510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number

K252102

B Applicant

Abbott Molecular Inc.

C Proprietary and Established Names

Alinity m HCV

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
MZP	Class II	21 CFR 866.3170 - Nucleic Acid-Based Hepatitis C Virus Ribonucleic Acid Tests	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To obtain clearance for the modification of a previously approved device, Alinity m HCV assay, which allows the use of an alternative DNA polymerase and reverse transcriptase.

B Measurand:

Hepatitis C virus (HCV) RNA.

C Type of Test:

Quantitative in vitro reverse transcription-polymerase chain reaction (RT-PCR) assay.

III Intended Use/Indications for Use:

A Intended Use(s):

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 Sustained Virologic Response (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.

B Indication(s) for Use:

See Intended Use above.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

For use with the Alinity m System.

IV Device/System Characteristics:

A Device Description:

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 Sustained Virologic Response (SVR) determination.

The Alinity m HCV assay requires 3 separate assay specific kits:

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

B Principle of Operation:

The Alinity m HCV assay utilizes RT-PCR with fluorescence labeled probes 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, RT-PCR assembly, amplification, detection, and result calculation and reporting. All steps of the Alinity m HCV assay procedures 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.

HCV RNA from human plasma or serum is extracted automatically on-board in the Alinity m System 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 nucleic acids are 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 targets.
At the beginning of the Alinity m HCV sample preparation process, a lyophilized unit-dose IC on the AMP Tray is rehydrated by the Alinity m System and delivered into each sample preparation reaction. The IC is then processed through the entire sample preparation and real-time PCR procedure along with the specimens, calibrators, and controls to demonstrate proper sample processing and 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 determination of 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.

Calculation
Quantitative viral load results are reported for patient specimens with HCV viral concentrations within the assay's quantitation range. The concentration of HCV RNA in a specimen is calculated from the calibration curve by the system software. The Alinity m System reports the results in International Units as IU/mL or Log [IU/mL]. Refer to the Alinity m System Operations Manual for configuration of result units. For specimens tested with the Specimen Dilution Procedure, the Alinity m System calculates and reports the neat concentration (ie, prior to dilution), by using the dilution factor selected by the user.

Results and Interpretation

Alinity M System Report **User Performed**

Result	Interpretation	Interpretation Additional Information	Clinical Interpretation
Not Detected	HCV RNA not detected		No active HCV infection. For HCV Diagnosis: No further testing indicated. For Viral Load Assessment: Routine clinical follow-up according to national HCV guidelines.
<LLoQ	HCV RNA detected but not quantified	HCV RNA concentration is below the Lower Limit of Quantitation (LLOQ) of the assay (12 IU/mL).	Low level HCV viremia may indicate previous spontaneous or treatment-related resolution of HCV infection. For HCV Diagnosis: Results must be interpreted within the context of all relevant clinical and laboratory findings. For Viral Load Assessment: Routine clinical follow-up according to national HCV guidelines.
12 to <25 IU/mL (1.08 to <1.40 Log IU/mL)	HCV RNA detected and quantified	HCV RNA concentration is within the linear range of the assay (≥12 IU/mL and <25 IU/mL).	Low level HCV viremia may indicate previous spontaneous or treatment-related resolution of HCV infection. For HCV Diagnosis and Viral Load Assessment: Provide patient with appropriate counseling and link to care and treatment according to current national HCV treatment guidelines.
25 IU/mL to ≤ULOQ (1.40 Log IU/mL to ≤ULOQ)	HCV RNA detected and quantified	HCV RNA concentration is within the linear range of the assay (≥25 IU/mL to ≤ULOQ).	Active HCV infection. For HCV Diagnosis and Viral Load Assessment: Provide patient with appropriate counseling and link to care and treatment according to current national HCV treatment guidelines.
>ULoQ	HCV RNA detected	HCV RNA concentration is above the Upper Limit of Quantitation (ULOQ) of the assay.	Active HCV infection. For HCV Diagnosis and Viral Load Assessment: Provide patient with appropriate counseling and link to care and treatment according to current national HCV treatment guidelines.

C Instrument Description Information:

1. Instrument Name:

   Alinity m System

2. Specimen Identification:

   Hepatitis C virus (HCV) RNA in human plasma (EDTA, Acid Citrate Dextrose) or serum, from HCV antibody positive individuals.

3. Specimen Sampling and Handling:

   For the Alinity m HCV assay, only use collection tubes as described: Plasma including Acid Citrate Dextrose (ACD), K_2 EDTA, K_3 EDTA, Plasma Preparation Tube (PPT), and serum including serum separator tubes (SST) and serum rapid-clot tubes.

4. Calibration:

   Alinity m HCV CAL Kit consists of 2 calibrator levels, each supplied as liquid in single-use tubes.

5. Quality Control:

   Alinity m HCV CTRL Kit consisting of negative controls, low-positive controls, and high-positive controls, each supplied as liquid in single-use tubes.

V Substantial Equivalence Information:

A Predicate Device Name(s):
Alinity m HCV

B Predicate 510(k) Number(s):
P190025

C Comparison with Predicate(s):

Device & Predicate Device(s):	P190025	K252102
Device Trade Name	Alinity m HCV	Alinity m HCV
Regulation No./Product Code	21 CFR 814 / MZP	21 CFR 866.3170 / MZP
Device Class	Class III	Class II
General Device Characteristic Similarities		
Intended Use/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 Sustained Virologic Response (SVR) determination. The results from the Alinity m HCV assay must be interpreted within the context of all relevant clinical and laboratory findings.	Same

	The Alinity m HCV assay is not intended to be used in screening blood, plasma, serum, tissue or tissue donors for HCV.	
Assay Type	Quantitative	Same
Assay Target	A highly conserved sequence within the 5' untranslated region (5′utr) of the HCV genome	Same
Specimen Types	Human Plasma (EDTA, Acid Citrate Dextrose) or Serum	Same
Specimen Collection and Transport	The following blood collection tubes may be used: <u>Plasma</u> • K_2 EDTA • K_3 EDTA • Acid Citrate Dextrose (ACD) • Plasma Preparation Tube (PPT) <u>Serum</u> • Serum Tubes • Serum Separator Tubes (SST) • Serum Rapid-Clot Tubes <u>Plasma or Serum may be stored in:</u> • Primary tubes (with or without gel)	Same
Principles of the Procedure	See above for description of principles of procedure	Same
Instrument and System Components	Alinity m System: Fully integrated and automated diagnostics analyzer which utilizes real-time PCR technology	Same
Sample Preparation Instrument Components	Automated liquid handling and robotic manipulation platform	Same
Assay Controls	• Negative Control • Low Positive Control • High Positive Control • Internal Control (IC)	Same
Results Reporting	• Not Detected • Detected < LLoQ • Detected > ULoQ • Concentration from LLoQ to ≤ ULoQ, reported in IU/mL or Log IU/mL For low volume specimens tested with the Specimen Dilution Procedure, quantitative	Same

	results generated on the Alinity m System represent the HCV RNA concentration in the specimen prior to dilution.	
Reagents and Storage Conditions	Alinity m HCV AMP Kit: 2°C to 8°C Alinity m HCV CTRL Kit: –25°C to –15°C Alinity m HCV CAL Kit: –25°C to –15°C	Same
General Device Characteristic Differences		
Amplification Enzymes	Original DNA Polymerase is the enzyme used for DNA amplification. Original Reverse Transcriptase is the enzyme that converts the target RNA to complimentary DNA (cDNA).	Original DNA Polymerase or alternative DNA polymerase is the enzyme used for DNA amplification. Original Reverse Transcriptase or alternative Reverse Transcriptase is the enzyme that converts the target RNA to complimentary DNA (cDNA).

VI Standards/Guidance Documents Referenced:

1. Special Control (86 FR 66169), labeling requirements under 21 CFR 809.10(b), the information for special controls pertaining to Nucleic Acid-Based Hepatitis C Virus Ribonucleic Acid Tests (21 CFR 866.3170).
2. CLSI EP05-A3: Clinical Laboratory Standards Institute. *Evaluation of the Precision of Quantitative Measurement Procedures; Approved Guideline* – Third Edition. CLSI Guideline EP05-A3, CLSI: Wayne, PA, 2014.
3. CLSI EP35: Clinical Laboratory Standards Institute. *Assessment of Equivalence or Suitability of Specimen Types for Medical Laboratory Measurement Procedures; Approved Guideline* – First Edition. CLSI Guideline EP35. CLSI: Wayne, PA: 2019.
4. CLSI EP09c: Clinical and Laboratory Standards Institute. *Measurement Procedure Comparison and Bias Estimation Using Patient Sample* – Third Edition. CLSI Guideline EP09c. Wayne, PA: CLSI; 2018.
5. CLSI EP06: Clinical Laboratory Standards Institute. *Evaluation of Linearity of Quantitative Measurement Procedures; Approved Guideline* – Second Edition. CLSI Guideline EP06, CLSI: Wayne, PA, 2020.
6. CLSI EP17-A2: Clinical Laboratory Standards Institute. *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline* – Second Edition. CLSI Guideline EP17-A2, CLSI: Wayne, PA, 2012.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Precision:
Precision of Alinity m HCV assay with alternative reverse transcriptase and DNA polymerase was determined by testing 8 panel members with HCV concentrations that span the range of approximately 12 IU/mL to 200,000,000 IU/mL (1.08 to 8.30 Log IU/mL). A high titer specimen and an HCV Armored RNA stock representing the most common HCV genotype (Genotype 1) and a second non-1 genotype (Genotype 2), were used to prepare panel members in negative human plasma. Each panel member was tested in 3 replicates, twice each day for 15 days on 1 Alinity m system operated by 3 operators using 3 lots of HCV AMP kit, for a total of 270 replicates per panel member. The study design and analysis were derived from recommendations in CLSI Guideline EP05-A3, 3rd Edition.

The precision study results are shown in **Table 1**.

Table 1. Precision Analysis (All lots)

Panel	N	Mean Conc. (Log IU/mL)	Within-Run		Between-Run		Between-Day		Within-Laboratory[a]		Between-Lot		Total[b]	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
01	269	0.96	0.24	24.6	0.00	0.00	0.10	10.2	0.26	26.6	0.09	9.1	0.27	28.2
02	270	1.61	0.14	8.8	0.06	3.5	0.00	0.0	0.15	9.5	0.08	4.8	0.17	10.6
03	270	1.80	0.11	6.2	0.06	3.1	0.00	0.0	0.13	7.0	0.07	4.1	0.15	8.1
04	270	2.88	0.09	3.0	0.05	1.6	0.00	0.0	0.10	3.4	0.07	2.3	0.12	4.1
05	270	3.78	0.07	1.9	0.04	1.1	0.00	0.0	0.08	2.2	0.06	1.6	0.10	2.7
06	270	5.85	0.06	1.1	0.02	0.3	0.03	0.5	0.07	1.2	0.05	0.9	0.09	1.5
07	270	6.89	0.04	0.5	0.02	0.2	0.00	0.0	0.04	0.6	0.06	0.9	0.07	1.1
08	270	8.26	0.04	0.5	0.01	0.1	0.02	0.2	0.05	0.6	0.10	1.2	0.11	1.3

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

Reproducibility:
Reproducibility of Alinity m HCV assay with alternative reverse transcriptase and DNA polymerase was determined by testing 10 panel members with HCV concentrations that span the range of approximately 12 IU/mL (the assay LLoQ) to 100,000,000 IU/mL and one HCV negative member, using 1 lot, 3 external sites, 1 instrument per site, 5 days, 2 runs per day, and 4 replicates per run. HCV genotype 1a and genotype 2 were used for the reproducibility study. The study design and analysis were derived from recommendations in CLSI Guideline EP05-A3, 3rd Edition.

The reproducibility results are summarized in **Table 2**.

Table 2. Reproducibility for Positive Panel Members

Panel	Genotype	N	Mean Conc. (Log IU/mL)	Within-Run		Between-Run		Between-Day		Within-Laboratory[a]		Between-site		Total[b]	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	1a	118	7.71	0.20	2.6	0.08	1.1	0.10	1.4	0.24	3.1	0.06	0.8	0.25	3.2
2	1a	115	5.72	0.06	1.1	0.05	0.8	0.00	0.0	0.08	1.4	0.06	1.1	0.10	1.8
3	1a	115	3.53	0.06	1.6	0.04	1.1	0.03	0.9	0.08	2.2	0.03	0.9	0.08	2.4
4	1a	119	1.84	0.11	5.9	0.07	4.1	0.00	0.0	0.13	7.2	0.06	3.0	0.14	7.8
5	1a	116	0.89	0.21	23.8	0.07	7.4	0.01	1.4	0.22	24.9	0.10	11.5	0.24	27.5
6	1a	116	0.80	0.25	31.5	0.04	5.5	0.00	0.0	0.26	31.9	0.06	8.1	0.26	32.9
7	2	120	6.97	0.06	0.9	0.03	0.5	0.05	0.6	0.08	1.2	0.02	0.3	0.09	1.3
8	2	116	2.99	0.08	2.6	0.02	0.8	0.03	1.1	0.09	2.9	0.03	0.8	0.09	3.0
9	2	118	1.65	0.14	8.5	0.00	0.0	0.05	2.8	0.15	9.0	0.06	3.7	0.16	9.7
10	2	119	1.05	0.22	21.3	0.00	0.0	0.06	5.9	0.23	22.1	0.07	6.5	0.24	23.1

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

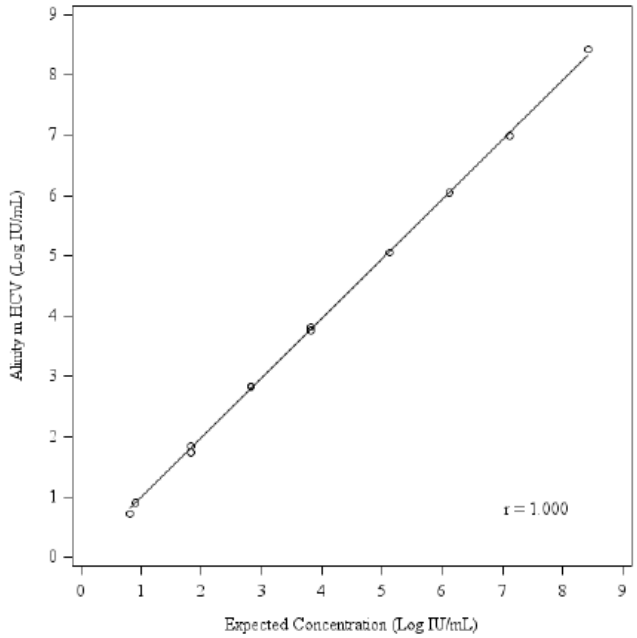
For the negative panel member, the overall negative percent agreement with the expected result was 100.0% (120/120) All three external sites had 100.0% negative percent agreement with the expected results.

2. Linearity:

Linearity was evaluated by testing 12 panel members that spanned the intended dynamic range of the assay (12 to 100,000,000 IU/mL), including a member below the expected Lower Limit of Quantification (LLoQ) at 10 IU/mL, and a member exceeding the expected Upper Limit of Quantification (ULoQ) at 200,000,000 IU/mL. Panels with different specimen types were designed to have at least 2 Log IU/mL overlap with adjacent sample types. Linearity study design and evaluation were based on the recommendations in CLSI EP06. The study was conducted using 1 lot of Alinity m HCV on 1 instrument with a minimum of 24 replicates for each panel member.

Alinity m HCV was linear across the quantitative range from 12 to 100,000,000 IU/mL (1.08 to 8.00 Log IU/mL). Results for the Alinity m HCV linearity performance are shown in **Figure 1**.

Figure 1. Alinity m HCV Linearity



3. Analytical Specificity/Interference:

   Refer to P190025.

4. Assay Reportable Range:

   Refer to P190025. The analytical measuring interval of the Alinity m HCV assay is from the lower limit of quantitation (LLoQ) of 12 IU/mL (1.08 Log IU/mL) to the upper limit of quantitation (ULoQ) of 100,000,000 IU/mL (7.00 Log IU/mL).

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   Traceability to internation standard material:
   Refer to P190025.

   Reagent Stability:
   The data submitted support the following storage conditions for the Alinity m HCV assay with alternative DNA polymerase and alternative reverse transcriptase.

   Table 3. Alinity m HCV AMP kit stability

Stability Study	Expiration
Reagent On-board	30 days
Reagent Shelf-life	18 months

6. Detection Limit:

   The limit of detection (LoD) was assessed by testing dilutions of the international standard material for HCV RNA (code 18/184) prepared in HCV-negative human plasma. The three

concentration panel members (9.0 IU/mL, 12.0 IU/mL, and 16.0 IU/mL) were selected based on LoD of the on-market Alinity m HCV (12 IU/mL). Testing for each HCV RNA concentration was performed with 3 lots of reagents across multiple days. LoD study design and analysis follow CLSI EP17-A2 and demonstrate a detection rate of ≥ 95.0% for samples at the claimed LoD of 12.0 IU/mL (1.08 Log IU/mL) and above.

7. Lower Limit of Quantitation (LLoQ):

   LLoQ is defined as the lowest concentration at which HCV RNA is reliably quantitated within an acceptable total error. The LLoQ was evaluated based on the recommendation in CLSI EP17-A2. Total Analytical Error (TAE) and Total Error in difference between two measurements (TE) were calculated for each HCV panel members from the LoD study, Linearity study and Precision study at the LLoQ claim concentrations of 12 IU/mL, as well as panel members higher and lower than the claimed LoD.

   The results are presented in **Table 4**.

Table 4. Alinity m HCV TAE and TE (overall)

Study	Panel	N	Target (IU/mL)	Target (Log IU/mL)	Mean (Log IU/mL)	Bias (Log IU/mL)	SD	TAE	TE
Linearity	11	24	12	1.08	0.90	-0.18	0.27	0.72	0.76
	12	24	10	1.00	0.72	-0.28	0.32	0.93	0.92
Precision	01	269	12	1.08	0.96	-0.12	0.26	0.63	0.73
LoD	1	138	9	0.95	0.77	-0.18	0.26	0.70	0.74
	2	141	12	1.08	0.85	-0.23	0.27	0.77	0.76
	3	140	16	1.20	1.06	-0.15	0.21	0.57	0.59

   The panel members with the target concentration at the assay's LLoQ of 12 IU/mL (1.08 Log IU/mL) have TAE and TE less than or equal to 1.00 Log IU/mL.

8. Accuracy (Instrument):

   Refer to P190025.

9. Carry-Over:

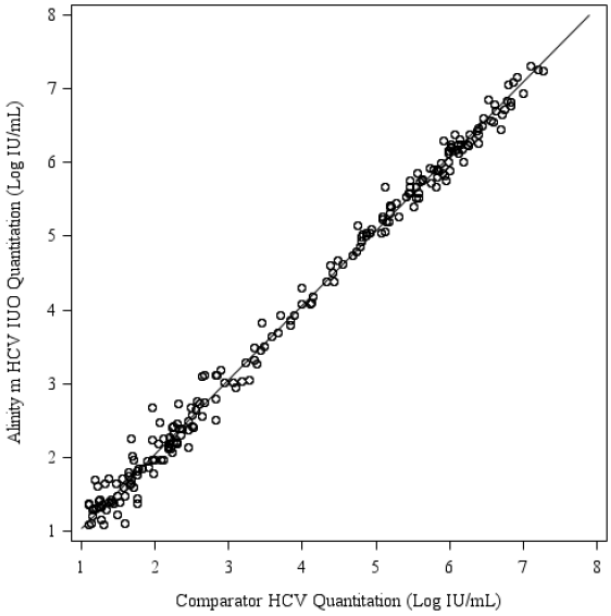
   Refer to P190025.

B Comparison Studies:

1. Method Comparison with Predicate Device:
   The Alinity m HCV clinical specimen method comparison testing was conducted at one internal testing site using 3 reagent lots of the Alinity m HCV with alternative enzymes and 1 lot of the on-market Alinity m HCV. The study used de-identified plasma and serum specimens and samples were selected such the HCV levels in the samples span the measuring range of the Alinity m HCV assay. To ensure coverage of the measuring range, clinical specimens were either tested neat or samples were created as needed by spiking individual negative clinical specimens with individual positive clinical specimens to reach the necessary target concentrations.

Figure 2 shows the Deming regression performed using the on-market Alinity m HCV assay result as the independent variable and the respective Alinity m HCV with alternative enzymes assay result as the dependent variable on 222 clinical samples. The Deming regression analysis results have correlation coefficient (r) of 0.996, a slope of 1.01 (95% CI of 1.00, 1.02), and intercept of 0.03 (95% CI of -0.02, 0.09).

Figure 2. Deming Regression Plot – All data



Systemic Difference was calculated for selected viral load levels (1.08, 1.40, 3.60, and 5.80 Log IU/mL) along with the two-sided 95% confidence intervals (**Table 5**).

Table 5. Systemic Difference at Selected Viral Load Levels

Target Viral Load Levels	Systemic Difference	95% CI
1.08 Log IU/mL	0.04 Log IU/mL	(-0.00, 0.09)
1.40 Log IU/mL	0.05 Log IU/mL	(0.00, 0.09)
3.60 Log IU/mL	0.06 Log IU/mL	(0.04, 0.09)
5.80 Log IU/mL	0.08 Log IU/mL	(0.05, 0.11)

The Alinity m HCV assay with alternative DNA polymerase and alternative reverse transcriptase has viral load values with a mean bias relative to the comparator assay of less than or equal to 0.25 Log IU/mL within the quantitation range.

2. Matrix Comparison:

Refer to P190025.

C Clinical Studies:

1. Clinical Sensitivity:

Refer to P190025

2. Clinical Specificity:

   Refer to P190025

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

   N/A

D Clinical Cut-Off:

Refer to P190025.

E Expected Values/Reference Range:

Refer to P190025.

F Other Supportive Instrument Performance Characteristics Data:

N/A

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.