Alinity m HCV Assay: plasma preparation tubes (PPT), K2 EDTA, K3 EDTA, ACD, serum tubes, and serum separation tubes (SST). To generate the positive samples aliquots of each of the 25 sets were spiked with a high positive clinical sample at a concentration of (a) 3x LLoQ and (b) 4 log IU/mL HCV.

Serum (serum and SST) and plasma collection tubes (K2 EDTA, K3 EDTA, ACD and PPT) demonstrated 100% positive and negative agreement both at 3x LLoQ and at 4 log IU/mL. For the 3x LLoQ samples the mean difference between the test condition and the control serum tube across all tube types ranged from -0.04 to 0.05 log IU/mL. For HCV positive samples targeted to 10,000 IU/mL the largest 95% confidence interval of the mean difference between each test condition and the control condition was -0.17, 0.11 Log IU/mL with a maximum standard deviation of 0.34 log IU/mL. The largest mean difference between the test condition and the control serum tube across all tube types was 0.08 log IU/mL. The results are summarized in **Table 11** below.

Table 11: Matrix Equivalency Study - Summary

Panel Member	Collection Tube	Test Condition			Mean Difference (Test-Control)	SD of Mean Difference	95% CI of Mean Difference
		N	Mean	SD			
Low Positive	Serum (Control Plasma)	25	**1.53**	0.165	N/A	N/A	N/A
	Serum (Control Serum)	25	**1.52**	0.119	N/A	N/A	N/A
	ACD-A	25	1.57	0.102	0.05	0.156	N/P
	K3 EDTA	25	1.56	0.146	0.04	0.191	N/P
	K2 EDTA	25	1.53	0.160	0.01	0.157	N/P
	PPT	25	1.49	0.131	-0.03	0.129	N/P
	Serum Rapid-Clot Tube	25	1.56	0.167	0.03	0.163	N/P
	SST	25*	1.49	0.163	-0.04	0.232	N/P

Panel Member	Collection Tube	Test Condition			Mean Difference (Test-Control)	SD of Mean Difference	95% CI of Mean Difference
		N	Mean	SD			
High Positive	Serum (Control Plasma)	25	**3.94**	0.186	N/A	N/A	N/A
	Serum (Control Serum)	25	**3.89**	0.088	N/A	N/A	N/A
	ACD-A	25	**3.97**	0.065	0.08	0.108	(0.03, 0.12)
	K3 EDTA	25	**3.89**	0.161	0.00	0.179	(-0.07, 0.08)
	K2 EDTA	25	**3.93**	0.108	0.04	0.115	(-0.00, 0.09)
	PPT	25	**3.95**	0.096	0.05	0.140	(-0.00, 0.11)
	Serum Rapid-Clot Tube	25	**3.91**	0.228	-0.03	0.340	(-0.17, 0.11)
	SST	25	**3.98**	0.065	0.04	0.172	(-0.03, -0.11)

N/P = Not provided; N/A – Not Applicable.

* one replicate was detected but below the LLoQ, however, the software returned a value that was used in calculation of the mean and SD (such a result in the final software would not be reported with a value)

11. Validation of Dilution Procedure

a. Confirmation of the LLoQ in Diluted Specimens

The LLOQ for the Alinity m HCV assay in diluted samples was confirmed in serum and plasma by testing 2 panel members for each dilution factor targeting HCV concentrations at the assay's LLOQ and a level above LLOQ. HCV positive clinical specimen(s) were used to prepare panel members in HCV negative serum and plasma. Each panel member was diluted using the Specimen Diluent Kit per the instructions for use. For each specimen type and each panel member, the detection rate, the mean, standard deviation (SD), bias, Error in Difference of two measurements (TE) and Total Analytical Error (TAE) were calculated. Results are summarized in **Table 12**.

Table 12: LLoQ Verification for Specimens in Specimen Diluent

Matrix	Panel Member	Target Concentration Undiluted (Log IU/mL)	Dilution Factor	Target Concentration Diluted (Log IU/mL)	Tested	Detected	Mean Concentration (Log IU/mL)	Bias (Log IU/mL)	SD	TAE	TE
Plasma	1	1.48	2.5	1.08	44	44*	1.55	0.07	0.19	0.45	0.53
Plasma	2	1.57	2.5	1.18	45	45	1.91	0.34	0.15	0.64	0.42
Plasma	3	2.78	50	1.08	45	45	2.71	-0.07	0.2	0.48	0.57
Plasma	4	2.88	50	1.18	43	43	2.84	-0.04	0.22	0.48	0.62
Serum	1	1.48	2.5	1.08	45	45	1.55	0.07	0.16	0.4	0.46
Serum	2	1.57	2.5	1.18	45	45	1.64	0.07	0.16	0.39	0.46
Serum	3	2.78	50	1.08	45	45	2.72	-0.06	0.2	0.47	0.58
Serum	4	2.88	50	1.18	45	45	2.9	0.02	0.22	0.45	0.62

* Without considering the dilution factor one replicate was detected but not quantifiable per the assays established LLOQ (i.e., < 1.08 log IU/mL). All other replicates were above 1.08 log IU/mL though some replicates were below a concentration of 1.48 log IU/mL – which is equivalent to the final concentration of a sample that returns a result equal to the LLoQ when diluted by factor 2.5 (i.e., 1.08 log IU/mL x 2.5 = 1.48 log IU/mL or 30 IU/mL)

b. Quantitation of Manually Diluted Specimens

This study was designed to demonstrate that the Alinity m HCV assay provides accurate quantitation of specimens when tested using the manual dilution procedures of 1:2.5 and 1:50 as recommended as an option in the package insert.

Ten plasma panel members and 10 serum panel members, ranging from 2.0 to 8.3 Log IU/mL, were tested for each of the dilution procedures. Panel members were prepared by spiking unique HCV negative plasma/serum with a high titer HCV patient specimen or HCV Armored RNA to a final concentration ranging from 2.0 Log IU/mL to 8.3 Log IU/mL. HCV Armored RNA was only used for panel members with high concentrations (≥4.5 Log IU/mL). Panel members were then manually diluted in the Specimen Diluent buffer provided in the Alinity m Specimen Dilution Kit. Dilutions of 1:2.5 and/or 1:50 were performed according to the instructions for use. For each HCV panel member, 5 undiluted replicates and 5 replicates diluted in Specimen Diluent were tested and compared.

Note that not all panel members were tested with both dilutions.

Correct quantitation was assessed by calculating the difference between the mean quantitation results from the diluted and the undiluted replicates, and the two-sided 95% confidence interval (CI) of the difference in means. Results are summarized in **Table 13**.

The standard deviation of the panel members in both matrices was consistent with the precision of the Alinity m HCV (i.e., was <0.2 log IU/mL) with the exception of the 3.0 log IU/mL panel member in plasma that showed a slightly larger difference of +0.38 log IU/mL and a standard deviation of 0.25 log IU/mL (slightly above the SD of the precision panel member with the lowest precision). However, considering the relatively small number of replicates in this study (versus the number of replicates in the precision study) and the concentration of panel member 3, the observed differences are clinically insignificant. This study demonstrates that the Alinity m HCV can accurately quantify specimens diluted 1:2.5 and 1:50 as recommended in the package insert (i.e., the obtained viral load of diluted samples is comparable to the viral load as determined in the neat, undiluted specimen).

Table 13: Quantitation of Manual Diluted Plasma and Serum Samples

Dilution Factor	Panel	Diluted			Undiluted (Neat)			Mean Difference (Test - Control)	95% CI of Mean Difference
		N	Mean (Log IU/mL)	SD	N	Mean (Log IU/mL)	SD		
PLASMA									
2.5	0	5	2.06	0.123	5	1.98	0.077	0.08	(-0.07, 0.23)
	1	5	2.51	0.068	5	2.42	0.155	0.09	(-0.08, 0.26)
	2	5	3.05	0.084	5	3.00	0.045	0.05	(-0.05, 0.15)
	4	5	3.59	0.090	5	3.52	0.066	0.07	(-0.04, 0.18)
	5	5	4.05	0.077	5	4.15	0.058	-0.09	(-0.19, 0.01)
	6	5	4.56	0.053	5	4.72	0.027	-0.17	(-0.23, -0.11)
	7	5	5.07	0.057	4 [a]	5.24	0.044	-0.17	(-0.25, -0.09)
	8	5	5.59	0.057	5	5.71	0.048	-0.11	(-0.19, -0.04)
	9	5	6.61	0.083	5	6.77	0.041	-0.16	(-0.26, -0.07)
	10	5	7.45	0.055	5	7.42	0.038	0.03	(-0.04, 0.10)
50	2	5	2.83	0.268	5	3.00	0.045	-0.17	(-0.50, 0.16)
	3	5	3.31	0.173	5	3.33	0.101	-0.02	(-0.23, 0.18)
	4	5	3.42	0.206	5	3.52	0.066	-0.1	(-0.36, 0.15)
	5	5	3.96	0.121	5	4.15	0.058	-0.18	(-0.32, -0.04)
	6	5	4.4	0.079	5	4.72	0.027	-0.33	(-0.41, -0.24)
	7	5	4.9	0.062	4 [a]	5.24	0.044	-0.34	(-0.43, -0.25)
	8	5	5.44	0.061	5	5.71	0.048	-0.26	(-0.34, -0.18)
	9	5	6.46	0.086	5	6.77	0.041	-0.32	(-0.41, -0.22)
	10	5	7.23	0.087	5	7.42	0.038	-0.19	(-0.29, -0.10)
	11	5	8.31	0.08	5	8.67	0.015	-0.35	(-0.45, -0.25)

SERUM									
2.5	1	5	2.13	0.048	4 [a]	1.86	0.099	0.26	(0.15, 0.38)
	2	5	2.53	0.041	5	2.39	0.079	0.14	(0.05, 0.23)
	3	5	3.04	0.063	5	2.66	0.252	0.38	(0.07, 0.68)
	4	5	3.51	0.144	5	3.52	0.075	-0.02	(-0.19, 0.15)
	5	5	4.05	0.068	5	3.95	0.023	0.1	(0.03, 0.17)
	6	5	4.61	0.076	5	4.61	0.175	0	(-0.20, 0.20)
	7	5	5.09	0.033	5	5.14	0.033	-0.06	(-0.10, -0.01)
	8	4 [a]	6.44	0.026	5	6.63	0.065	-0.19	(-0.27, -0.11)
	9	5	7.45	0.039	5	7.38	0.089	0.07	(-0.03, 0.17)
	7b	5	5.56	0.068	5	5.65	0.043	-0.09	(-0.17, -0.01)
50	3	4 [a]	3.04	0.029	5	2.66	0.252	0.38	(0.07, 0.69)
	4	5	3.53	0.151	5	3.52	0.075	0	(-0.17, 0.18)
	5	5	3.92	0.078	5	3.95	0.023	-0.04	(-0.13, 0.06)
	6	5	4.48	0.093	5	4.61	0.175	-0.13	(-0.34, 0.07)
	7	5	4.94	0.064	5	5.14	0.033	-0.21	(-0.28, -0.13)
	8	5	6.51	0.084	5	6.63	0.065	-0.13	(-0.24, -0.02)
	9	5	7.26	0.057	5	7.38	0.089	-0.12	(-0.23, -0.01)
	10	5	8.33	0.034	5	8.59	0.135	-0.27	(-0.43, -0.10)
	3b	5	3.15	0.147	5	3.24	0.054	-0.09	(-0.26, 0.07)
	7b	5	5.42	0.07	5	5.65	0.043	-0.23	(-0.32, -0.15)

CI = Confidence Interval

[a] The missing replicate was invalid and was not retested

c. Precision of Diluted Specimens

Precision was evaluated in both plasma and serum by testing 3 panel members (see **Table 14**), that were prepared with HCV concentrations, such that when diluted in Specimen Diluent, the concentrations (target concentration in Specimen Diluent) were within the linear range of the Alinity m HCV assay. Plasma and serum panel members were prepared by diluting a high titer HCV genotype 1 patient specimen (panel member 1) or HCV genotype 1 Armored RNA stock (panel members 2 and 3) into HCV negative human plasma and serum. Testing was performed with 3 Diluent lots (1lot per Instrument/Operator). Results are summarized in **Table 15**.

Table 14: Panel Target Concentrations and Testing Plan for Diluted Specimen Precision

Panel Member	Concentration (Log IU/mL)	Dilution Factor Tested	Concentration after Specimen Dilution (Log IU/ mL)	Minimum Replicates per Run	Minimum Replicates per Day	Minimum Days	Minimum Replicates per Specimen Diluent Lot	Total Replicates
1	2.9	1:2.5	2.5	3	2	12	72	216
2	7.2	1:50	5.5	3	2	12	72	216
3	5.7	1:50	4.0	3	2	12	72	216

Table 15: Precision of Diluted Plasma and Serum Specimens

Matrix	Panel	N	Mean Concentration (Log IU/mL)	Within-Run Component		Between-Run Component		Between-Day Component		Within-Laboratory[a]		Between-Instrument Component		Total[b]	
				SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Plasma	01	276	2.95	0.09	3.1	0.04	1.3	0.23	7.7	0.25	8.5	0.06	2.0	0.26	8.7
	02	284	7.20	0.08	1.1	0.01	0.1	0.02	0.3	0.08	1.1	0.06	0.9	0.10	1.4
	03	283	5.65	0.12	2.1	0.00	0.0	0.01	0.2	0.12	2.1	0.07	1.3	0.14	2.5
Serum	01	283	2.97	0.12	3.9	0.05	1.7	0.04	1.2	0.13	4.5	0.05	1.7	0.14	4.8
	02	282	7.13	0.12	1.6	0.02	0.2	0.03	0.4	0.12	1.7	0.09	1.2	0.15	2.1
	03	280	5.57	0.09	1.7	0.02	0.4	0.02	0.3	0.10	1.8	0.08	1.5	0.13	2.3

[a] Within-Laboratory includes Within-Run, Between-Run and Between-Day Components.

[b] Total includes Within-Run, Between-Run, Between-Day and Between-Instrument Components.

d. On-Board Stability of Diluted Specimens

Onboard/off-board stability of the diluted sample was evaluated in both serum and plasma using some of the panel members described in **Table 13** (above). The panel members were tested undiluted onboard the Alinity m System for a minimum of 4 hours prior to testing (control) and diluted and stored as follows: For each dilution factor, the undiluted panel member was placed onboard for a minimum of 4 hours, then diluted in Specimen Diluent (using factors 2.5 and 50), placed off-board (i.e., at 15°C to 30°C) for a minimum of 2 hours, and then placed onboard for a minimum of another 4 hours prior to testing.

The difference between the mean quantitation results from testing diluted specimens with onboard/off-board storage (test conditions 1 and 2) and the mean quantitation results from testing undiluted specimens (control condition) across all panel members, dilution factors and matrices ranged from -0.09 Log IU/mL to +0.26 Log IU/mL with standard deviations that were consistent with the precision of the test. The study confirmed on-board stability for diluted serum and plasma specimens.

12. Specimen Stability

Specimen stability studies demonstrated that, for the Alinity m HCV Assay, specimens should be stored as listed in **Table 16** and can be stored in primary or secondary tubes.

Table 16: Final Sample Storage Claims

Specimen	Temperature	Max. Storage Time	Special Instructions
Whole Blood	2 to 8°C	0-72 hours	Whole blood may be stored between draw and plasma/serum separation. **Whole blood storage plus separated plasma/serum storage at 2 to 8°C must not exceed a combined total of 72 hours.**
	15 to 30°C	4 hours	
Plasma/ Serum	2 to 8°C	0-72 hours	Plasma/Serum may be stored in primary tubes (with or without gel) or secondary tubes after separation from blood cells (plasma) or clot (serum). Plasma/Serum may further stay onboard Alinity m System for up to 4 hours prior to processing.
	15 to 30°C	20 hours	
	-20°C	60 days	Plasma/Serum may be stored frozen in primary gel tubes or secondary tubes after separation from blood cells (plasma) or clot (serum). Plasma can be subjected to at most 2 freeze-thaw cycles. Serum can be subjected to at most 3 freeze-thaw cycles. Defrosted samples may be stored at 2 to 8°C for up to 6 hours prior to loading on Alinity m System. Plasma/Serum may further stay onboard Alinity m System for up to 4 hours prior to processing.
	-70°C	Long term	Plasma/Serum may be stored frozen in primary gel tubes or secondary tubes after separation from blood cells (plasma) or clot (serum). Plasma can be subjected to at most 2 freeze-thaw cycles. Serum can be subjected to at most 3 freeze-thaw cycles. Defrosted samples may be stored at 2 to 8°C for up to 6 hours prior to loading on Alinity m System. Plasma/Serum may further stay onboard Alinity m System for up to 4 hours prior to processing.

13. Reagent Stability

a. Shelflife

Realtime stability studies were performed to establish the shelf-life for the Alinity m HCV assay. Three (3) lots of the following reagent kits were stored at the intended storage temperature indicated in **Table 17** and then tested at various time points throughout the study. Performance was assessed against clinically relevant acceptance criteria using controls and calibrators and an internal stability panel consisting of three panel members between 3xLLOQ of the Alinity m HCV and 5 log IU/mL.

The Shelflife study included the assessment of an inverted condition as well as a condition that simulated fluctuating (hot/cold) temperature extremes during shipping.

Study results demonstrate that reagents are stable at their intended storage condition and continue to meet acceptance criteria fifteen (15) months after the date of manufacture including when shipped upon exposure to fluctuating temperature extremes. Shelflife conditions are summarized in **Table 17**.

b. On-Board Storage

The effect of the On-Board Storage (OBS) on reagent performance was assessed by testing one lot of reagents at the maximum on-board temperature/humidity conditions allowed by the Alinity m Instrument System (i.e., 30ºC [±2 ºC], 65% [±10%] relative humidity [RH]) for the intended OBS of the reagents. Results of the OBS conditions were compared to the results when the reagents were stored at their intended storage condition.

Study results demonstrate that reagents are stable on-board the Alinity m Instrument and continue to meet acceptance criteria for the intended on-board storage time. On-board storage conditions are summarized in **Table 17**

Table 17: Reagent Shelf Life and On-Board Stability for the Alinity m HCV and Alinity m Accessory Kits

Kit/Accessory	Intended Storage Condition (Shelf Life)	On-Board Storage
HCV AMP Kit	15 months 2°C to 8°C	30 days
HCV CTRL Kit	15 months -15°C to -25°C	4 hours
HCV CAL Kit	15 months -15°C to -25°C	4 hours
Sample Prep Kit 2 (Elution Buffer 2, Microparticles 2)	12 months 15°C to 30°C	10 days
System Solutions (Lysis Solution, Diluent Solution, Vapor Barrier Solution)	15 months 15°C to 30°C	Lysis Solution: 30 days Diluent Solution: 30 days Vapor Barrier Solution: until expiration
Specimen Dilution Kit I	18 months	None (single unit packaged)

Expiration dating for the Alinity m HCVassay has been established and approved at 15 months when reagents are stored at the intended storage conditions.

14. Antimicrobial Effectiveness (AET)
Testing was performed for the Alinity m HCV assay for all reagents that contained preservatives. Effectiveness of the preservative for each tested organism was classified as cidal, static or neither cidal nor static depending on the microbial count at the testing time point. Nine different bacteria and yeast were tested. Results of the study demonstrated the effectiveness of the preservatives in the preservative containing reagents.

B. Animal Studies

None

C. Additional Studies

Table 18: Electromagnetic Compatibility (EMC) Testing

Test	Acceptance Criteria	Results
Radiated Emissions Testing	Met Class A, Group 1 limits	Passed
AC Mains conducted emissions	Met Class A, Group 1 limits	Passed
Electrostatic Discharge	Contact: at ±4 kV, both polarities	Passed
	Contact: at ±8 kV, both polarities	Passed
	Air: up to 15 kV, both polarities	Passed
Radiated Radio-Frequency, Electromagnetic Immunity	Frequencies up to 2700 MHz	Passed
Conducted Disturbances, RF Electromagnetic Immunity	up to 6 Vrms in the ISM bands.	Passed

X. SUMMARY OF PRIMARY CLINICAL STUDIES

The applicant performed the following two clinical studies to establish a reasonable assurance of safety and effectiveness for HCV testing with the Alinity m HCV assay when used as an aid in the diagnosis of active HCV infection in individuals with antibody evidence of HCV infection, and to aid in the management of patients with known active HCV infection, including SVR determination:

- **Diagnostic Utility Study:**
  Evaluation of the ability of the assay to correctly diagnose anti-HCV positive subjects with active HCV infection (Section 2, below)
- **Patient Management Study:**
  Evaluation of the ability of the assay to predict clinical outcome in patients undergoing treatment (Section 1, below)

Data from these clinical studies were the basis for the PMA approval decision. A summary of the clinical studies is presented below.

DIAGNOTIC UTILITY STUDY

A. Study Design

Patient samples were tested between June 2019 and August 2019. The database for this PMA reflected data collected through August 19, 2019, and included a total of 522 patients. There were three investigational sites.

The study was designed to evaluate the ability of the assay to correctly diagnose HCV antibody positive subjects with active HCV infection. The study consisted of two parts:

1. Testing HCV antibody positive subjects of unknown HCV RNA status (Diagnostic Population) to determine whether they have active HCV infection, and
2. Testing subjects with non-HCV related liver disease to determine specificity of the Alinity m HCV for HCV related liver disease.

a. Testing of HCV Antibody Positive Subjects of Unknown HCV RNA Status

Plasma or serum specimens were collected from 307 subjects who were at risk for HCV and tested positive for HCV antibodies with an FDA approved HCV antibody test (i.e. individuals with antibody evidence of HCV with evidence of liver disease, individuals suspected to be actively infected with HCV antibody evidence, individuals at risk for HCV infection with antibodies to HCV). 5 subjects were excluded. All specimens were tested with a FDA approved HCV nucleic acid test to establish the patient infected status (PIS). All specimens were then tested across four Alinity m Systems and three clinical sites.

According to AASLD guidelines[1], an FDA-approved NAAT with a detection level of 25 IU/mL or lower should be used to confirm a positive HCV infection status following a reactive HCV antibody test result. The patient infected status (PIS) for the tested cohort was defined as "Active HCV Infection" if the HCV antibody was positive and the FDA approved HCV nucleic acid test result was ≥25 IU/mL. The PIS was defined as "Resolved HCV Infection" if the HCV antibody was positive and the FDA approved HCV nucleic acid test result was <25 IU/mL. Specimens with Alinity m HCV result ≥25 IU/mL were considered positive. Specimens with Alinity m HCV <25 IU/mL were considered negative.

b. Testing of Subjects with Non-HCV Related Liver Diseases

The cross-reactivity of Alinity m HCV was evaluated with 215 plasma and serum specimens from subjects with hepatitis not caused by HCV. Specimens were retrospectively collected by specimen vendors using vendor IRB approved consent forms and collection protocols or were de-identified remnant specimens not requiring

consent. All the specimens were tested across 4 Alinity m Systems and 3 clinical sites. Six (6) samples were excluded.

Clinical Endpoints

With regard to safety, as an in vitro diagnostic test, the Alinity m HCV test involves taking a sample of plasma or serum from a patient. The test, therefore, presents no more safety hazard to an individual being tested than other tests where blood samples are drawn. Safety issues regarding false positive and negative test results are discussed in section VIII.

With regard to effectiveness, the clinical performance of the Alinity m HCV in this study was evaluated versus the HCV infected status of patients actively infected with HCV. Patient infected status was determined by FDA approved tests for antibodies to HCV and HCV viral load.

B. Accountability of PMA Cohort

The following Figure accounts for subjects in the Diagnostic Study (Dx).

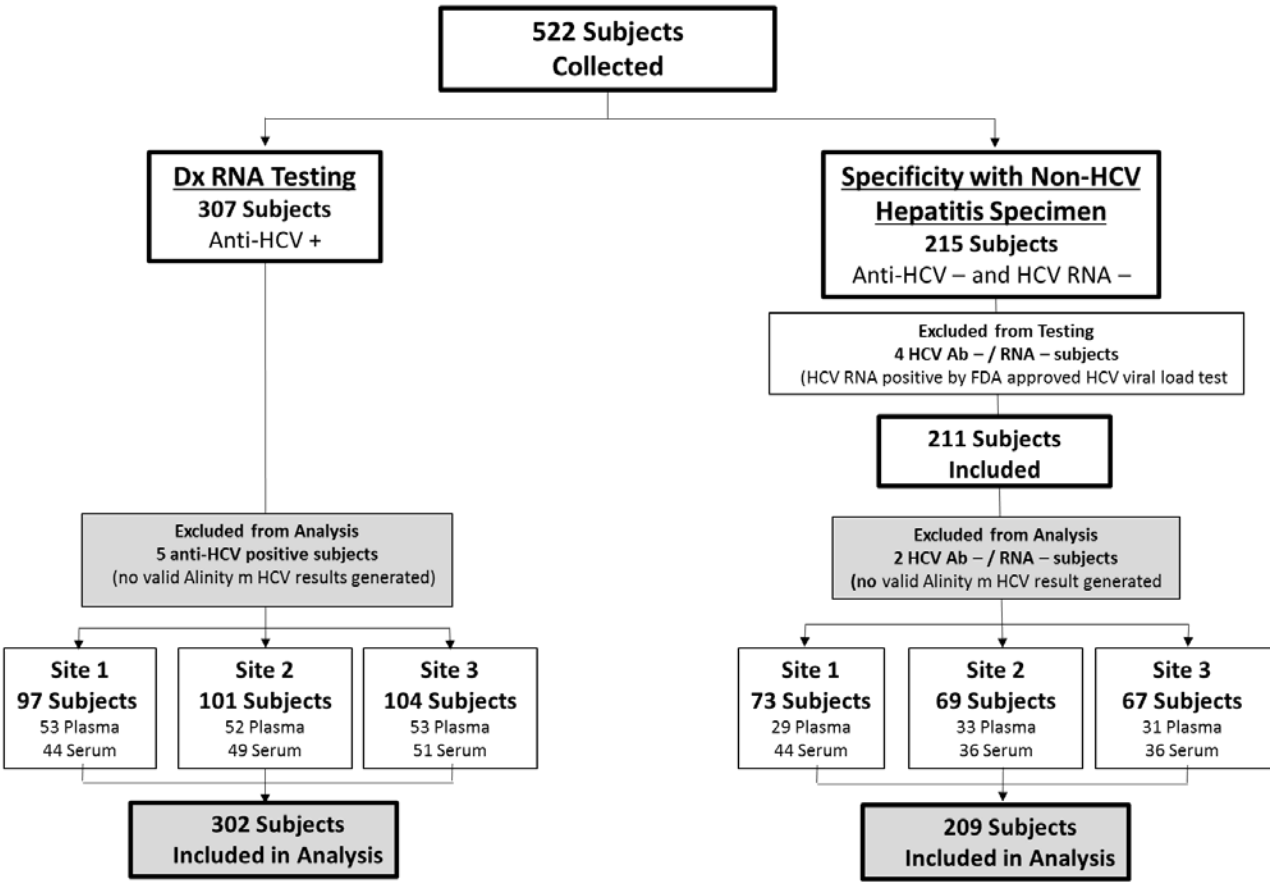

Figure 4: Accountability Diagnostic Study Cohort

a. Testing of HCV Antibody Positive Subjects of Unknown HCV RNA Status
At the time of database lock, of 307 patients enrolled in the PMA study, 98% (302) patients were available for analysis at the completion of the study.

b. Testing of Subjects with Non-HCV Related Liver Diseases
At the time of database lock 215 subjects were enrolled in the PMA study. Four subjects did not meet the inclusion criteria (i.e., being negative for HCV) and wer excluded. A total of 211 Anti-HCV – /HCV RNA – subjects were available for testing with the Alinity m HCV. Of these 211 subjects 209 subjects produced a valid result with the Alinity m HCV test and were included. Therefore, 97.2% (209) of subjects were available for analysis at the completion of the study.

C. Study Population Demographics and Baseline Parameters

The demographics of the study population are typical for a HCV Diagnostic Study performed in the US. Demographics and baseline characteristics of the Diagnostic Study subjects are presented in **Table 19** for the Diagnostic population and **Table 20** for the subjects with non-HCV related liver disease.

Table 19: Demographics of HCV Antibody Positive Subjects (Diagnostic Population)

Characteristics	Category	N (%)
Age (years)	Mean	50.2
	Median	52.0
	SD	11.42
	<21	3 (1.0)
	21 - 49	118 (38.4)
	50 - 70	183 (59.6)
	> 70	3 (1.0)
Gender	Female	80 (26.1)
	Male	227 (73.9)
Race	Asian	1 (0.3)
	Black	159 (51.8)
	White	62 (20.2)
	Mixed	2 (0.7)
	Other	83 (27.0)
Ethnicity	Hispanic/Latino	83 (27.0)
	Non-Hispanic/Latino	224 (73.0)

Characteristics	Category	N (%)
Risk Factor	Baby Boomers (Born: 1945 - 1965)	28 (9.1)
	IV Drug Users	116 (37.8)
	Baby Boomers and IV Drug Users	153 (49.8)
	Other*	10 (3.3)
HCV Infection Status	HCV RNA positive	249 (81.1)
	HCV RNA negative	58 (18.9)
Matrix	Plasma	163
	Serum	144
Total		**307**

* neither Baby Boomers nor IV Drug Users

Table 20: Demographics of Subjects with Non-HCV Related Liver Diseases

Characteristics	Category	N (%)
Age (years)	Mean	56.0
	Median	58.0
	SD	13.52
	21 - 49	63 (29.9)
	50 - 70	117 (55.5)
	> 70	30 (14.2)
	Not Available	1 (0.5)
Gender	Female	125 (59.2)
	Male	86 (40.8)
Race	Asian	42 (19.9)
	Black	19 (9.0)
	White	149 (70.6)
	Mixed	1 (0.5)
Ethnicity	Hispanic/Latino	13 (6.2)
	Non-Hispanic/Latino	194 (91.9)
	Not Available	4 (1.9)
Baby Boomers (Born: 1945 - 1965)	Yes	105 (49.8)
	No	70 (33.2)
	Not Available	36 (17.1)

Characteristics	Category	N (%)
Hepatitis Conditions not Caused by HCV	Autoimmune hepatitis	30 (14.2)
	Alcoholic liver disease	49 (23.2)
	HBV	44 (20.9)
	Primary biliary cirrhosis	29 (13.7)
	Nonalcoholic steatohepatitis (NASH)	30 (14.2)
	Fatty liver disease	29 (13.7)
Total		**211**

D. Safety and Effectiveness Results

1. Safety Results

   The analysis of safety was based on the tested cohort of 511 analyzable patients in the Diagnostic Utility Study available for evaluation. There were no adverse effects during the study. As an in vitro diagnostic test, the Alinity m HCV test involves taking a sample of plasma or serum from a patient. The test, therefore, presents no more safety hazard to an individual being tested than other tests where blood samples are drawn.

2. Effectiveness Results

   The analysis of effectiveness was based on the cohort of 511 patients in the Diagnostic Utility Study available for evaluation. Key effectiveness outcomes are presented in **Table 21** and **Table 22**. The analysis of effectiveness of the Alinity m HCV assay assessed whether the Alinity m HCV can accurately diagnose active HCV infection in RNA positive and negative plasma and serum specimens from patients that are HCV antibody positive. The testing cohort included two sub-cohorts for which the analysis is shown below.

   a. Testing of HCV Antibody Positive Subjects of Unknown HCV RNA Status

   Results of the Testing of HCV antibody positive subjects for whom the RNA status (i.e., the status of an acute infection) is unknown is summarized in **Table 21** below.

Table 21: Agreement between Alinity m HCV and Patient Infected Status for HCV Antibody Positive Subjects Using the AASLD Recommended Threshold of 25* IU/mL

		Patient Infected Status		Total
		Active HCV Infection	**Resolved HCV Infection**	
Alinity m HCV	**Positive***	226	4**	230
	Negative*	0	72	72
Total		**226**	**76**	**302**
% Positive Agreement (95% CI)		**100.0%** (98.3%, 100.0%)		
% Negative Agreement (95% CI)		**94.7%** (87.2%, 97.9%)		

CI: Confidence Interval (Score Method)

* "Negative" if HCV RNA was detected but < 25 IU/mL (i.e., 1.40 log10 IU/mL); otherwise, assay result interpretation was defined as "Positive"

** All 4 samples were detected below 25 IU/mL by the FDA approved HCV viral load test but were detected above 25 IU/mL with the Alinity m HCV (between 1.72 and 2.2 log IU/mL).

This study demonstrates the clinical utility of the Alinity m HCV assay to correctly diagnose subjects with ongoing active HCV RNA infections and to distinguish them from subjects with inactive infections in a population with prior exposure to HCV (HCV antibody-positive serology).

b. Testing of Subjects with Non-HCV Related Liver Diseases

Table 22 shows the Alinity m HCV assay specificity by liver disease and the distribution of test results across viral load categories. Using the AASLD recommended threshold of 25 IU/mL, the specificity was 100% in subjects with chronic hepatitis B virus (HBV), primary biliary cirrhosis, nonalcoholic steatohepatitis (NASH), and multiple liver diseases reported. HCV RNA was detected at very low levels (<LLoQ) in 1 subject with alcoholic liver disease and in 1 subject with chronic repatis B virus infection.

Table 22: Distribution of Results in Subjects with Non-HCV Related Liver Diseases

Liver Disease	% Specificity (95% CI[a])	Not Detected	<12 IU/mL (LLoQ)	12 to <25 IU/mL	25 IU/mL to 8 log10 IU/mL	>8 log10 IU/mL	Total
Autoimmune hepatitis	100 (88.3-100)	30 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	30
Alcoholic liver disease	98 (89.5-99.7)	49 (98)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	49
Chronic HBV	97.7 (88.2-99.6)	43 (100)	1 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	44
Primary biliary cirrhosis	100 (88.3-100)	29 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	29
NASH	100 (88.7-100)	30 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	30
Fatty Liver Disease	100 (87.6-100)	27 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	27
Total	**99.0 (96.6-99.7)**	**207 (99.0)**	**2 (1.0)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**209***

* Specimens from two subjects did not produce a valid Alnity HCV result and were excluded

3. Subgroup Analyses
   The study design enabled an assessment of assay performance by subgroup as depicted in **Table 22** above.

4. Pediatric Extrapolation
   In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population.

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.

PATIENT MANAGEMENT STUDY

A. Study Design

Clinical specimen testing was conducted between April 15, 2019 and August 20, 2019. The database for this PMA reflected testing data collected through August 20, 2019 and included serum and plasma specimens from 232 patients (61 patients with plasma samples; 171 patients with serum samples). There were 3 external investigational sites in the U.S. that collected and tested samples.

The study was a prospectively archived, multi-center clinical study. The study subjects had been treated with 8-week or 12-week therapeutic regimens containing Sofosbuvir or Mavyret. For each subject, specimens were collected from the following timepoints: Baseline, Week 4 On Treatment, End of Treatment, and End of Follow-up. Depending on the therapeutic regimen, the End of Follow-up timepoint was 12 or 24 weeks after the completion of the treatment Specimens were tested across 4 Alinity m Systems and 3 clinical sites.

The distribution of subjects in different treatment regimens and with different genotypes is presented in **Table 23**; the table excludes four subjects whose genotype (GT) could not be verified (i.e., two GT 1 subjects, one GT 3 subject and one GT 4 subject were excluded per accountability section below).

Table 23: Patient Management Study - Genotype/Treatment Distribution

Treatment*	GT 1	GT2	GT3	GT4	GT5	Total
Sofosbuvir Containing	100	31	43	1	4	**179**
– Harvoni ± Ribavirin	100	-	-	1	-	**101**
– Epclusa ± Ribavirin	-	25**	40	-	-	**65****
– Sovaldi	-	6	3	-	-	**9**
– Daklinza	-	-	-	-	4	**4**
Mavyret	-	20**	33**	-	-	**53****
Total	**100**	**51**	**76**	**1**	**4**	**232**

* Harvoni (Sofosbuvir + Ledipasvir); Epclusa (Sofosbuvir + Velpatasvir); Sovaldi (Sofosbuvir + Ribavirin); Daklinza (Sofosbuvir + daclatasvir); Mavyret (Glecaprevir + Pibrentasvir)
** One Epclusa treated subjects and 3 Mavyret treated subjects (one Gt 2 and two Gt 3) were excluded from SVR analysis in
Table 25 below because they were missing the EOF sample needed to determine SVR.

Clinical Endpoints

With regard to safety, as an in vitro diagnostic test, the Alinity m HCV test involves taking a sample of plasma or serum from a patient. The test, therefore, presents no more safety hazard to an individual being tested than other tests where blood samples are

drawn. Safety issues regarding false positive and negative test results are discussed in section VIII.

With regard to effectiveness, the clinical performance of the Alinity m HCV in the Patient Management Study was evaluated by determining sustained virological response (SVR) of patients actively infected with HCV and undergoing antiviral treatment with Direct Acting Antiviral agents. Clinical truth (i.e., SVR or Non-SVR) was established by testing with an FDA approved HCV viral load test.

B. Accountability of PMA Cohort

The following Figure accounts for all subjects in the Patient Management Study.

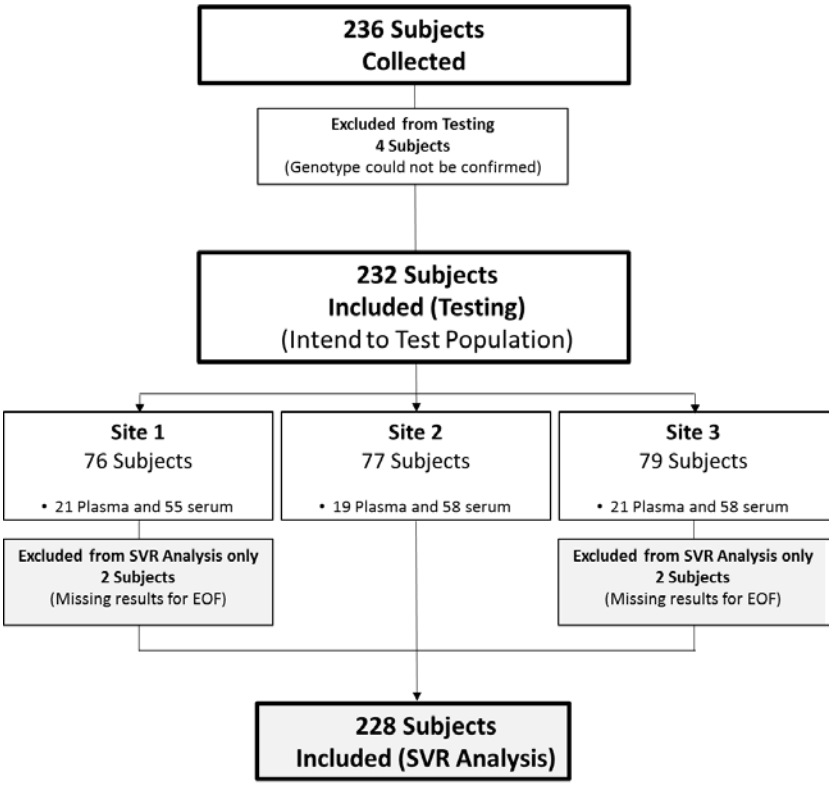

Figure 5: Accountability Patient Management Cohort

At the time of database lock, of 236 chronic HCV infected patients enrolled in the PMA study, four subjects were excluded because their genotype could not be verified. A total of 98.3% (232) of patients were available for testing and 96.6% (228) of the patients were available for SVR analysis at the completion of the study.

C. Study Population Demographics and Baseline Parameters

The demographics of the study population are typical for a HCV Patient Management study performed in the US. Demographics and baseline characteristics of the patient management study subjects are presented in **Table 24**.

Table 24: Demographics of Actively HCV Infected Subjects (Management Population)

Characteristics	Category	N (%)
Age	< 40 years	44 (19.0)
	≥ 40 years	188 (81.0)
Gender	Female	88 (37.9)
	Male	144 (62.1)
Race	Asian	9 (3.9)
	Black	24 (10.3)
	White	172 (74.1)
	Mixed	11 (4.7)
	Other	16 (6.9)
Genotype	1	100 (43.1)
	2	51 (22.0)
	3	76 (32.8)
	Non 1-3	5 (2.2)
Baseline Viral Load	≤ 800,000 IU/mL	109 (47.0)
	> 800,000 IU/mL	104 (44.8)
	Not Available	19 (8.2)
Baseline Liver Biopsy	Cirrhotic	8 (3.4)
	Non-Cirrhotic	126 (54.3)
	Not Available	98 (42.2)
Treatment Status	Naïve	164 (70.7)
	Experienced	39 (16.8)
	Not Available	29 (12.5)
Total		232 (100.0)

D. Safety and Effectiveness Results

1. Safety Results

   The analysis of safety was based on the tested cohort of 232 patients in the Patient Management Study available for evaluation. There were no adverse effects during the study.

2. Effectiveness Results

The analysis of effectiveness was based on the tested cohort of 232 patients in the Patient Management Study. Key effectiveness outcomes are presented in

Table 25 and **Table 26**. The analysis of effectiveness of the Alinity m HCV assay assessed whether the Alinity m HCV can correctly determine viral response to treatment at relevant on-treatment time points as well as sustained virological response (SVR) at the end of treatment follow-up by accurately quantitating HCV viral load in patients serum and plasma specimens.

a. Analysis of Sustained Virologic Response (SVR)

Table 25 demonstrates the percent of subjects with virologic response (VR, defined as Alinity m HCV results < LLoQ) by on-treatment week 4 visit and at the end of treatment (EOT) for each genotype and drug regimen. While virological response at week 4 was achieved in only 46.5% to 64% of patients, most subjects progressed to virological response at EOT (i.e., 89.8 to 100%) and further to SVR. The analysis of Sustained Virologic Response (SVR) assesses the response to Antiviral Therapy 12 or 24 weeks after discontinuation of treatment (i.e., at the EOF), depending on the treatment regimen. **Table 25** shows the overall frequency of SVR and SVR non-response (NSVR) by genotype and drug regimen in comparison to the FDA approved test that was used for clinical management of the subjects. Most subjects achieved SVR across genotypes.

Note that four subjects were missing the EOF sample and were excluded from SVR analysis per **Figure 5** (please refer to **Table 25** footnotes 6, 8, 11, and 12).

Table 25: Probability of Achieving SVR Across On-Treatment WK4 and EOT

GT	Regimen	Treatment Time [weeks]	N	SVR[1] Response % (n)	Non-SVR[1] Response % (n)	Virologic Response [2] by Visit % (n)		
						Week 4	EOT	EOF (SVR)
1	Harvoni ± RBV	8	28	**100%** (28/28)	**0%** (0/28)	**57.1%** (16/28)	**92.9%** 26/28	**100%** (28/28)
		12	54	**98%** (53/54)	**2%** (1/54)	**49%** (26/53)[3]	**93%** (50/54)	**98.1%** [4] (53/54)
		24	18	**83%** (15/18)	**17%** (3/18)	**33.3%** (6/18)	**94.4%** (17/18)	**83.3%** [5] (15/18)
2	Epclusa ± RBV	12	24 [6]	**95.8%** (23/24)	**4.2%** (1/24)	**62.5%** (15/24)	**87.5%** (21/24)	**95.8%** (23/24)
	SOF±RBV	12	5	**100%** (5/5)	**0%** (0/5)	**60%** (3/5)	**100%** (5/5)	**80%** [7] (4/5)
		24	1	**100%** (1/1)	**0%** (0/1)	**0%** (0/1)	**100%** (1/1)	**100%** (1/1)
	Mavyret	8	6	**100%** (6/6)	**0%** (0/6)	**66.7%** (4/6)	**83.3%** (5/6)	**100%** (6/6)
		12	13 [6]	**100%** (13/13)	**0%** (0/13)	**53.8%** (7/13)	**92.3%** (12/13)	**100%** (13/13)
3	Epclusa ± RBV	12	40	**97.5%** (39/40)	**2.5%** (1/40)	**47.5%** (19/40)	**92.5%** (37/40)	**97.5%** [8] (39/40)
	SOF±RBV	24	3	**66.6%** (2/3)	**33.3%** (1/3)	**33.3%** (1/3)	**100%** (3/3)	**66.7%** [9] (2/3)
	Mavyret	8	25 [6]	**100%** (25/25)	**0%** (0/25)	**72%** (18/25)	**100%** (25/25)	**100%** (25/25)
		12	6 [6]	**100%** (6/6)	**0%** (0/6)	**83.3%** (5/6)	**100%** (6/6)	**100%** (6/6)
4	Harvoni	24	1	**100%** (1/1)	**0%** (0/1)	**0%** (0/1)	**100%** (1/1)	**100%** (1/1)
5	Daklinza	24	4	**100%** (4/4)	**0%** (0/4)	**75%** (3/4)	**100%** (4/4)	**100%** (4/4)

[1] SVR based on the FDA approved HCV viral load test of record with SVR defined as ≤ 25 IU/mL per AASLD Guidelines

[2] VR defined as <1.08 log10 IU/mL by Alinity m HCV (i.e., 12 IU/mL or LLoQ)

[3] One subject did not generate a valid Alinity m HCV result and was excluded

[4] One subject had a viral response at EOT but did not achieve SVR at EOF (HCV viral load at 1.25 Log IU/mL). This subject was treatment naïve with no cirrhosis.

[5] Two subjects had a viral response at EOT but did not achieve SVR at EOF (HCV viral load at 5.81 Log IU/mL and 4.27 Log IU/mL). These subjects were both treatment experienced with unknown cirrhosis status.

[6] One of the subjects from **Table 23** was missing the EOF timepoint and was excluded from SVR analysis in this row

[7] One subject had a viral response at EOT but did not achieve SVR at EOF (HCV viral load at 5.76 Log IU/mL). This subject was treatment naïve with unknown cirrhosis status.

[8] One subject had a viral response at EOT but did not achieve SVR at EOF (HCV viral load at 6.04 Log IU/mL). This subject was treatment naïve with no cirrhosis
[9] One subject had a viral response at EOT but did not achieve SVR at EOF (HCV viral load at 6.24 Log IU/mL). This subject was treatment naïve with unknown cirrhosis status.

Most subjects who were not entirely negative at the EOT still achieved SVR as long as their viral load was low (e.g., <2 log10). Only patients with significant high viral loads (i.e., those above 5 log10 IU/mL) at the EOT did not achieve SVR. Virological responses at on-treatment week 4 and EOT as well as SVR appear to be regimen dependent rather then test dependent.

The Patient Management Study demonstrates the effectiveness of Alinity m HCV assay across all genotypes to determine on-treatment viral response and Sustained Virological Response at the End of Follow-Up in chronically HCV-infected subjects undergoing direct-acting antiviral (DAA) HCV therapies.

b. Association Between SVR and Baseline Covariates

The association between SVR and Baseline Covariates (based on vendor data) included Age, Gender, Race, Genotype, Baseline Viral Load, Baseline Liver Biopsy, and Treatment Status (**Table 26**). SVR was determined with an FDA approved HCV viral load test. None of the characteristics were significant in association with SVR for the patient management subjects.

Table 26: Association Between SVR and Baseline

Characteristics	Category	Odds Ratio (95% CI)
Age	< 40 years	1.4 (0.2, 12.1)
	≥ 40 years	
Gender	Male	0.6 (0.1, 3.4)
	Female	
Race	White	0.6 (0.1, 4.9)
	Non-White	
Genotype	Non-1	1.8 (0.4, 8.2)
	1	
Baseline Viral Load	≤ 800,000 IU/mL	0.8 (0.2, 3.6)
	> 800,000 IU/mL	
Baseline Liver Biopsy	Non-Cirrhotic	N/A
	Cirrhotic	
Treatment Status	Naïve	3.3 (0.7, 15.5)
	Experienced	

Cirrhosis Status:

There was no genotype or regimen specific analysis by cirrhosis status performed across the entire cohort because the total number of known cirrhotic patients was very low (i.e., 3.4% of subjects) and the total number of patients with unknown cirrhosis status was high (i.e., 42% of subjects) - see demographics **Table 24**. However, SVR was achieved in all cirrhotic patients indicating that the cirrhosis status did not impact performance of the device.

Prior Treatment:

A specific analysis of patients by treatment experienced and treatment naïve status could not be performed across the entire cohort because the information on prior treatment was missing for 12.5% of patients and only about 17% of patients were treatment experienced. More than 70% of patients were treatment naïve. 92.3% of treatment experienced and 96.9% of treatment naïve subjects achieved SVR by the candidate device and the FDA approved HCV viral load test. Considering the different patients numbers in these sub population the difference in SVR is not likely to reflect a different performance of the Alinity m HCV with treatment naïve and treatment experienced patients.

3. Subgroup Analyses

   The study design enabled an assessment of assay performance by subgroup as described in the tables and text above which show subjects stratified by treatment regimen and baseline characteristics/covariates.

4. Pediatric Extrapolation

   In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population.

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)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Microbiology Devices panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Safety Conclusions

The risks of the device are based on nonclinical laboratory studies as well as data collected in the clinical studies conducted to support PMA approval as described above. Based on the results of these studies the Alinity m HCV when used according to the manufacturer's instructions is safe to aid the physician in the diagnosis of active HCV infection and in the management of chronically infected HCV patients undergoing antiviral treatment.

For diagnosing HCV in the intended use population, the positive agreement of the assay is 100.0% with a two-sided 95% confidence interval (CI) of 98.3%, 100.0% and the negative percent agreement is 94.7% with a two-sided 95% CI of (87.2%, 97.9%).

For determining sustained virological response (SVR) to treatment, using the AASLD recommended threshold of 25 IU/mL the Alinity m HCV correctly determined SVR in 220 out of 221 subjects for whom SVR was determined by the FDA approved test. One patient was determined to achieve SVR with the FDA approved test but yielded a viral load of more than 5 log IU/mL with the Alinity m HCV. In the context of the entire study cohort a false positive rate of less than 0.5% is consistent with the overall performance of the test and does not raise safety concerns beyond what has been observed for other HCV viral load tests.

The Alinity m HCV correctly determined NSVR in 6 out of 7 patients. For one patient the Alinity m HCV determined SVR when the FDA approved test had determined NSVR. The Alinity m HCV determined viral load for that patient was close to the LLoQ of the Alinity m HCV. The difference in viral load between the Alinity m HCV and the FDA approved test was <0.5 log IU/mL. When using two different viral load tests on a sample with low viral load right at the AASLD recommended 25 IU/mL threshold difference in outcome can clinically be expected due to the different assay designs and their inherent precision. Therefore, it is generally recommended in the labeling not to switch between viral load tests when monitoring the treatment responses of patients undergoing treatment.

B. Effectiveness Conclusions

The clinical and analytical studies performed to validate the Alinity m HCV test demonstrate the effectiveness of the Alinity m HCV in correctly diagnosing HCV antibody positive subjects with active HCV RNA infection, to distinguish them from subjects with inactive infections and to determine on-treatment viral response and Sustained Virological Response (SVR) at the End of Follow-Up in chronically HCV-infected subjects undergoing direct-acting antiviral (DAA) HCV therapies. The Alinity m HCV test demonstrated effectiveness in the context of all main HCV genotypes (i.e., Genotypes 1 to 6).

C. Benefit-Risk Determination

The benefit of the assay is aiding in the diagnosis and management of Hepatitis C Virus (HCV) infection. Accurate diagnosis of HCV infection will guide further clinical decisions, including initiation of appropriate antiviral medications, and improve patient knowledge regarding the condition. Appropriate treatment of HCV can eradicate the HCV virus and mitigate the consequences of HCV infection, including symptom alleviation, decreases in all-cause mortality, liver disease-related complications and death, hepato-cellular carcinoma rates, and need for liver transplantation. Additionally, diagnosis and appropriate treatment can potentially decrease transmission and disease burden in the general population as well as in populations at high risk for hepatitis C infection. Accurate diagnosis of HCV infection also leads clinicians to evaluate and subsequently treat patients for human immunodeficiency virus (HIV) and hepatitis B virus (HBV) if indicated as these viruses share common modes of transmission with HCV, and patients are often coinfected. Management of HCV infection using the assay will aid clinicians in assessing adherence to antiviral regimens and document treatment course and virologic responses, which are evaluated due to the potential for viral resistance.

The risks associated with the device, when used as intended, are those related to the risk of false test results, failure to correctly interpret the test results, and failure to correctly operate the device. Risk of a false positive test include subjecting the patient to unnessesary treatment, repeat hepatits C testing and additional imaging and laboratory evaluations. A false positive test may result in anxiety for the patient. Risks of a false negative test includes the failure to treat HCV infection and unnessesary additional diagnostic procedures in pursuit of a different cause for the patient's hepatitis. Failure to treat HCV infection could lead to increased all-cause mortality, liver disease-related complications and death, hepatocellular carcinoma, and liver transplantation. A failure to diagnose HCV infection could also lead to an increase in transmission and disease burden. A false negative result may not provide a clinician the opportunity to evaluate and potentially treat a patient who is co-infected with HIV or HBV as these viruses share common modes of transmission with HCV, and patients are often co-infected.

1. Patient Perspectives
   This submission did not include specific information on patient perspectives for this device.

In conclusion, given the available information above, the data support that for the indication for use of the Alinity m HCV the probable benefits outweigh the probable risks.

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 indications for use. The probable clinical benefits outweigh the potential risks for the proposed assay considering the performance of the device in the clinical trial and the low risk and associated risk mitigations in clinical practice. The proposed assay labelling will facilitate accurate assay implementation and interpretation of results. The clinical performance observed in the prospective and retrospective clinical trials suggests that erroneous results will be uncommon and that the assay may provide substantial benefits to patients as an accurate and sensitive aid in the diagnosis of active hepatitis C virus infection.

XIII. CDRH DECISION

CDRH issued an approval order on March 23, 2020.

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. AASLD/IDSA HCV Guidance: Recommendations for Testing, Managing, and Treating Hepatitis C (Last Updated: November 6, 2019, www.hcvguidelines.org)