Aptima® HCV Quant Dx Assay

Instructions for Use
For *in vitro* diagnostic use
For U.S. export only

General Information **2**
Intended Use 2
Summary and Explanation of the Test 2
Principles of the Procedure 3
Summary of Safety and Performance 4
Warnings and Precautions 4
Reagent Storage and Handling Requirements 7
Specimen Collection and Storage 9
Samples Onboard the Panther System 12
Specimen Transport 12
Panther System **13**
Reagents and Materials Provided 13
Materials Required but Available Separately 14
Optional Materials 15
Panther System Test Procedure 16
Procedural Notes 20
Quality Control **21**
Assay Calibration 21
Negative and Positive Controls 21
Internal Calibrator/Internal Control 21
Interpretation of Results **22**
Limitations **23**
Analytical Performance **24**
Limit of Detection (LoD) Using the WHO 2nd International Standard 24
Limit of Detection Across HCV Genotypes 24
Linear Range 25
Linearity Across HCV Genotypes 26
Lower Limit of Quantitation Using the WHO 2nd International Standard 27
Determination of the Lower Limit of Quantitation (LLoQ) Across HCV Genotypes 28
Traceability to the WHO Standard 30
Precision 30
Potentially Interfering Substances 31
Performance with HCV Negative Specimens 33
Analytical Specificity 33
Clinical Samples Containing Viruses Other Than HCV 34
Matrix Equivalency 35
Sample Dilution Using Aptima Specimen Diluent (1:3) 35
Sample Dilution Using Aptima Specimen Diluent (1:100) 36
Confirmation of the LLoQ in Clinical Specimens Diluted in Specimen Diluent 37
Precision of Diluted Samples 38
Carryover 38
Seroconversion Panel 38
Reproducibility Study 39
Clinical Performance **41**
Method Correlation 41
Clinical Utility Study 41
Response to Antiviral Therapy 45
Diagnostic Utility Study 49
Cross-Reactivity in Subjects With Non-HCV Related Liver Disease 51
Bibliography **53**
Contact Information and Revision History **54**

General Information

Intended Use

The Aptima® HCV Quant Dx assay is a real-time transcription-mediated amplification test. This assay is used for both detection and quantitation of hepatitis C virus (HCV) RNA in fresh and frozen human serum and plasma from HCV-infected individuals.

Plasma may be prepared in ethylenediaminetetraacetic acid (EDTA), anticoagulant citrate dextrose (ACD) solution, and plasma preparation tubes (PPT). Serum may be prepared in serum tubes and serum separator tubes (SST). Specimens are tested using the Panther™ System for automated specimen processing, amplification, detection, and quantitation. Specimens containing HCV genotypes 1 to 6 are validated for detection and quantitation in the assay.

The Aptima HCV Quant Dx assay is indicated for use as an aid in the diagnosis of HCV infection. The assay can be used to confirm active HCV infection in patients with a positive HCV antibody result. Detection of HCV RNA indicates that the virus is replicating and therefore, is evidence of active infection.

The Aptima HCV Quant Dx assay is indicated for use as an aid in the management of HCV infected patients undergoing HCV antiviral drug therapy. The assay measures HCV RNA levels at baseline, during treatment, and after treatment to determine sustained virological response (SVR). The results from the Aptima HCV Quant Dx assay must be interpreted within the context of all relevant clinical and laboratory findings.

The Aptima HCV Quant Dx assay is not intended for use as a screening test for the presence of HCV in blood or blood products.

Summary and Explanation of the Test

HCV is a blood-borne pathogen and a worldwide public health burden with up to 170 million people infected globally and 350,000 annual deaths due to HCV related conditions, including cirrhosis and liver cancer.[1,2] Transmission of HCV is through exposure to blood, blood products, or activities with potential for percutaneous exposure.[3,4] Genetically, HCV contains a positive-strand RNA genome of approximately 9500 nucleotides encoding structural proteins (core, E1 and E2 glycoproteins, p7 ion channel protein) and non-structural proteins (NS2, NS3, NS4A/B, NS5A/B), the latter being key viral replicative proteins and targets of direct acting antivirals.[4,5] Two untranslated regions (UTR) of the genome, 5'UTR and 3'UTR, function in genome translation and replication/packaging roles, respectively.[5] The 5'-UTR is the most highly conserved genomic region across the six major HCV genotypes.[6]

Clinically, there is a high prevalence of asymptomatic HCV infection, and, chronic HCV infection occurs in up to 75% of patients.[2] HCV laboratory testing algorithms require diagnosis of active HCV infections in antibody positive individuals through detection of HCV RNA in plasma or serum to allow appropriate link to care.[7,8,9]

Quantitation of HCV RNA (viral load) has played a pivotal role in defining and monitoring successful HCV treatment. Sustained virological response (SVR), defined as undetected HCV RNA after successful therapy, is a key marker for an HCV cure.[10,11] In interferon-based therapy, early virological response (EVR), defined as 2 log or greater decrease in HCV viral load after 12 weeks of therapy, and a rapid virological response (RVR), defined as undetectable levels of HCV RNA after 4 weeks of therapy, were shown to be positive predictors for SVR.[10,12,13] These markers of viral kinetics are utilized in response-guided approaches tailoring treatment options for stopping or extending therapy to achieve SVR.[14]

Furthermore, long term follow-up studies demonstrated durability of SVR after successful treatment and viral eradication prevents progression of liver disease.[10]

In the era of direct-acting antivirals (DAAs), quantitation of HCV RNA (viral load) has played a pivotal role in defining and monitoring successful HCV treatment. Sustained virological response (SVR) is defined as undetectable HCV RNA (with an assay that has a limit of detection of <25 IU/mL) after therapy.[10,11] Recent guidelines from the AASLD suggest testing HCV RNA not only at baseline, but also periodically during treatment (i.e., 4 weeks) and at 12 weeks following completion of treatment.[10,12,13]

Principles of the Procedure

The Aptima HCV Quant Dx assay is a nucleic acid amplification test that uses real-time transcription-mediated amplification (TMA) technology to detect and quantitate HCV RNA for aiding diagnosis or to establish baseline viral load, as well as to measure on-treatment and post-treatment responses. The assay targets a conserved region of the HCV genome, detecting and quantitating genotypes 1, 2, 3, 4, 5, and 6. The assay is standardized against the 2nd WHO International Standard for Hepatitis C Virus (NIBSC Code 96/798).[12]

The Aptima HCV Quant Dx assay involves three main steps, which all take place in a single tube on the Panther system: target capture, target amplification by TMA, and detection of the amplification products (amplicon) by the fluorescent labeled probes (torches).

During target capture, viral RNA is isolated from specimens. The specimen is treated with a detergent to solubilize the viral envelope, denature proteins, and release viral genomic RNA. Capture oligonucleotides hybridize to highly conserved regions of HCV RNA, if present, in the test specimen. The hybridized target is then captured onto magnetic microparticles that are separated from the specimen in a magnetic field. Wash steps remove extraneous components from the reaction tube.

Target amplification occurs via TMA, which is a transcription-mediated nucleic acid amplification method that utilizes two enzymes, Moloney murine leukemia virus (MMLV) reverse transcriptase and T7 RNA polymerase. The reverse transcriptase is used to generate a DNA copy (containing a promoter sequence for T7 RNA polymerase) of the target sequence. T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template. The Aptima HCV Quant Dx assay utilizes the TMA method to amplify a portion of the 5' UTR of the HCV genome. Amplification of this region is achieved using specific primers which are designed to amplify HCV genotypes 1, 2, 3, 4, 5, and 6.

Detection is achieved using single-stranded nucleic acid torches that are present during the amplification of the target and that hybridize specifically to the amplicon in real time. Each torch has a fluorophore and a quencher. When the torch is not hybridized to the amplicon, the quencher is in close proximity of the fluorophore and suppresses the fluorescence. When the torch binds to the amplicon, the quencher is moved farther away from the fluorophore and it will emit a signal at a specific wavelength when excited by a light source. As more torches hybridize to amplicon a higher fluorescent signal is generated. The time taken for the fluorescent signal to reach a specified threshold is proportional to the starting HCV concentration. Each reaction has an internal calibrator/internal control (IC) that controls for variations in specimen processing, amplification, and detection. The concentration of a sample is determined by the Panther system software using the HCV and IC signals for each reaction and comparing them to calibration information.

Summary of Safety and Performance

The SSP (Summary of Safety and Performance) is available in the European database on medical devices (Eudamed), where it is linked to the device identifiers (Basic UDI-DI). To locate the SSP for Aptima HCV Quant Dx assay, refer to the Basic Unique Device Identifier (BUDI): 54200455DIAGAPTHCVAJ.

Warnings and Precautions

A. For *in vitro* diagnostic use.

B. For professional use.

C. To reduce the risk of invalid results, carefully read the entire package insert and the *Panther*/*Panther Fusion*™ *System Operator's Manual* for procedural information prior to performing the assay on the Panther system.

D. For additional specific warnings, precautions and procedures to control contamination, consult the *Panther/Panther Fusion System Operator's Manual*.

Laboratory Related

E. CAUTION: The controls for this assay contain human plasma. The plasma is nonreactive for hepatitis B surface antigen (HBsAg), antibodies to HCV, antibodies to HIV-1 and HIV-2, and HIV antigen when tested with US Food and Drug Administration licensed procedures. In addition, the plasma is nonreactive for HCV RNA and HIV-1 RNA when tested with licensed nucleic acid tests using pooled samples. All human blood sourced materials should be considered potentially infectious and should be handled with Universal Precautions.[14,15,16]

F. Only personnel adequately trained in the use of the Aptima HCV Quant Dx assay and in handling potentially infectious materials should perform this procedure. If a spill occurs, immediately disinfect following appropriate site procedures.

G. Use only supplied or specified disposable laboratory ware.

H. Use routine laboratory precautions. Do not pipet by mouth. Do not eat, drink or smoke in designated work areas. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

I. Work surfaces, pipettes, and other equipment must be regularly decontaminated with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution.

J. Dispose of all materials that have come in contact with specimens and reagents according to local, state, and federal regulations.[14,15,16,17] Thoroughly clean and disinfect all work surfaces.

K. The controls contain sodium azide as a preservative. Do not use metal tubing for reagent transfer. If solutions containing sodium azide compounds are disposed of in a plumbing system, they should be diluted and flushed with generous amounts of running water. These precautions are recommended to avoid accumulation of deposits in metal piping in which explosive conditions could develop.

L. Good standard practices for molecular laboratories include environmental monitoring. To monitor a laboratory's environment, the following procedure is suggested:

1. Obtain a cotton-tipped swab and pair with the Aptima Specimen Aliquot Tube (SAT).
2. Label each SAT appropriately.
3. Fill each SAT with 1 mL of Aptima Specimen Diluent.
4. To collect the surface samples, lightly moisten a swab with nuclease-free deionized water.
5. Swab the surface of interest using a top to bottom vertical motion. Rotate the swab approximately one-half turn while swabbing the location.
6. Immediately place the swab sample into the tube and gently swirl the swab in the diluent to extract potential swabbed materials. Press the swab on the side of the transport tube to extract as much liquid as possible. Discard the swab and cap the tube.
7. Repeat steps for remaining swab samples.
8. Test swab with molecular assay.

Specimen Related

M. Specimens may be infectious. Use Universal Precautions[14,15,16] when performing this assay. Proper handling and disposal methods should be established according to local regulations.[17] Only personnel adequately trained in the use of the Aptima HCV Quant Dx assay and trained in handling infectious materials should perform this procedure.

N. Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.

O. Avoid cross-contamination during the specimen handling steps. Be especially careful to avoid contamination by the spread of aerosols when loosening or uncapping specimens. Specimens can contain extremely high levels of organisms. Ensure that specimen containers do not contact one another, and discard used materials without passing over open containers. Change gloves if they come in contact with specimen.

Assay Related

P. Do not use the reagent kit, the calibrator, or the controls after the expiration date.

Q. Do not interchange, mix, or combine assay reagents from kits with different master lot numbers. Assay fluids can be from different lot numbers. Controls and the calibrator can be from different lot numbers.

R. Avoid microbial and nuclease contamination of reagents.

S. Cap and store all assay reagents at specified temperatures. The performance of the assay may be affected by use of improperly stored assay reagents. See *Reagent Storage and Handling Requirements* and *Panther System Test Procedure* for more information.

T. Do not combine any assay reagents or fluids without specific instruction. Do not top off reagents or fluids. The Panther system verifies reagent levels.

U. Some reagents in this kit are labeled with risk and safety symbols.

Note: *Hazard communication reflects the EU Safety Data Sheets (SDS) classifications. For hazard communication information specific to your region, refer to the region specific SDS on the Safety Data Sheet Library at www.hologicsds.com. For more information on the symbols, refer to the symbol legend on www.hologic.com/package-inserts.*

EU Hazard Information	
—	**Amplification Reagent** *MAGNESIUM CHLORIDE 60-65%* — H412 – Harmful to aquatic life with long lasting effects. P273 – Avoid release to the environment. P501 – Dispose of contents/container to an approved waste disposal plant.
—	**Promoter Reagent** *MAGNESIUM CHLORIDE 60-65%* — H412 – Harmful to aquatic life with long lasting effects. P273 – Avoid release to the environment. P501 – Dispose of contents/container to an approved waste disposal plant.
—	**Enzyme Reagent** *Triton X-100 1-5%* *HEPES 1-5%* — H412 – Harmful to aquatic life with long lasting effects. P273 – Avoid release to the environment. P501 – Dispose of contents/container to an approved waste disposal plant.
	**HCV VL Kit Controls** *SODIUM AZIDE <1%* — **DANGER** H300 – Fatal if swallowed. H410 – Very toxic to aquatic life with long lasting effects. P264 – Wash face, hands and any exposed skin thoroughly after handling. P273 – Avoid release to the environment. P301 + P310 - IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician. P321 – Specific treatment (see supplemental first aid instructions on this label). P330 – Rinse mouth. P391 – Collect spillage.
—	**HCV VL Kit Calibrators** *HEPES 15-20%* *Lauryl Sulfate Lithium Salt 5-10%* *Lithium Hydroxide, Monohydrate 1-5%* *Succinic Acid 1-5%* — H412 – Harmful to aquatic life with long lasting effects. P273 – Avoid release to the environment. P501 – Dispose of contents/ container to an approved waste disposal plant.

—	**Enzyme Reconstitution Solution** *Glycerol 20-25%* *Triton X-100 5-10%* *HEPES 1-5%* — H412 – Harmful to aquatic life with long lasting effects. P273 – Avoid release to the environment. P501 – Dispose of contents/ container to an approved waste disposal plant.
—	**Target Capture Reagent** *HEPES 15-20%* *Lauryl Sulfate Lithium Salt 5-10%* *Lithium Hydroxide, Monohydrate 1-5%* *Succinic Acid 1-5%* — H412 – Harmful to aquatic life with long lasting effects. P273 – Avoid release to the environment. P501 – Dispose of contents/ container to an approved waste disposal plant.

Reagent Storage and Handling Requirements

A. The following table shows the storage conditions and stability for reagents, controls, and calibrator.

Reagent	Unopened Storage	Open Kit (Reconstituted)	
		Storage	Stability
qHCV Amplification Reagent	2°C to 8°C		
qHCV Amplification Reconstitution Solution	2°C to 8°C	2°C to 8°C	30 days[a]
qHCV Enzyme Reagent	2°C to 8°C		
qHCV Enzyme Reconstitution Solution	2°C to 8°C	2°C to 8°C	30 days[a]
qHCV Promoter Reagent	2°C to 8°C		
qHCV Promoter Reconstitution Solution	2°C to 8°C	2°C to 8°C	30 days[a]
qHCV Target Capture Reagent	2°C to 8°C	2°C to 8°C	30 days[a]
qHCV NC CONTROL – (Negative Control)	-15°C to -35°C	15°C to 30°C	Single use vial Use within 24 hours
qHCV LPC CONTROL + (Low Positive Control)	-15°C to -35°C	15°C to 30°C	Single use vial Use within 24 hours
qHCV HPC CONTROL + (High Positive Control)	-15°C to -35°C	15°C to 30°C	Single use vial Use within 24 hours
qHCV PCAL (Positive Calibrator)	-15°C to -35°C	15°C to 30°C	Single use vial Use within 24 hours

[a] When reagents are removed from the Panther system, they should be immediately returned to their appropriate storage temperatures.

B. Discard any unused, reconstituted reagents and target capture reagent (TCR) after 30 days or after the Master Lot expiration date, whichever comes first.

C. Reagents stored onboard the Panther system have 72 hours of onboard stability. Reagents can be loaded onto the Panther system up to 8 times. The Panther system logs each time the reagents are loaded.

D. After thawing the calibrator, the solution must be clear, i.e., not cloudy or have precipitates.



E. The promoter reagent and reconstituted promoter reagent are photosensitive. Protect these reagents from light during storage and preparation for use.

Specimen Collection and Storage

Note: *Handle all specimens as if they contain potentially infectious agents. Use Universal Precautions.*

Note: *Take care to avoid cross-contamination during sample handling steps. For example, discard used material without passing over open tubes.*

Note: *Only plastic secondary tubes are recommended for storage.*

Whole blood specimens collected in the following glass or plastic tubes may be used:

- Tubes containing ethylenediaminetetraacetic acid (EDTA) or acid citrate dextrose (ACD) anticoagulants or
- Plasma preparation tubes (PPTs)
- Serum tubes
- Serum separator tubes (SSTs)

For serum, allow the clot to form before further processing.

A. Specimen Collection

Whole blood can be stored at 2°C to 30°C and must be centrifuged within 6 hours of specimen collection. Separate the plasma or serum from the pelleted red blood cells following the manufacturer's instructions for the tube used. Plasma or serum can be tested on the Panther system in a primary tube or transferred to a secondary tube such as the Aptima Specimen Aliquot Tube. To obtain the 500 µl reaction volume, the minimum volume of plasma or serum for primary collection tubes is up to 1200 µL and for secondary tubes, the minimum volume is 700 µL. The following table identifies dead volume requirements for each primary and secondary tube type.

Tube (Size and Type)	Dead Volume on Panther
Aptima Sample Aliquot Tube (SAT)	0.2 mL
12x75 mm	0.5 mL
13x100 mm	0.5 mL
13x100 mm with Gel	0.3 mL
16x100 mm with Gel	0.7 mL

If not tested immediately, plasma and serum can be stored in accordance with the specifications below. If transferred to a secondary tube, plasma or serum may be frozen at -20°C. Do not exceed 3 freeze–thaw cycles. Do not freeze specimens in EDTA, ACD, or serum primary collection tubes.

B. Specimen Storage Conditions

1. EDTA and ACD Plasma Specimens

Whole blood can be stored at 2°C to 30°C and must be centrifuged within 6 hours of specimen collection. Plasma may then be stored under one of the following conditions:

- In the primary collection tube or secondary tube at 2°C to 25°C for up to 24 hours,
- In the primary collection tube or secondary tube at 2°C to 8°C for up to 5 days, or
- In the secondary tube at -20°C for up to 60 days.

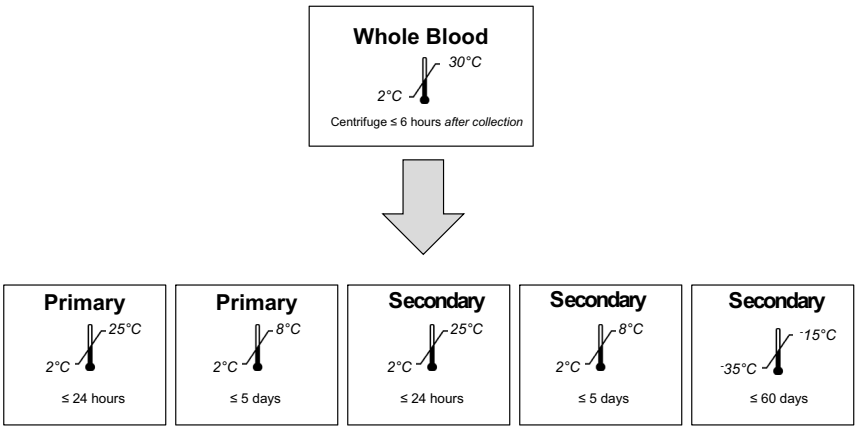


Figure 1. Storage Conditions for EDTA/ACD Tubes

2. PPT Specimens

Whole blood can be stored at 2°C to 30°C and must be centrifuged within 6 hours of specimen collection. Plasma may then be stored under one of the following conditions:

- In the primary collection tube or secondary tube at 2°C to 25°C for up to 24 hours,
- In the primary collection tube or secondary tube at 2°C to 8°C for up to 5 days, or
- In the primary collection tube or secondary tube at -20°C for up to 60 days.

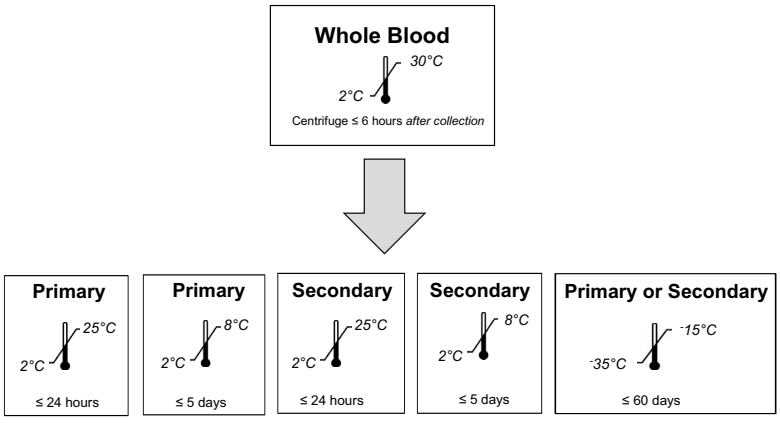


Figure 2. Storage Conditions for PPTs

3. Serum Tube Specimens

   Whole blood can be stored at 2°C to 30°C and must be centrifuged within 6 hours of specimen collection. Serum may then be stored under one of the following conditions:

   - In the primary collection tube or secondary tube at 2°C to 30°C for up to 24 hours,
   - In the primary collection tube or secondary tube at 2°C to 8°C for up to 5 days, or
   - In the secondary tube at -20°C for up to 60 days.

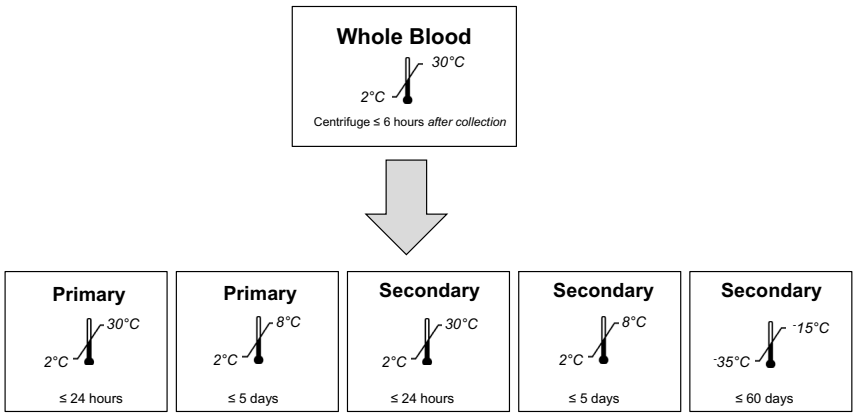


Figure 3. Storage Conditions for Serum Tubes

4. SST Specimens

   Whole blood can be stored at 2°C to 30°C and must be centrifuged within 6 hours of specimen collection. Serum may then be stored under one of the following conditions:

   - In the primary collection tube or secondary tube at 2°C to 30°C for up to 24 hours,
   - In the primary collection tube or secondary tube at 2°C to 8°C for up to 5 days, or
   - In the primary collection tube or secondary tube at -20°C for up to 60 days.

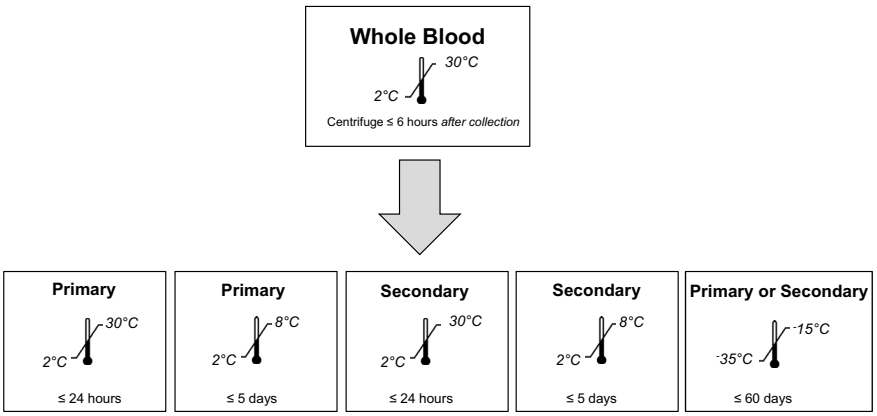


Figure 4. Storage Conditions for SSTs

C. Long Term Frozen Storage

   Plasma or serum samples may be stored at -65°C to -70°C for up to 60 days in SATs.

D. Dilution of Plasma and Serum Specimens

Plasma and serum specimens may be diluted in the SAT or secondary tube for testing on the Panther system. See *Panther System Test Procedure*, step E.6 below for more information.

⚠ *A diagnostic interpretation should not be made from a "Not Detected" result for serum or plasma specimens that have been diluted. Obtain a new, undiluted specimen and retest.*

Note: *If a specimen is diluted, it should be tested immediately after dilution. Do not freeze a diluted specimen.*

Samples Onboard the Panther System

Samples may be left on the Panther system uncapped for up to 8 hours. Samples may be removed from the Panther system and tested as long as the total time onboard does not exceed 8 hours prior to the pipetting of the sample by the Panther system.

Specimen Transport

Maintain sample storage conditions as described in *Specimen Collection and Storage*.

Note: *Specimens must be shipped in accordance with applicable national, international, and regional transportation regulations.*

Panther System

Reagents for the Aptima HCV Quant Dx assay are listed below for the Panther system. Reagent identification symbols are also listed next to the reagent name.

Reagents and Materials Provided

Aptima HCV Quant Dx Assay Kit, 100 tests Cat. No. PRD-03506
(1 assay box, 1 calibrator kit, and 1 controls kit)
Additional calibrators and controls can be ordered separately. See the respective catalog numbers below.

Aptima HCV Quant Dx Assay Box
(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity
A	**qHCV Amplification Reagent** *Non-infectious nucleic acids dried in buffered solution.*	1 vial
E	**qHCV Enzyme Reagent** *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution.*	1 vial
PRO	**qHCV Promoter Reagent** *Non-infectious nucleic acids dried in buffered solution.*	1 vial
AR	**qHCV Amplification Reconstitution Solution** *Aqueous solution containing glycerol and preservatives.*	1 x 7.2 mL
ER	**qHCV Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 x 5.8 mL
PROR	**qHCV Promoter Reconstitution Solution** *Aqueous solution containing glycerol and preservatives.*	1 x 4.5 mL
TCR	**qHCV Target Capture Reagent** *Nucleic acids in a buffered salt solution containing solid phase, non-infectious nucleic acids, and Internal Calibrator.*	1 x 72.0 mL
	Reconstitution Collars	3
	Master Lot Barcode Sheet	1 sheet

Aptima HCV Quant Dx Calibrator Kit (Cat. No. PRD-03507)
(store at -15°C to -35°C upon receipt)

Symbol	Component	Quantity
PCAL	**qHCV Positive Calibrator** *Transcript in buffered solution.*	5 x 2.5 mL
	Calibrator Barcode Label	—

Aptima HCV Quant Dx Controls Kit (Cat. No. PRD-03508)
(store at -15°C to -35°C upon receipt)

Symbol	Component	Quantity
NC	**qHCV Negative Control** *HCV negative defibrinated human plasma containing gentamicin and 0.2% sodium azide as preservatives.*	5 x 0.8 mL
LPC	**qHCV Low Positive Control** *Non-infectious HCV Armored RNA in defibrinated human plasma containing gentamicin and 0.2% sodium azide as preservatives.*	5 x 0.8 mL
HPC	**qHCV High Positive Control** *Non-infectious HCV Armored RNA in defibrinated human plasma containing gentamicin and 0.2% sodium azide as preservatives.*	5 x 0.8 mL
	Control Barcode Label	—

Materials Required but Available Separately

Note: *Materials available from Hologic have catalog numbers listed, unless otherwise specified.*

Material	Cat. No.
Panther™ System	303095
Panther Fusion™ System	PRD-04172
Panther™ System, Continuous Fluid and Waste (Panther Plus)	PRD-06067
Aptima® HCV Quant Dx Controls Kit	PRD-03508
Aptima® HCV Quant Dx Calibrator Kit	PRD-03507
Panther Run Kit for Real Time Assays (for real time assays only)	PRD-03455 (5000 tests)
Aptima® Assay Fluids Kit (also known as Universal Fluids Kit) *contains Aptima® Wash Solution, Aptima® Buffer for Deactivation Fluid, and Aptima® Oil Reagent*	303014 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther™ Waste Bag Kit	902731
Panther™ Waste Bin Cover	504405
Or, Panther™ System Run Kit *(when running non-real time-TMA assays in parallel with real time-TMA assays) contains MTUs, waste bags, waste bin covers, auto detect, and assay fluids*	303096 (5000 tests)
Tips, 1000 µL, filtered, liquid sensing, conductive, and disposable *Not all products are available in all regions. Contact your representative for region-specific information.*	901121 (10612513 Tecan) 903031 (10612513 Tecan) MME-04134 (30180117 Tecan) MME-04128
Bleach, 5% to 8.25% (0.7 M to 1.16 M) sodium hypochlorite solution	—
Disposable, powderless gloves	—

Material	Cat. No.
Replacement non-penetrable caps	103036A
Reagent replacement caps	
Amplification, Enzyme, and Promoter reagent reconstitution bottles	CL0041 (100 caps)
TCR bottle	CL0040 (100 caps)
Plastic-backed laboratory bench covers	—
Lint-free wipes	—
Pipettor	—
Tips	—
Primary collection tube options:	
13 mm x 100 mm	—
13 mm x 75 mm	—
16 mm x 100 mm	—
Centrifuge	—
Vortex mixer	—

Optional Materials

Material	Cat. No.
Secondary tube options:	
12 mm x 75 mm	—
13 mm x 100 mm	—
16 mm x 100 mm	—
Aptima Specimen Aliquot Tubes (SATs) (100 pack)	FAB-18184
Transport Tube Cap (100 pack) *cap for SAT*	504415
Aptima® Specimen Diluent	PRD-03003
Aptima® Specimen Diluent Kit *contains specimen diluent, 100 SATs, and 100 caps*	PRD-03478
Transfer pipets	—
Commercially available panels	—
Cotton-tipped swabs	—
Tube rocker	PRD-03488

Panther System Test Procedure

Note: *See the Panther/Panther Fusion System Operator's Manual for additional procedural information.*

A. Work Area Preparation

1. Clean work surfaces where reagents will be prepared. Wipe down work surfaces with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and then follow with a deionized (DI) water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface with clean, plastic-backed absorbent laboratory bench covers.
2. Clean a separate work surface where samples will be prepared. Use the procedure described above (step A.1).
3. Clean any pipettors. Use the cleaning procedure described above (step A.1).

B. Calibrator and Controls Preparation

Allow the calibrator and controls to reach 15°C to 30°C prior to processing as follows:

1. Remove the calibrator and controls from storage (-15°C to -35°C) and place at 15°C to 30°C. Throughout the thawing process, gently invert each tube to mix thoroughly. Ensure tube contents are fully thawed prior to use.

   Option. Calibrator and control tubes may be placed on a tube rocker to mix thoroughly. Ensure tube contents are fully thawed prior to use.

   Note: *Avoid creating excessive foam when inverting the calibrator and controls. Foam compromises the level-sensing by the Panther system.*
2. When the tube contents have thawed, dry the outside of the tube with a clean, dry disposable wipe.
3. To prevent contamination, do not open the tubes at this time.

C. Reagent Reconstitution/Preparation of a New Kit

Note: *Reconstitution of reagents should be performed prior to beginning any work on the Panther system.*

1. To prepare Target Capture Reagent (TCR), perform the following:
   a. Remove the TCR from storage (2°C to 8°C). Check the lot number on the TCR bottle to make sure that it matches the lot number on the Master Lot Barcode Sheet.
   b. Immediately shake the TCR bottle vigorously 10 times. Allow the TCR bottle to remain at 15°C to 30°C to warm for at least 45 minutes. During this period, swirl and invert the TCR bottle at least every 10 minutes.

      Option. The TCR bottle may be prepared on a tube rocker by following these instructions: Remove the TCR from storage (2°C to 8°C) and immediately shake vigorously 10 times. Place the TCR bottle on a tube rocker and leave the TCR at 15°C to 30°C to warm for at least 45 minutes.
   c. Ensure all precipitate is in solution and the magnetic particles are suspended before use.

2. To reconstitute Amplification, Enzyme, and Promoter Reagents, perform the following:
   a. Remove the lyophilized reagents and corresponding reconstitution solutions from storage (2°C to 8°C). Pair each reconstitution solution with its lyophilized reagent.
   b. Ensure that the reconstitution solution and lyophilized reagent have matching label colors. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired.
      i. Open the lyophilized reagent vial by removing the metallic seal and rubber stopper.
      ii. Firmly insert the notched end of the reconstitution collar (black) onto the vial (Figure 5, Step 1).
      iii. Open the matching reconstitution solution bottle, and set the cap on a clean, covered work surface.
      iv. Place the reconstitution solution bottle on a stable surface (i.e., bench). Then, invert the lyophilized reagent vial over the reconstitution solution bottle and firmly attach the collar to the reconstitution solution bottle (Figure 5, Step 2).
      v. Slowly invert the assembled bottles (vial attached to solution bottle) to allow the solution to drain into the glass vial (Figure 5, Step 3).
      vi. Pick up the assembled bottles, and swirl the assembled bottles for at least 10 seconds (Figure 5, Step 4).
      vii. Wait for at least 30 minutes for the lyophilized reagent to go into solution.
      viii. After the lyophilized reagent has gone into solution, swirl the assembled bottles for at least 10 seconds and then slightly rock the solution within the glass vial back and forth to mix thoroughly.
   c. Slowly tilt the assembled bottles again to allow all of the solution to drain back into the reconstitution solution bottle (Figure 5, Step 5).
   d. Carefully remove the reconstitution collar and glass vial (Figure 5, Step 6).
   e. Recap the bottle. Record operator initials and reconstitution date on the label (Figure 5, Step 7).
   f. Discard the reconstitution collar and glass vial (Figure 5, Step 8).

Warning: *Avoid creating excessive foam when reconstituting reagents. Foam compromises the level-sensing by the Panther system.*

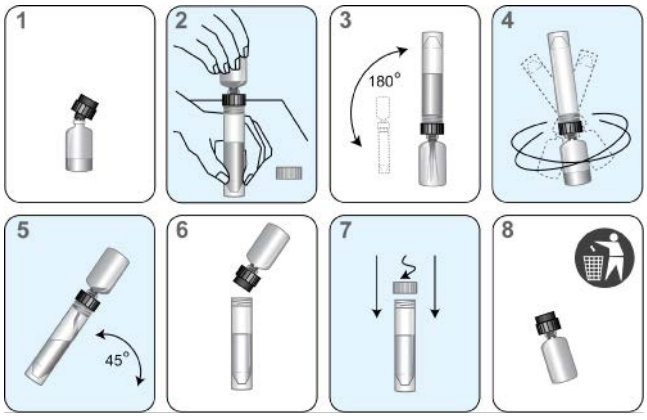


Figure 5. Reagent Reconstitution Process

D. Reagent Preparation for Previously Prepared Reagents

1. Remove the previously prepared reagents from storage (2°C to 8°C).
2. Previously prepared Amplification, Enzyme, Promoter reagents, and TCR must reach 15°C to 30°C prior to the start of the assay.
3. For previously prepared TCR, perform step C.1 above prior to loading on the system.
4. Swirl and invert the Amplification, Enzyme, and Promoter reagents to mix thoroughly prior to loading on the system. Avoid creating excessive foam when inverting reagents.

   Option: The previously prepared reagents may be prepared on a tube rocker by following these instructions: Remove the reagents from storage (2°C to 8°C). Place the reagents on a tube rocker and leave at 15°C to 30°C to warm for at least 30 minutes.
5. Do not top off reagent bottles. The Panther system will recognize and reject bottles that have been topped off.

E. Specimen Handling

1. Ensure that processed specimens in primary tubes or undiluted specimens in secondary tubes have been stored properly per *Specimen Collection and Storage*.
2. Ensure frozen specimens are thoroughly thawed. Vortex the thawed specimens for 3 to 5 seconds to mix thoroughly.
3. Allow the specimens to reach 15°C to 30°C prior to processing. See *Samples Onboard the Panther System* for additional onboard information.
4. Ensure each primary collection tube contains up to 1200 µL of specimen or each SAT contains at least 700 µL of specimen. Refer to the table provided in *Specimen Collection* to identify dead volume requirements for each primary and secondary tube type. If specimen dilution is necessary, see step E.5 below for additional information.
5. Dilute a plasma or serum specimen 1:3 in a SAT or 1:100 in a secondary tube.

   A specimen may be diluted in a secondary tube for testing on the Panther system.

⚠ *A diagnostic interpretation should not be made from a "Not Detected" result for serum or plasma specimens that have been diluted. Obtain a new, undiluted specimen and retest.*

Note: *If a specimen is diluted, it must be tested immediately after dilution.*

a. Dilution of low-volume specimens

   The volume of specimens may be increased to the minimum volume required (700 µL) using Aptima Specimen Diluent. Specimens with at least 240 µL may be diluted with two parts specimen diluent (1:3) as follows:

   i. Place 240 µL of specimen in the SAT.

   ii. Add 480 µL of Aptima Specimen Diluent.

   iii. Cap the tube.

   iv. Gently invert 5 times to mix.

   Specimens diluted 1:3 can be tested using the 1:3 option on the Panther system (see the *Panther/Panther Fusion System Operator's Manual* for more information). The software will automatically report the neat result by applying the dilution factor. These specimens will be flagged as diluted specimens.

b. Dilution of high-titer specimens

If a specimen's result is above the upper limit of quantitation (ULoQ), it may be diluted with 99 parts of Aptima Specimen Diluent (1:100) as follows:

i. Place 30 µL of specimen in the SAT or a secondary tube.

ii. Add 2970 µL of Aptima Specimen Diluent.

iii. Cap the tube.

iv. Gently invert 5 times to mix.

Specimens diluted 1:100 can be tested using the 1:100 option on the Panther system (see *Panther/Panther Fusion System Operator's Manual* for more information). The software will automatically report the neat result by applying the dilution factor. These specimens will be flagged as diluted specimens.

Note: *For diluted specimens with neat concentrations greater than the ULoQ , results will be reported using scientific notation.*

6. Just prior to loading specimens into a Sample Rack, centrifuge each specimen at 1000 to 3000*g* for 10 minutes. Do not remove caps. Bubbles in the tube compromise the level-sensing by the Panther system.

   See *System Preparation*, step F.2 below, for information about loading the rack and removing the caps.

F. System Preparation

1. Set up the system according to the instructions in the *Panther/Panther Fusion System Operator's Manual* and *Procedural Notes.* Make sure that the appropriately sized reagent racks and TCR adapters are used.
2. Load samples into the Sample Rack. Perform the following steps for each sample tube (specimen, and, when necessary, calibrator and controls):
   a. Loosen one sample tube cap, but do not remove it yet.

      Note: *Be especially careful to avoid contamination by the spread of aerosols. Gently loosen caps on samples.*

   b. Load the sample tube into the Sample Rack.

   c. Repeat steps 2.a and 2.b for each remaining sample.

   d. After the samples have been loaded into the Sample Rack, remove and discard each sample tube cap in one Sample Rack. To avoid contamination, do not pass a cap over any other Sample Racks or sample tubes.

   e. If necessary, use a new, disposable transfer pipet to remove any bubbles or foam.

   f. When the last cap has been removed, load the Sample Rack into a Sample Bay.

      Note: *If running other assays and sample types at the same time, secure the Sample Retainer prior to loading the Sample Rack into a Sample Bay.*

   g. Repeat steps 2.a to 2.f for the next Sample Rack.

Procedural Notes

A. Calibrator and Controls

1. The qHCV positive calibrator, the qHCV low positive control, qHCV high positive control, and qHCV negative control tubes can be loaded in any position in the Sample Rack and in any Sample Bay Lane on the Panther system. Specimen pipetting will begin when one of the following two conditions has been met:
   a. The calibrator and controls are currently being processed by the system.
   b. Valid results for the calibrator and controls are registered on the system.
2. Once the calibrator and control tubes have been pipetted and are processing for the Aptima HCV Quant Dx assay reagent kit, specimens can be tested with the associated, reconstituted kit for up to 24 hours **unless:**
   a. The calibrator result or control results are invalid.
   b. The associated assay reagent kit is removed from the system.
   c. The associated assay reagent kit has exceeded stability limits.
3. The calibrator and each control tube can be used once. Attempts to use the tube more than once can lead to processing errors.

B. Glove Powder

As in any reagent system, excess powder on some gloves may cause contamination of opened tubes. Powderless gloves are recommended.

Quality Control

A run or specimen result may be invalidated by an operator if technical, operator, or instrument difficulties are observed while performing the assay and are documented. In this case, specimens must be retested.

Assay Calibration

To generate valid results, an assay calibration must be completed. A single positive calibrator is run in triplicate each time a reagent kit is loaded on the Panther system. Once established, the calibration is valid for up to 24 hours. Software on the Panther system alerts the operator when a calibration is required. The operator scans a calibration coefficient found on the Master Lot Barcode Sheet provided with each reagent kit.

During processing, criteria for acceptance of the calibrator are automatically verified by the software on the Panther system. If less than two of the calibrator replicates are valid, the software automatically invalidates the run. Samples in an invalidated run must be retested using a freshly prepared calibrator and freshly prepared controls.

Negative and Positive Controls

To generate valid results, a set of assay controls must be tested. One replicate of the negative control, the low positive control, and the high positive control must be tested each time a reagent kit is loaded on the Panther system. Once established, the controls are valid for up to 24 hours. Software on the Panther system alerts the operator when controls are required.

During processing, criteria for acceptance of controls are automatically verified by software on the Panther system. To generate valid results, the negative control must give a result of “Not Detected” and the positive controls must give results within predefined parameters (LPC Nominal Target: 2.36 $\log_{10}$ IU/mL, HPC Nominal Target: 5.36 $\log_{10}$ IU/mL). If any one of the controls has an invalid result, the software automatically invalidates the run. Samples in an invalidated run must be retested using a freshly prepared calibrator and freshly prepared controls.

Internal Calibrator/Internal Control

Each sample contains an internal calibrator/internal control (IC). During processing, IC acceptance criteria are automatically verified by the Panther system software. If an IC result is invalid, the sample result is invalidated. Every sample with an invalid IC result must be retested to obtain a valid result.

The Panther system software is designed to accurately verify processes when procedures are performed following the instructions provided in this package insert and the *Panther/ Panther Fusion System Operator's Manual*.

Interpretation of Results

The Panther system automatically determines the concentration of HCV RNA for specimens and controls by comparing the results to a calibration curve. HCV RNA concentrations are reported in IU/mL and $\log_{10}$ IU/mL. The interpretation of results is provided in Table 1. If the 1:3 or 1:100 dilution is used for diluted specimens, the Panther system automatically calculates the HCV RNA concentration for the neat specimen by multiplying the diluted concentration by the dilution factor and diluted specimens are flagged as diluted.

Note: *For diluted specimens, results listed as "Not Detected" or "<10 detected" may be generated by diluting a specimen with a concentration above, but close to the LoD or LLoQ (limit of detection or lower limit of quantitation). It is recommended to collect and test another neat specimen if a quantitative result is not obtained.*

The Panther system does not provide a qualitative result (i.e., "Reactive" or "Non-reactive") for diagnostic use. The operator must interpret the reported HCV RNA concentration into a qualitative result (Table 1). Specimens with results listed as "Not Detected" are nonreactive for HCV RNA. Specimens with results listed as "<10 detected" with results listed within the linear range, and >100,000,000 (upper limit of quantitation) indicate HCV RNA was detected and these specimens are reactive for HCV RNA.

Table 1: Result Interpretation

Reported Aptima HCV Quant Dx Assay Result		HCV RNA Concentration Interpretation	User's Diagnostic Qualitative Interpretation[a]
IU/mL	$\log_{10}$ Value[b]		
Not Detected	Not Detected	HCV RNA not detected.	Non-reactive for HCV RNA
< 10 detected	< 1.00	HCV RNA is detected but at a level below the LLoQ	Reactive for HCV RNA
10 to 100,000,000	1.00 to 8.00	HCV RNA concentration is within the linear range of 10 to 100,000,000 IU/mL	Reactive for HCV RNA
> 100,000,000	> 8.00	HCV RNA concentration is above the ULoQ	Reactive for HCV RNA
Invalid[c]	Invalid[c]	There was an error in the generation of the result. Specimen should be retested	Invalid

[a] A diagnostic interpretation may be made from either serum or plasma specimens that have not been diluted.
[b] Value is truncated to two decimal places.
[c] Invalid results are displayed in blue colored font.

The acceptance criteria for each of the Aptima HCV Quant Dx assay controls are outlined in Table 2.

Note: *The recovery range listed below shifts based on the assigned value of each specific lot. Refer to the assigned concentration listed on the Control Barcode Sheet insert provided with each Control box.*

Table 2: Acceptance Criteria for Recovery Range for Aptima HCV Quant Dx Assay Controls

Component	Recovery Range for Valid Runs
Negative Control	N/A
Low Positive Control	+/- 0.5 $\log_{10}$ IU/mL
High Positive Control	+/- 0.5 $\log_{10}$ IU/mL

Limitations

A. Use of this assay is limited to personnel who have been trained in the procedure. Failure to follow the instructions given in this package insert may result in erroneous results.

B. Reliable results are dependent on adequate specimen collection, transport, storage, and processing.

C. Though rare, mutations within the highly conserved regions of the viral genome covered by the primers and/or probes in the Aptima HCV Quant Dx assay may result in failure to detect the virus.

Analytical Performance

Limit of Detection (LoD) Using the WHO 2nd International Standard

The limit of detection (LoD) of the assay is defined as the concentration of HCV RNA that is detected at 95% or greater probability according to CLSI EP17-A2.[18]

The LoD was determined by testing panels of the WHO 2nd International Standard for Hepatitis C Virus RNA (NIBSC 96/798 genotype 1) diluted in HCV negative human EDTA plasma and serum. A minimum of 36 replicates of each dilution were tested with each of three reagent lots for a minimum of 108 replicates per dilution. Probit analysis was performed to generate the predicted detection limits. The LoD values are the results from the reagent lot with the highest predicted detection limit. The 95% LoD for the Aptima HCV Quant Dx assay using the WHO 2nd International Standard is 4.3 IU/mL for plasma and 3.9 IU/mL for serum.

Limit of Detection Across HCV Genotypes

The LoD was determined by testing dilutions of HCV positive clinical specimens for genotypes 1, 2, 3, 4, 5 and 6 in HCV negative human plasma and serum. Concentrations were determined using an approved assay. A minimum of 20 replicates of each panel member were tested with each of three reagent lots for a minimum of 60 replicates per panel member. Probit analysis was performed to generate 95% predicted detection limits. The LoD values shown in Table 3 are the results from the reagent lot with the highest predicted detection limit.

Table 3: Limit of Detection Across HCV Genotypes Using Clinical Specimens

Genotype	Predicted Detection Limit	Concentration (IU/mL)	
		Plasma	Serum
1	95%	4.0	5.2
2	95%	2.8	4.0
3	95%	4.3	3.4
4	95%	4.8	2.3
5	95%	2.1	3.3
6	95%	4.1	3.9

Linear Range

The linear range was established by testing panels of HCV 1a Armored RNA (aRNA) diluted in HCV negative human plasma and serum according to CLSI EP06-A.[19] Panels ranged in concentration from 1.0 log_{10} IU/mL to 8.2 log_{10} IU/mL. The Aptima HCV Quant Dx assay demonstrated linearity across the range tested, with an upper limit of quantitation (ULoQ) of 8.0 log_{10} IU/mL, as shown in Figure 6.

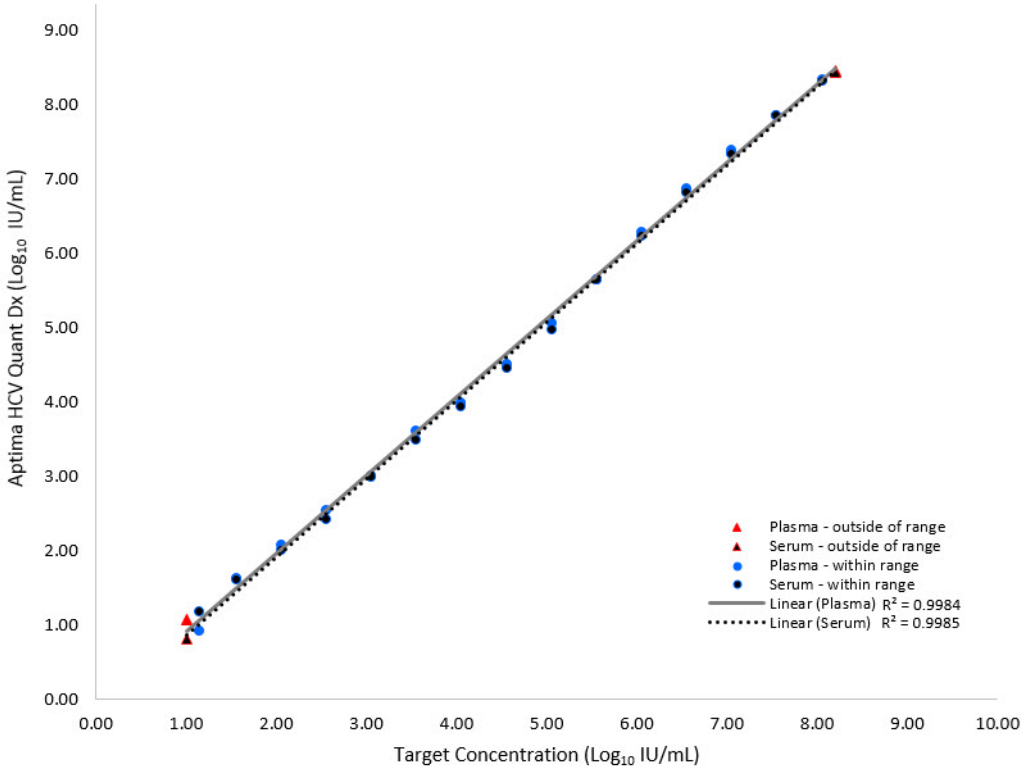


Figure 6. Linearity in Plasma and Serum

Linearity Across HCV Genotypes

The linearity and linear range of HCV genotypes 1 (1a for Plasma and 1b for Serum) through 6 was established by testing diluted individual clinical positives, one for each HCV genotype for the lower range of the assay combined with aRNA (HCV 1a) for the higher range of the assay (above 100,000 IU/mL). Dilutions in both negative human plasma and serum were tested for all genotypes. Linearity was demonstrated for all six genotypes across the range tested as shown in Figure 7 for plasma and Figure 8 for serum.

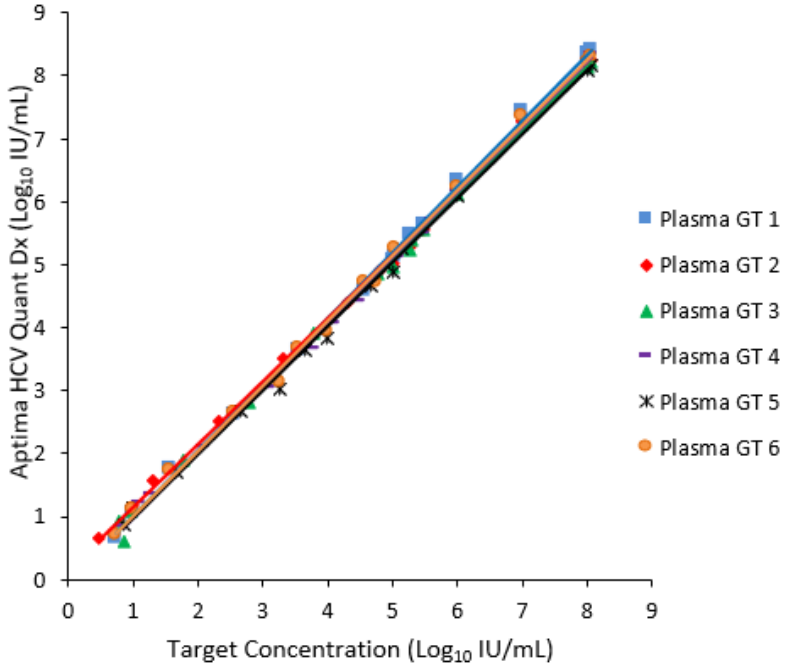


Figure 7. Linear Range and Linearity (Plasma)

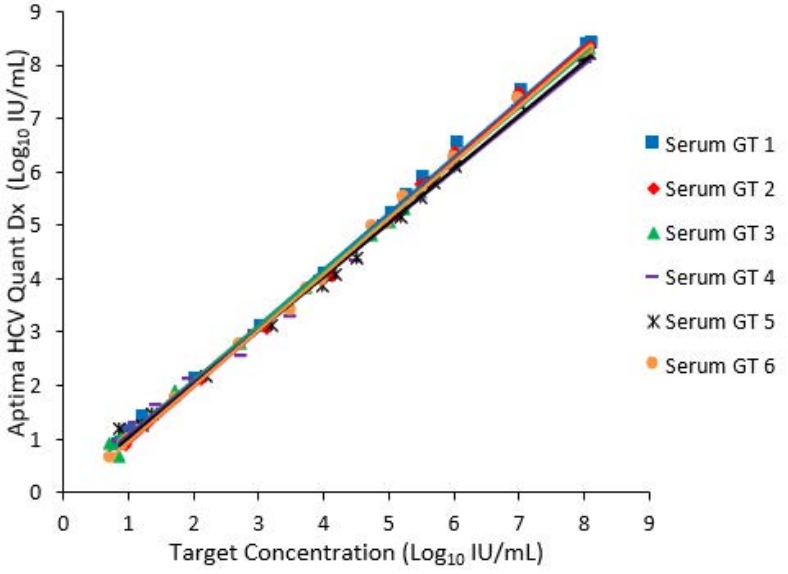


Figure 8. Linear Range and Linearity (Serum)

Lower Limit of Quantitation Using the WHO 2nd International Standard

The lower limit of quantitation (LLoQ) is defined as the lowest concentration at which HCV RNA is reliably quantitated within a total error, according to CLSI EP17-A2.[18] Total error was estimated by two methods: Total Analytical Error (TAE) = |bias| + 2SD, and Total Error (TE) = SQRT(2) x 2SD. To ensure accuracy and precision of measurements, the total error of the Aptima HCV Quant Dx assay was set at 1 $\log_{10}$ IU/mL (i.e., at the LLoQ, the difference between two measurements of more than 1 $\log_{10}$ IU/mL is statistically significant).

The LLoQ was determined by testing panels of the WHO 2nd International Standard for Hepatitis C Virus RNA (NIBSC 96/798, genotype 1) diluted in HCV negative human plasma and serum. Thirty-six (36) replicates of each dilution were tested with each of three reagent lots for a minimum of 108 replicates per dilution. The results from the reagent lot with the highest concentration equal to or greater than the LoD and meeting the TE and TAE requirements are shown in Table 4 for plasma and Table 5 for serum. The LLoQ for the WHO 2nd International Standard is 7 IU/mL (0.82 $\log_{10}$ IU/mL) for plasma and 9 IU/mL (0.93 $\log_{10}$ IU/mL) for serum as summarized in Table 6. The LLoQ was established across genotypes (see *Determination of the Lower Limit of Quantitation (LLoQ) Across HCV Genotypes*). This genotype data establishes the overall LLoQ for the assay as 10 IU/mL.

Table 4: LLoQ Using the WHO 2nd International Standard for HCV Diluted in Plasma

Reagent Lot	Aptima HCV Quant Dx (IU/mL)	Aptima HCV Quant Dx ($\log_{10}$ IU/mL)	SD ($\log_{10}$ IU/mL)	Bias ($\log_{10}$ IU/mL)	Calculated TE ($\log_{10}$ IU/mL)	Calculated TAE ($\log_{10}$ IU/mL)
1	3	0.53	0.30	0.05	0.85	0.65
	5	0.73	0.31	-0.04	0.88	0.67
	12	1.08	0.23	0.18	0.65	0.64
2	2	0.37	0.32	-0.11	0.92	0.75
	7	0.82	0.27	0.04	0.78	0.59
	9	0.96	0.26	0.06	0.74	0.58
3	3	0.46	0.25	-0.01	0.71	0.52
	6	0.76	0.34	-0.02	0.95	0.69
	8	0.89	0.26	-0.01	0.73	0.53

SD=standard deviation

Table 5: LLoQ Using the WHO 2nd International Standard for HCV Diluted in Serum

Reagent Lot	Aptima HCV Quant Dx (IU/mL)	Aptima HCV Quant Dx ($\log_{10}$ IU/mL)	SD ($\log_{10}$ IU/mL)	Bias ($\log_{10}$ IU/mL)	Calculated TE ($\log_{10}$ IU/mL)	Calculated TAE ($\log_{10}$ IU/mL)
1	4	0.65	0.33	0.17	0.94	0.84
	8	0.93	0.32	0.15	0.90	0.79
	12	1.08	0.28	0.18	0.80	0.74
2	3	0.52	0.36	0.04	1.02	0.76
	8	0.89	0.32	0.11	0.90	0.75
	10	1.01	0.21	0.11	0.60	0.53
3	3	0.47	0.39	-0.01	1.11	0.79
	5	0.71	0.30	-0.06	0.86	0.67
	9	0.95	0.29	0.05	0.83	0.63

SD=standard deviation

Table 6: Summary of the LLoQ Using the WHO 2nd International Standard for HCV

Reagent Lot	Plasma LLoQ		Serum LLoQ	
	IU/mL	log_{10} IU/mL	IU/mL	log_{10} IU/mL
1	5	0.73	8	0.93
2	7	0.82	8	0.89
3	6	0.76	5	0.71

Determination of the Lower Limit of Quantitation (LLoQ) Across HCV Genotypes

The LLoQ was determined by testing dilutions of HCV positive clinical specimens for genotypes 1, 2, 3, 4, 5 and 6 in HCV negative human plasma and serum. Assignment of the concentration for clinical specimens was determined using a comparator assay. Thirty-six (36) replicates of each panel member were tested with each of three reagent lots for a minimum of 108 replicates per panel member. The results from the reagent lot with the highest concentration equal to or greater than the LoD and meeting the TE and TAE, calculated according to CLSI EP 17-A2,[18] requirements are shown in Table 7 for plasma and Table 8 for serum. Only one result greater than and less than the LLoQ per genotype are reported in Table 7 and Table 8 below, as these results are most reflective of the expected LLoQ. The LLoQ for genotypes 1 to 6 in plasma and serum are summarized in Table 9. Genotype 6 had the highest LLoQ for serum at 10 IU/mL. This established the overall LLoQ for the assay as 10 IU/mL.

Table 7: Determination of LLoQ Across Genotypes in Plasma

Genotype	Aptima HCV Quant Dx[a] (IU/mL)	Aptima HCV Quant Dx[a] (log_{10} IU/mL)	SD (log_{10} IU/mL)	Bias (log_{10} IU/mL)	Calculated TE (log_{10} IU/mL)	Calculated TAE (log_{10} IU/mL)
1	4	0.65	0.38	0.17	1.08	0.94
	8	0.88	0.35	0.11	1.00	0.82
	10	0.99	0.24	0.09	0.68	0.56
2	4	0.63	0.40	0.16	1.13	0.95
	6	0.76	0.29	0.15	0.81	0.73
	13	1.12	0.30	0.34	0.86	0.94
3	3	0.52	0.30	-0.26	0.85	0.86
	6	0.80	0.21	-0.20	0.59	0.62
	8	0.89	0.26	-0.19	0.74	0.71
4	4	0.61	0.39	-0.29	1.11	1.07
	7	0.82	0.31	-0.18	0.87	0.80
	10	1.01	0.29	-0.07	0.83	0.65
5	4	0.57	0.37	0.10	1.06	0.85
	7	0.87	0.31	0.09	0.89	0.72
	14	1.15	0.16	0.15	0.44	0.46
6	5	0.66	0.36	0.36	1.02	1.08
	6	0.79	0.28	0.31	0.80	0.87
	14	1.14	0.26	0.36	0.74	0.89

SD=standard deviation

[a]Additional levels were run but this data is not reported in the table.

Table 8: Determination of LLoQ Across Genotypes in Serum

Genotype	Aptima HCV Quant Dx[a] (IU/mL)	Aptima HCV Quant Dx[a] (log_{10} IU/mL)	SD (log_{10} IU/mL)	Bias (log_{10} IU/mL)	Calculated TE (log_{10} IU/mL)	Calculated TAE (log_{10} IU/mL)
1	6	0.75	0.36	-0.03	1.02	0.75
	8	0.88	0.32	-0.02	0.89	0.65
	11	1.04	0.29	0.04	0.81	0.61
2	3	0.48	0.35	0.00	0.99	0.70
	6	0.80	0.31	0.02	0.86	0.63
	11	1.04	0.25	0.04	0.72	0.54
3	3	0.45	0.25	-0.33	0.72	0.84
	5	0.67	0.22	-0.33	0.63	0.78
	8	0.92	0.19	-0.16	0.54	0.54
4	2	0.19	0.27	0.19	0.77	0.73
	4	0.65	0.32	0.35	0.91	0.99
	4	0.65	0.34	0.17	0.96	0.85
5	3	0.48	0.37	0.02	1.04	0.76
	5	0.72	0.29	-0.04	0.81	0.61
	11	1.04	0.27	0.10	0.77	0.65
6	4	0.58	0.37	0.11	1.04	0.84
	10	0.99	0.22	0.21	0.61	0.64
	18	1.25	0.22	0.25	0.63	0.70

SD=standard deviation

[a]Additional levels were run but this data is not reported in the table.

Table 9: Summary of LLoQ Across Genotypes in Plasma and Serum

HCV Genotype	Plasma LLoQ		Serum LLoQ	
	IU/mL	log_{10} IU/mL	IU/mL	log_{10} IU/mL
1	8	0.88	8	0.88
2	6	0.76	6	0.80
3	6	0.80	5	0.67
4	7	0.82	4	0.65
5	7	0.87	5	0.72
6	6	0.79	10	0.99

Traceability to the WHO Standard

A series of secondary standards with known concentrations were used throughout product development and product manufacturing to establish traceability to the WHO standard. The concentrations tested for the HCV WHO standard were between 4.00 and 2.30 $\log_{10}$ IU/mL, the secondary standards ranged in concentration from 1.50 to 8.46 $\log_{10}$ IU/mL. The Aptima HCV Quant Dx assay controls and calibrators were also tested along with the secondary standards and the WHO standard. All of the panels had similar results, and they were distributed linearly across the assay's linear range, as presented in Figure 9.

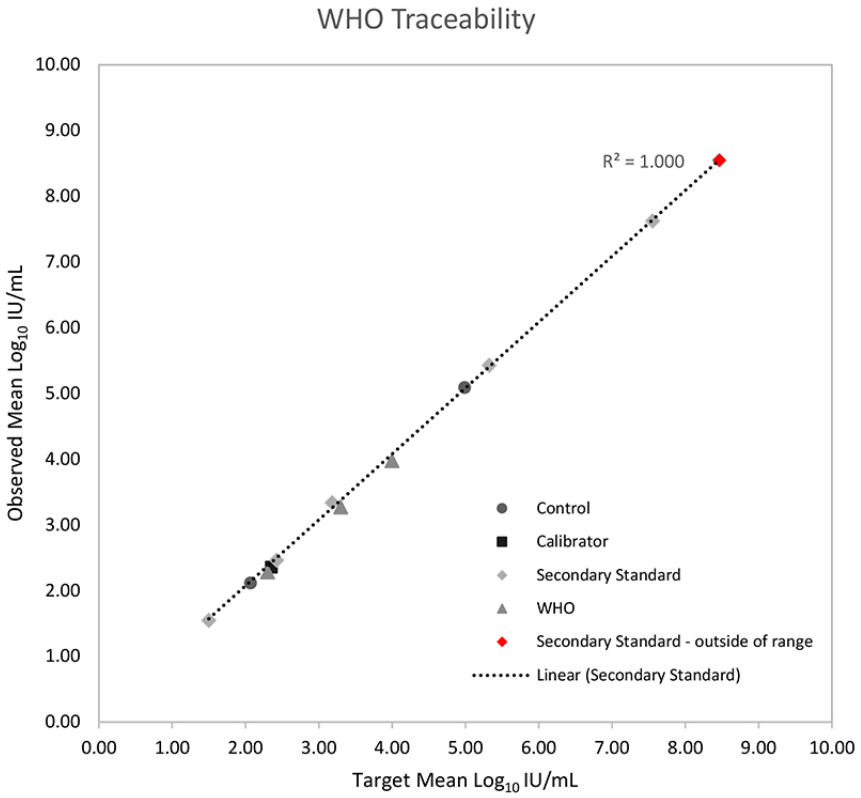


Figure 9. Traceability Between the 2nd HCV Who Standard Target Concentrations and Observed Concentration From the Aptima HCV Quant Dx Assay

Precision

The Aptima HCV Quant Dx precision panel was built by diluting HCV-positive clinical plasma and HCV 1a aRNA into HCV-negative clinical plasma (the two highest panel members were aRNA). Seven positive panel members spanned the range of the assay (1.86 $\log_{10}$ IU/mL to 7.68 $\log_{10}$ IU/mL), and were tested in three replicates per run by one operator, using one pilot lot of reagents on one Panther system over 21 test days, two runs a day.

Table 10 shows the precision of assay results (in $\log_{10}$ IU/mL) between runs, within runs, and overall. Total variability was ≤ 0.14 across all panel members, primarily due to intra-run variability (i.e., random error).

Table 10: Precision of the Aptima HCV Quant Dx Assay

N	Mean Concentration (IU/mL)	Mean Concentration (log_{10} IU/mL)	Inter-Day SD	Inter-Run SD	Intra-Run SD	Total SD
126	72	1.86	0.02	0.06	0.12	0.14
126	182	2.26	0.00	0.07	0.07	0.10
126	617	2.79	0.00	0.06	0.08	0.10
126	1,950	3.29	0.00	0.07	0.05	0.08
126	8,128	3.91	0.00	0.06	0.07	0.10
126	616,595	5.79	0.03	0.04	0.06	0.08
125[a]	47,863,009	7.68	0.05	0.05	0.06	0.09

SD=standard deviation

[a] Number of valid results within linear range of the assay.

Note: Variability from some factors may be numerically negative, which can occur if the variability due to those factors is very small. When this occurs, SD is shown as 0.00.

Potentially Interfering Substances

The susceptibility of the Aptima HCV Quant Dx assay to interference by elevated levels of endogenous substances or by drugs commonly prescribed to HCV infected individuals was evaluated. HCV negative plasma samples and samples spiked with HCV to a concentration of approximately 15 IU/mL (1.2 log_{10} IU/mL) or 1995 IU/mL (3.3 log_{10} IU/mL) of HCV RNA were tested.

No interference in the performance of the assay was observed in the presence of albumin (90 mg/mL), hemoglobin (5 mg/mL), triglycerides (30 mg/mL), or unconjugated bilirubin (0.2 mg/mL).

Clinical plasma specimens from patients with elevated levels of defined substances or from patients with the diseases, ten samples for each substance, listed in Table 11 were tested with the Aptima HCV Quant Dx assay. No interference in the performance of the assay was observed.

Table 11: Tested Clinical Specimen Types

	Clinical Specimen Types
1	Rheumatoid factor (RF)
2	Antinuclear antibody (ANA)
3	Anti-Jo-1 antibody (JO-1)
4	Systemic lupus erythematosus (SLE)
5	Rheumatoid arthritis (RA)
6	Multiple sclerosis (MS)
7	Hyperglobulinemia
8	Elevated alanine aminotransferase (ALT)
9	Elevated aspartate aminotransferase (AST)
10	Alcoholic cirrhosis (AC)
11	Multiple myeloma (MM)
12	Lipemic (elevated lipid)
13	Icteric (elevated bilirubin)
14	Hemolyzed (elevated hemoglobin)
15	Elevated protein albumin
16	HBV antibodies
17	HIV-1 antibodies
18	HIV-2 antibodies

No interference in the performance of the assay was observed in the presence of the exogenous substances listed in Table 12 at concentrations at least three times the C_{max} (human plasma).

Table 12: Exogenous Substances

Exogenous Substance Pool	Exogenous Substances Tested
1	Telaprevir, clarithromycin, interferon alpha-2a, dolutegravir, azithromycin
2	Simeprevir, sofosbuvir
3	Efavirenz, boceprevir, pegylated interferon alpha-2b, emtricitabine, raltegravir, amoxicillin
4	Abacavir sulfate, ribavirin, dasabuvir, rilpivirine, rifampin/rifampicin
5	Lopinavir, tenofovir, lamivudine, valganciclovir
6	Heparin, EDTA, sodium citrate

Performance with HCV Negative Specimens

Specificity was determined using 198 fresh and 538 frozen HCV negative clinical specimens. A total of 370 plasma and 366 serum specimens were confirmed to be HCV negative using approved assays. Specificity was calculated as the percentage of HCV negative samples with results of "Not Detected." HCV RNA was not detected in all 736 samples. Specificity was 100% (736/736, 95% CI: 99.6 -100%).

Table 13: Performance in HCV Negative Plasma and Serum Clinical Specimens

	Fresh Plasma	Frozen Plasma	Plasma Total	Fresh Serum	Frozen Serum	Serum Total	Combined
Valid replicates (n)	100	270	370	98	268	366	736
Not Detected	100	270	370	98	268	366	736
Specificity (95% CI)	100%	100%	100%	100%	100%	100%	100%
	(97.1-100)	(98.9-100)	(99.2-100)	(97.0-100)	(98.9-100)	(99.2-100)	(99.6-100)

CI=confidence interval

Analytical Specificity

Potential cross-reactivity to the pathogens listed in Table 14 was evaluated in HCV negative human plasma in the presence or absence of approximately 15 IU/mL (1.2 $\log_{10}$ IU/mL) or 1995 IU/mL (3.3 $\log_{10}$ IU/mL) HCV. No cross-reactivity was observed. No interference was observed in the presence of the pathogens.

Table 14: Pathogens Tested for Analytical Specificity

Pathogen	Concentration	
Hepatitis A virus	100,000	copies/mL
Hepatitis B virus (HBV)	100,000	IU/mL[a]
Hepatitis G virus	1,470	genome/mL
HIV-1	100,000	copies/mL
HIV-2	100,000	PFU/mL[b]
Herpes simplex virus 1 (HSV-1)	100,000	PFU/mL
Herpes simplex virus 2 (HSV-2)	100,000	PFU/mL
Human herpes virus 6B	100,000	copies/mL
Human herpes virus 8	2,667	TCID50 U/mL[c]
Human T-cell lymphotropic virus-type 1 (HTLV-1)	100,000	vp/mL[d]
Human T-cell lymphotropic virus-type 2 (HTLV-2)	100,000	vp/mL
Parvovirus B19	100,000	IU/mL
West Nile virus	100,000	PFU/mL
Dengue virus 1	100,000	PFU/mL
Dengue virus 2	100,000	PFU/mL
Dengue virus 3	100,000	PFU/mL
Dengue virus 4	100,000	PFU/mL

Pathogen	Concentration	
Corynebacterium diphtheriae	1,000,000	CFU/mL[f]
Streptococcus pneumoniae	1,000,000	CFU/mL
Staphylococcus aureus	1,000,000	CFU/mL
Propionibacterium acnes	1,000,000	CFU/mL
Staphylococcus epidermidis	1,000,000	CFU/mL
Candida albicans	1,000,000	CFU/mL
Neisseria gonorrhoeae	1,000,000	CFU/mL
Chlamydia trachomatis	1,000,000	IFU/mL[g]
Trichomonas vaginalis	1,000,000	cells/mL

[a]IU/mL = International units per mL
[b]PFU/mL = Plaque forming units per mL
[c]TCID50 U/mL = Tissue culture infective dose units per mL
[d]vp/mL = Viral particles per mL
[e]LD50/mL = Lethal dose per mL
[f]CFU/mL = Colony forming units per mL
[g]IFU/mL = Inclusion forming units per mL

Table 14: Pathogens Tested for Analytical Specificity (continued)

Pathogen	Concentration		Pathogen	Concentration
Cytomegalovirus	100,000	PFU/mL		
Epstein-Barr virus	100,000	copies/mL		
Rubella virus	100,000	PFU/mL		
Human papillomavirus	100,000	cells/mL		
Adenovirus type 5	100,000	TCID50 U/mL		
Influenza A virus	100,000	TCID50 U/mL		
Japanese encephalitis virus	NA	NA		
St. Louis encephalitis virus	NA	NA		
Murray Valley encephalitis virus	2,643	LD50/mL[e]		
Yellow fever virus	100,000	cells/mL		

Clinical Samples Containing Viruses Other Than HCV

The pathogens listed in Table 15 were evaluated by obtaining individual naturally infected clinical specimens. These were tested in the presence or absence of approximately 15 IU/mL (1.2 $\log_{10}$ IU/mL) or 1995 IU/mL (3.3 $\log_{10}$ IU/mL) HCV virus. No cross-reactivity was observed. No interference was observed.

Table 15: Clinical Samples Tested for Analytical Specificity

Microorganism	Matrix	N (donors)
HBV	Serum	5
HBV	Plasma	5
Dengue virus	Plasma	10
Hepatitis A virus	Plasma	10
HTLV-1	Plasma	10
HTLV-2	Plasma	10
HIV-1	Plasma	10
West Nile virus	Plasma	10

Matrix Equivalency

One hundred seventy-six sample sets of matched blood collection tubes (serum tubes, SST, PPT, ACD, K2 EDTA, K3 EDTA) were assessed for matrix equivalency. Of these sample sets, 68 were naturally infected HCV-positive, and 108 were HCV-negative sets. Twenty-six of the 68 positives were further diluted with 13 negative samples of matched blood collection tubes at 1:10, 1:100, and 1:1000 or 1:100, 1:10,000 and 1:100,000, with concentrations across the assay's dynamic range (146 positive replicates per collection tube). Correlation was measured by using the serum collection tube as a comparator.

Table 16: Matrix Equivalency Study

Blood Collection Tube	Deming Regression	95% C.I. of Slope		95% C.I. of Intercept		R^2	Mean Difference (log_{10})
		Lower Limit	Upper Limit	Lower Limit	Upper Limit		
SST	y = 1.00x - 0.01	0.99	1.01	-0.05	0.07	0.998	0.00
K2 EDTA	y = 1.01x - 0.03	0.99	1.02	-0.12	-0.07	0.995	0.00
K3 EDTA	y = 1.00x - 0.01	0.98	1.01	-0.10	0.07	0.996	-0.02
ACD	y = 1.00x - 0.14	0.99	1.01	-0.20	-0.08	0.997	-0.13
PPT	y = 1.00x - 0.01	0.98	1.01	-0.10	0.08	0.996	-0.01

Sample Dilution Using Aptima Specimen Diluent (1:3)

To assess the detection accuracy of HCV RNA in samples diluted with Aptima Specimen Diluent plasma or serum, samples from 1.67 log_{10} IU/mL to 6.34 log_{10} IU/mL were diluted 1:3 with Aptima Specimen Diluent (240 µL of sample combined with 480 µL of Aptima Specimen Diluent). Each sample was tested neat and diluted (1:3) in triplicate. Testing was performed using one lot of assay reagents on one Panther system with two Aptima Specimen Diluent lots. The difference between the neat and diluted test results was calculated for each sample set as shown in Table 17 for plasma and Table 18 for serum.

Table 17: Plasma Specimen 1:3 Dilution Matrix Comparison Summary of Log_{10} IU/mL

Plasma Matrix Average Reported Concentration (log_{10} IU/mL) n = 3	Diluent Average Reported Concentration (log_{10} IU/mL) n = 6	Difference Diluent from Plasma Matrix (log_{10} IU/mL)
1.74[a]	1.57[b]	-0.18
4.56	4.36	-0.20
5.62	5.46	-0.17
3.69	3.56	-0.13
2.65	2.60	-0.05
4.18	4.09	-0.09
3.25	3.28	0.03
4.12	4.06	-0.07
3.54	3.48	-0.06
6.20	5.91	-0.30

[a]Result from two replicates. One result "Detected" but not quantified.
[b]Result from four replicates. Two results "Detected" but not quantified.

Table 18: Serum Specimen 1:3 Dilution Matrix Comparison Summary of Log_{10} IU/mL

Serum Matrix Average Reported Concentration (log_{10} IU/mL) n = 3	Diluent Average Reported Concentration (log_{10} IU/mL) n = 6	Difference Diluent from Serum Matrix (log_{10} IU/mL)
1.50[a]	1.80	0.30
5.32	5.14	-0.18
3.65	3.59	-0.06
4.91	4.71	-0.20
3.66	3.72	0.06
3.81	3.74	-0.07
2.42	2.60	0.18
2.65	2.88	0.23
4.90	4.70	-0.21
6.17	6.00	-0.17

[a]Result from two replicates. One result "Detected" but not quantified.

Sample Dilution Using Aptima Specimen Diluent (1:100)

To assess the detection accuracy of HCV RNA in samples diluted with Aptima Specimen Diluent plasma or serum, five naturally infected plasma specimens and five naturally infected serum specimens, along with ten specimens each of HCV negative plasma and serum spiked with HCV aRNA targeting above the ULoQ, 8 log_{10} IU/mL at 8.30 log_{10} IU/mL were tested in triplicate. A 1:100 dilution was performed with one part sample and 99 parts Aptima Specimen Diluent just prior to testing. Testing was performed using one lot of assay reagents on one Panther system with two Aptima Specimen Diluent lots. The difference between the neat and diluted test results was calculated for each sample set as shown in Table 19 for plasma and Table 20 for serum.

Table 19: Plasma Specimen 1:100 Dilution Matrix Comparison Summary of Log_{10} IU/mL

Matrix	Dilution	Plasma Matrix Average Reported Concentration (log_{10} IU/mL) n = 3	Diluent Average Reported Concentration (log_{10} IU/mL) n = 6	Difference
Plasma	1:100	8.03[a]	7.95	-0.08
		8.07[a]	7.93	-0.15
		8.14[a]	7.97	-0.17
		8.16[a]	7.94	-0.22
		8.09[a]	7.96	-0.13
		8.38[a]	7.97	-0.42
		7.98[a]	7.89	-0.09
		8.08[a]	7.89	-0.19
		8.14[a]	7.92	-0.22
		8.08[a]	7.97	-0.11
		6.41	6.22	-0.19
		6.73	6.58	-0.15
		6.88	6.75	-0.14
		6.54	6.51	-0.03
		6.55	6.29	-0.27

[a]Spiked specimen

Table 20: Serum Specimen 1:100 Dilution Matrix Comparison Summary of Log_{10} IU/mL

Matrix	Dilution	Serum Matrix Average Reported Concentration (log_{10} IU/mL) n = 3	Diluent Average Reported Concentration (log_{10} IU/mL) n = 6	Difference
Serum	1:100	8.04[a]	7.94	-0.09
		8.07[a]	7.91	-0.16
		7.99[a]	7.91	-0.08
		8.03[a]	7.92	-0.11
		7.99[a]	7.89	-0.09
		8.05[a]	7.85	-0.20
		8.16[a]	7.93	-0.23
		8.16[a]	7.90	-0.26
		8.06[a]	7.89	-0.17
		8.26[a]	7.92	-0.34
		7.34	7.09	-0.25
		7.07	6.83	-0.24
		7.18	6.80	-0.38
		7.35[b]	7.12	-0.23
		7.07	6.68	-0.39

[a]Spiked specimen
[b]Result from two out of three replicates tested. One outlier replicate removed.

Confirmation of the LLoQ in Clinical Specimens Diluted in Specimen Diluent

The LLoQ of the Aptima HCV Quant assay was confirmed with HCV genotype 1 clinical specimens diluted into Aptima Specimen Diluent. Specimens were prepared in HCV negative human plasma and serum at 21, 30, and 45 IU/mL. Each panel was diluted 1:3 into Aptima Specimen Diluent just prior to testing to give final concentrations of approximately 7, 10, and 15 IU/mL. Thirty-six (36) replicates of each panel member were tested with one reagent lot across three days. A LLoQ ≤ 10 IU/mL for HCV plasma and serum diluted into Aptima Specimen Diluent was confirmed as shown in Table 21.

Table 21: Confirmation of LLoQ – Clinical Samples in Aptima Specimen Diluent

Matrix	Target Concentration (IU/ml)	Target Concentration (log_{10} IU/ml)	% Detected	Aptima HCV Quant Dx (IU/ml)	Aptima HCV Quant Dx (log_{10} IU/ml)	SD (log_{10} IU/ml)	Bias (log_{10} IU/ml)	Calculated TE (log_{10} IU/ml)	Calculated TAE (log_{10} IU/ml)
Plasma	7	0.85	100%	9	0.94	0.25	0.10	0.71	0.60
Serum	7	0.85	100%	9	0.96	0.21	0.12	0.60	0.54

Precision of Diluted Samples

The Aptima HCV Quant Dx precision panel was built by diluting HCV-positive clinical plasma and HCV aRNA into HCV-negative clinical plasma. These were further diluted in Aptima Specimen Diluent. Positive panels were diluted into Aptima Specimen Diluent. These were tested in three replicates per run by one operator, using one pilot lot of reagents on one Panther system over 21 test days, two runs a day.

Table 22 shows the precision of assay results (in $\log_{10}$ IU/mL) between runs, within runs, between days, and overall. Total variability was ≤ 0.15 across all panel members, primarily due to intra-run variability (i.e., random error).

Table 22: Precision of Panels Diluted in Aptima Specimen Diluent

Dilution	N	Mean Concentration ($\log_{10}$ IU/ml)	Standard Deviation			
			Inter-day	Inter-Run	Intra-Run	Total
1:3	126	2.24	0.00	0.05	0.13	0.14
1:100	126	3.86	0.06	0.05	0.12	0.15
1:100	126	7.31	0.03	0.10	0.05	0.12

[a]Number of valid results within linear range of the assay

Note: *Variability from some factors may be numerically negative, which can occur if the variability, due to those factors, is very small. When this occurs, SD and CV are shown as 0.*

Carryover

To establish that the Panther system minimizes the risk of false positive results arising from carryover contamination, a study was conducted using spiked panels on three Panther systems. Carryover was assessed using high titer aRNA spiked plasma samples (7 $\log_{10}$ IU/mL) interspersed between HCV negative samples in a checkerboard pattern. Testing was carried out over fifteen runs. The overall carryover rate was 0.14% (1/704)

Seroconversion Panel

Eleven HCV seroconversion panel sets, totaling 72 samples, were tested. Aptima HCV Quant Dx assay results were compared to HCV antibody test results. The number of days to first reactive result are listed in Table 23. The Aptima HCV Quant Dx assay detected the presence of HCV an average of 20 days earlier than antibody tests.

Table 23: Seroconversion Panel Data Summary

Panel ID	Number of Panel Members Tested	Number of Reactive Panel Members			Days to First Reactive Result			Difference in Days to First Reactive Result (Based on Bleed Date)	
		Aptima HCV Quant Dx	HCV Antibody Test1	HCV Antibody Test2	Aptima HCV Quant Dx	HCV Antibody Test1	HCV Antibody Test2	Days Earlier than HCV Antibody Test1	Days Earlier than HCV Antibody Test2
PHV911	4	4	3	3	3[a]	14	14	11	11
PHV913	4	4	0	2	0	9[b]	7	9	7
PH919	7	4	3	3	25	28	28	3	3
PH920	9	9	7	6	0[c]	13	16	13	16
PH921	11	11	9	7	0	7	18	7	18
PH923	6	6	2	2	0	21	21	21	21
PH924	6	6	3	3	0	59	59	59	59
PH925	5	5	1	1	0	27	27	27	27
PH926	5	5	1	0	0	14	14[b]	14	14

Table 23: Seroconversion Panel Data Summary (continued)

Panel ID	Number of Panel Members Tested	Number of Reactive Panel Members			Days to First Reactive Result			Difference in Days to First Reactive Result (Based on Bleed Date)	
		Aptima HCV Quant Dx	HCV Antibody Test1	HCV Antibody Test2	Aptima HCV Quant Dx	HCV Antibody Test1	HCV Antibody Test2	Days Earlier than HCV Antibody Test1	Days Earlier than HCV Antibody Test2
6227	7	4	2	2	42	74	74	32	32
6229	8	8	4	3	0	17	20	17	20
Total	72	66	35	32			Mean	19.36	20.73
							Median	14	18

HCV antibody test1 was completed with the Abbott Prism HCV assay

HCV antibody test2 was completed with the Ortho Enhanced SAVE assay, with the following exceptions:

Panels 6227 and 6229, which were both tested with the Ortho ELISA Anti-HCV 3.0 assay

[a] First bleed was not tested due to unavailability of sample from vendor.

[b] All bleeds in this panel were non-reactive for HCV antibody. The last bleed day was used as the “Days to First Reactive Result.”

[c] Second bleed was not tested due to unavailability of sample from vendor

Reproducibility Study

Reproducibility was evaluated on the Panther system at three external U.S. sites. Two operators performed testing at each site. Each operator performed two runs per day over three days, using 3 reagent lots over the course of testing. Each run had 3 replicates of each panel member. Overall, 108 replicates of each panel member were tested.

Reproducibility was tested using panel members made from HCV-negative plasma. The positive panel members were positive for HCV genotype 1, genotype 2, or genotype 3. HCV RNA concentrations spanned the linear range of the assay.

Table 24 shows the reproducibility and precision of assay results for each positive panel member between sites, between operators, between lots, between days, between runs, within runs, and overall, and the proportion of replicates with results outside the Aptima HCV Quant Dx assay's reportable range.

The coefficient of variation was calculated using the following equation where σ^2 is the sample variance of the data after $\log_{10}$ transformation.

$$\%CV = 100 \times \sqrt{(10^{\sigma^2 \ln(10)} - 1)}$$

For the HCV-negative panel member, 108 replicates were tested and HCV RNA was not detected in all 108 replicates (negative agreement=100%, 95% CI: 96.6% to 100%). For all HCV-positive panel members, agreement values were 100%.

Table 24: Reproducibility of Aptima HCV Quant Dx Assay HCV RNA Levels on the Panther System in Positive Panel Members

GT	N	Observed Mean IU/mL	Observed Mean Log_{10} IU/mL	Between Sites SD (%CV	Between Lots SD (%CV)	Between Operators/Days[a] SD (%CV)	Between Runs SD (%CV)	Within Runs SD (%CV	Total SD (%CV)	Below LLoQ n (%)	Above ULoQ n (%)
1	108	10.6	1.0	0.070 (16.22)	0.048 (10.98)	0.000 (0.00)	0.040 (9.20)	0.258 (64.93)	0.274 (69.94)	59 (54.6)	0 (0.0)
	107	19.5	1.3	0.034 (7.77)	0.035 (7.97)	0.009 (2.10)	0.064 (14.91)	0.169 (40.31)	0.187 (45.12)	9 (8.4)	0 (0.0)
	108	43.6	1.6	0.000 (0.00)	0.070 (16.11)	0.000 (0.00)	0.061 (14.13)	0.155 (36.82)	0.180 (43.39)	0 (0.0)	0 (0.0)
	108	602.7	2.8	0.042 (9.58)	0.035 (8.02)	0.022 (5.05)	0.023 (5.30)	0.075 (17.40)	0.098 (22.81)	0 (0.0)	0 (0.0)
	108	22710.9	4.3	0.075 (17.42)	0.045 (10.49)	0.044 (10.08)	0.058 (13.46)	0.071 (16.57)	0.135 (31.74)	0 (0.0)	0 (0.0)
	108	4195539.0	6.6	0.007 (1.65)	0.026 (6.08)	0.045 (10.43)	0.000 (0.00)	0.131 (30.97)	0.142 (33.50)	0 (0.0)	0 (0.0)
	108	58549271.2	7.8	0.010 (2.26)	0.000 (0.00)	0.022 (5.10)	0.035 (8.08)	0.105 (24.42)	0.113 (26.43)	0 (0.0)	1 (0.9)
2	108	11.3	1.0	0.062 (14.29)	0.075 (17.28)	0.112 (26.28)	0.000 (0.00)	0.290 (75.02)	0.326 (86.94)	53 (49.1)	0 (0.0)
	108	13.9	1.1	0.000 (0.00)	0.125 (29.36)	0.128 (30.18)	0.068 (15.83)	0.211 (51.69)	0.285 (73.45)	46 (42.6)	0 (0.0)
	108	62.6	1.8	0.000 (0.00)	0.044 (10.18)	0.101 (23.48)	0.069 (16.00)	0.124 (29.25)	0.180 (43.24)	0 (0.0)	0 (0.0)
	108	351.0	2.5	0.022 (5.18)	0.062 (14.38)	0.004 (1.01)	0.045 (10.31)	0.081 (18.70)	0.113 (26.56)	0 (0.0)	0 (0.0)
	108	14519.2	4.1	0.000 (0.00)	0.051 (11.78)	0.048 (11.01)	0.011 (2.42)	0.092 (21.50)	0.116 (27.25)	0 (0.0)	0 (0.0)
	108	5810012.3	6.8	0.000 (0.00)	0.017 (4.00)	0.041 (9.54)	0.015 (3.53)	0.104 (24.25)	0.114 (26.73)	0 (0.0)	0 (0.0)
	108	77499195.7	7.9	0.000 (0.00)	0.018 (4.24)	0.028 (6.56)	0.000 (0.00)	0.077 (17.77)	0.084 (19.46)	0 (0.0)	10 (9.3)
3	107	8.6	0.9	0.049 (11.32)	0.103 (24.05)	0.056 (12.95)	0.000 (0.00)	0.225 (55.47)	0.258 (65.16)	71 (66.4)	0 (0.0)
	108	14.3	1.1	0.157 (37.31)	0.000 (0.00)	0.090 (21.04)	0.000 (0.00)	0.178 (42.88)	0.254 (63.90)	36 (33.3)	0 (0.0)
	107	49.9	1.7	0.119 (27.96)	0.065 (15.08)	0.047 (10.80)	0.034 (7.81)	0.109 (25.42)	0.183 (44.15)	0 (0.0)	0 (0.0)
	108	218.3	2.2	0.324 (86.23)	0.043 (10.01)	0.139 (32.83)	0.063 (14.64)	0.082 (19.09)	0.370 (103.21)	0 (0.0)	0 (0.0)
	107	8541.1	3.9	0.248 (61.97)	0.056 (12.90)	0.151 (35.77)	0.048 (10.99)	0.078 (18.19)	0.309 (81.20)	0 0.0)	0 (0.0)
	107	4128761.4	6.6	0.044 (10.17)	0.000 (0.00)	0.000 (0.00)	0.042 (9.61)	0.126 (29.65)	0.140 (33.06)	0 (0.0)	0 (0.0)
	108	63813728.0	7.8	0.012 (2.84)	0.000 (0.00)	0.000 (0.00)	0.059 (13.76)	0.092 (21.32)	0.110 (25.71)	0 (0.0)	3 (2.8)

%CV=log-normal coefficient of variation, GT=genotype, SD=standard deviation (log_{10} IU/mL)

Note: Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, SD and CV are shown as 0.

[a] Between Operators may be confounded with Between Days; therefore, Between Operators and Between Days estimates are combined in Between Operators/Days.

Clinical Performance

Method Correlation

The performance of the Aptima HCV Quant Dx assay was assessed against a CE-marked comparator assay by testing undiluted clinical specimens from HCV infected patients on three Panther systems using four reagent lots. A total of 1058 plasma and serum samples (872 plasma, 186 serum) across all HCV genotypes within the linear range common to both assays were used for the linear regression as shown in Figure 10.

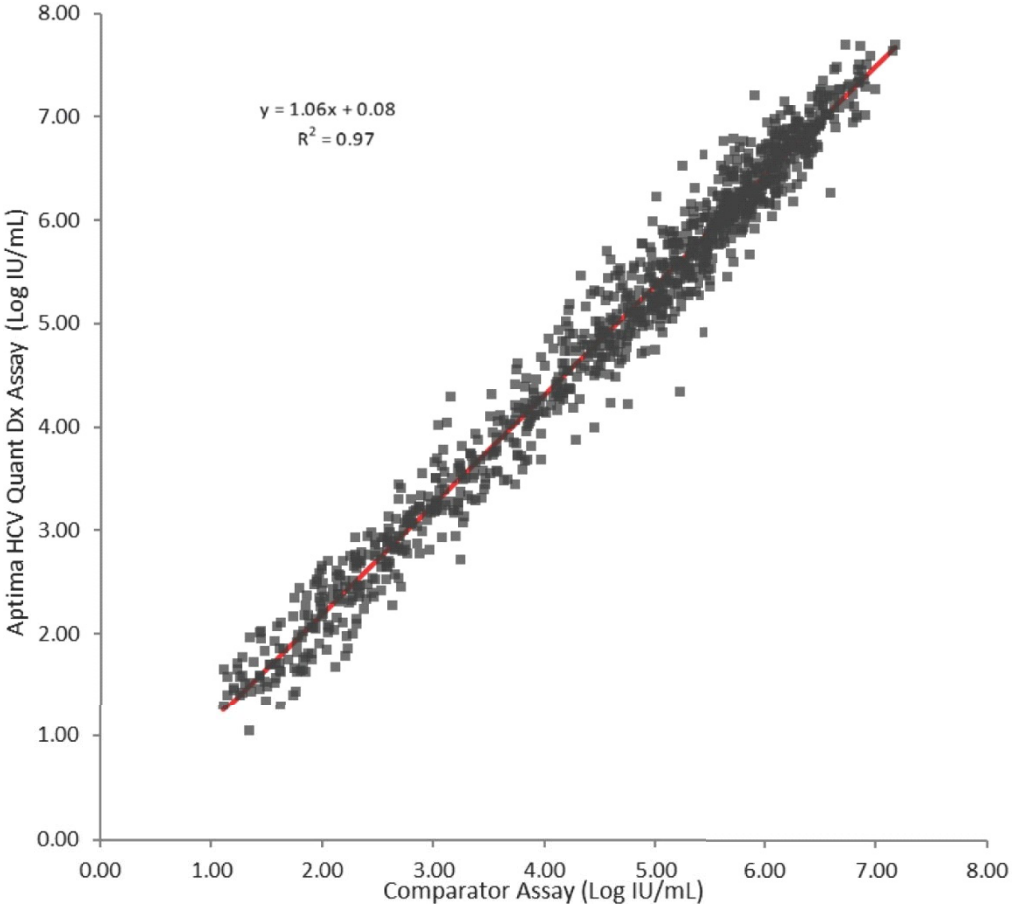


Figure 10. Correlation between the Aptima HCV Quant Dx Assay and Comparator Assay

Clinical Utility Study

The study was designed to demonstrate clinical utility by estimating the performance characteristics for achieving sustained virologic response (SVR) or nonsustained virologic response (NSVR) at established clinically relevant time points during 8-, 12-, or 24-week antiviral treatment. The study also assessed the impact of covariates (i.e., age, sex, race, baseline HCV RNA level, presence or absence of cirrhosis) on SVR response.

Specimens were prospectively collected from genotype 1, 2, and 3 subjects chronically infected with HCV who were initiating therapy with 8-, 12-, or 24-week sofosbuvir-based treatments using FDA-approved drugs prescribed in accordance with FDA-approved labeling and/or AASLD/IDSA HCV treatment guidelines.[20,21] HCV treatment-naive or treatment-experienced subjects, and subjects without cirrhosis or with compensated cirrhosis were eligible, as applicable for each treatment regimen.

Determination of HCV RNA viral load was performed at baseline, week 1, week 2, week 4, week 8, week 12, and week 24 using the Aptima HCV Quant Dx assay. End of treatment (EOT) and post-treatment week 4 and week 12 follow-up results were determined using the Aptima HCV Quant Dx assay and two approved quantitative HCV RNA assays. SVR was defined as an HCV RNA level < LLoQ as determined by an approved quantitative assay for the post-treatment week 12 sample.

Of the 352 subjects enrolled from 35 U.S. clinical sites, 274 subjects had a conclusive SVR status and were evaluable for analysis. 56 subjects had nonevaluable SVR status and were not included in the analysis. An additional 22 subjects had conclusive SVR status, but were nonevaluable due to missing pre-treatment/baseline results, all on-treatment results <LLoQ or not detected, or genotypes were not established with an approved assay. In this study, 90.5% (248/274) subjects achieved SVR and 9.5% (26/274) achieved NSVR (see Table 31 through Table 33 for counts by genotype and DRA). The highest baseline viral load was 7.58 $\log_{10}$ IU/mL, as determined by the Aptima HCV Quant Dx assay. All evaluable subjects completed therapy, with no on-treatment failures (e.g., nonresponse). A total of 2449 samples collected at different time points from each subject were tested at three U.S. testing sites.

Table 25 shows subject demographics and clinical characteristics for the 274 evaluable subjects.

Table 25: Demographics and Clinical Characteristics

Characteristics		
Total	N	274
Sex	Female, n (%)	112 (40.9)
	Male, n (%)	162 (59.1)
Age (years)	n	274
	Mean	55.1
	Median	56.0
	SD	10.40
	Minimum	23
	Maximum	83
	21-49, n (%)	61 (22.3)
	50-70, n (%)	203 (74.1)
	>70, n (%)	10 (3.6)
Ethnicity	n	274
	Hispanic or Latino, n (%)	65 (23.7)
	Not Hispanic or Latino, n (%)	202 (73.7)
	Unknown, n (%)	6 (2.2)
	Refused, n (%)	1 (0.4)
Race[a]	n	274
	White, n (%)	223 (81.4)
	Black or African American, n (%)	43 (15.7)
	Asian, n (%)	1 (0.4)
	American Indian/Alaska Native, n (%)	3 (1.1)
	Native Hawaiian/Pacific Islander, n (%)	0 (0.0)
	Other, n (%)	3 (1.1)
	Unknown, n (%)	1 (0.4)
	Refused, n (%)	0 (0.0)
Genotype	n	274
	Genotype 1, n (%)	158 (57.7)
	Genotype 2, n (%)	66 (24.1)
	Genotype 3, n (%)	50 (18.2)

Table 25: Demographics and Clinical Characteristics (continued)

Characteristics		
HCV treatment status	n	274
	Naive, n (%)	232 (84.7)
	Experienced, n (%)	42 (15.3)
	Relapsed, n (%)	32 (76.2)
	Null responder, n (%)	1 (2.4)
	Non-responder, n (%)	2 (4.8)
	Partial responder, n (%)	3 (7.1)
	Breakthrough, n (%)	1 (2.4)
	Rebound, n (%)	0 (0.0)
	Treatment response unknown, n (%)	3 (7.1)
	Missing, n (%)	0 (0.0)
Cirrhotic status	n	274
	Cirrhotic, n (%)	98 (35.8)
	Non-cirrhotic, n (%)	176 (64.2)

[a] Subjects may report multiple races

Table 26 shows the final drug regimen assignments for the 274 evaluable subjects.

Table 26: Summary of Subjects' Final Drug Regimen Assignments by Genotype

Drug Regimen Assignment	HCV Genotype n (%)		
	1	2	3
Sofosbuvir and Peginterferon and Ribavirin for 12 weeks	25 (15.8)	-	3 (6.0)
Sofosbuvir and Simeprevir and/or Ribavirin for 12 weeks	63 (39.9)	-	-
Sofosbuvir and Simeprevir and/or Ribavirin for 24 weeks	4 (2.5)	-	-
Sofosbuvir and Ribavirin for 12 weeks	-	66 (100)	-
Sofosbuvir and Ribavirin for 24 weeks	6 (3.8)	-	47 (94.0)
Ledipasvir and Sofosbuvir for 8 weeks	14 (8.9)	-	-
Ledipasvir and Sofosbuvir for 12 weeks	39 (24.7)	-	-
Ledipasvir and Sofosbuvir for 24 weeks	7 (4.4)	-	-
Total eligible subjects	158	66	50

Table 27 evaluates the association of baseline covariates (age, race, sex, baseline HCV viral load [binary variable], and cirrhotic status) and SVR using the odds ratio (OR) with univariate (unadjusted) logistic regression analysis. Results for the 274 evaluable subjects were evaluated by HCV genotype. For genotypes 1 and 2, cirrhotic subjects were less likely than non-cirrhotic subjects to achieve SVR (OR=0.16 [95% Wald CI: 0.04 to 0.63], P=0.009 and OR=0.13 [95% Wald CI: 0.02 to 0.70], P=0.017, respectively). The 95% CI values for other covariates for genotype 1 and 2, and all covariates for genotype 3, included the value 1 and were not statistically significant.

Table 27: Evaluation of Baseline Predictors of SVR at Baseline

GT	Category	N	Grouping	N(%)	Percent Achieving SVR	Odds Ratio (Wald 95% CI) Using Univariate LR Analysis	P Value
1	Age	158	≥ 40 years	146 (92.4)	91.8	0 [0 – ∞)	Not Calculable
			< 40 years	12 (7.6)	100		
	Sex	158	Male	92 (58.2)	90.2	0.44 (0.11 - 1.69)	0.231
			Female	66 (41.8)	95.5		
	Race	158	White	116 (73.4)	94.0	2.10 (0.63 - 7.03)	0.227
			Non-white	42 (26.6)	88.1		
	HCV RNA baseline viral load[a]	156	> 740,000 IU/mL	111 (71.2)	92.8	1.26 (0.36 - 4.40)	0.721
			≤ 740,000 IU/mL	45 (28.8)	91.1		
	Cirrhotic status	158	Cirrhotic	57 (36.1)	84.2	0.16 (0.04 - 0.63)	0.009
			Non-cirrhotic	101 (63.9)	97.0		
2	Age	66	≥ 40 years	60 (90.9)	88.3	1.51 (0.15 - 14.91)	0.722
			< 40 years	6 (9.1)	83.3		
	Sex	66	Male	36 (54.5)	86.1	0.69 (0.15 - 3.15)	0.631
			Female	30 (45.5)	90.0		
	Race	66	White	61 (92.4)	86.9	0 [0 – ∞)	Not Calculable
			Non-white	5 (7.6)	100		
	HCV RNA baseline viral load	66	> 740,000 IU/mL	44 (66.7)	84.1	0.25 (0.03 - 2.19)	0.211
			≤ 740,000 IU/mL	22 (33.3)	95.5		
	Cirrhotic status	66	Cirrhotic	22 (33.3)	72.7	0.13 (0.02 - 0.70)	0.017
			Non-cirrhotic	44 (66.7)	95.5		
3	Age	50	≥ 40 years	44 (88.0)	86.4	0 [0 – ∞)	NC
			< 40 years	6 (12.0)	100		
	Sex	50	Male	34 (68.0)	85.3	0.39 (0.04 - 3.62)	0.405
			Female	16 (32.0)	93.8		
	Race	50	White	46 (92.0)	87.0	0 [0 – ∞)	Not Calculable
			Non-white	4 (8.0)	100		
	HCV RNA baseline viral load[a]	49	> 740,000 IU/mL	30 (61.2)	83.3	0.28 (0.03 - 2.59)	0.261
			≤ 740,000 IU/mL	19 (38.8)	94.7		
	Cirrhotic status	50	Cirrhotic	19 (38.0)	78.9	0.26 (0.04 - 1.58)	0.143
			Non-cirrhotic	31 (62.0)	93.5		

CI=confidence interval, GT=genotype, LR=logistic regression

[a]The HCV RNA baseline viral load covariate does not include subjects that did not provide a quantitative HCV RNA viral load prior to initiation of therapy.

Response to Antiviral Therapy

Assay performance characteristics have been established for individuals treated with certain DAA regimens. No information is available on the assay's predictive value when other DAA combination therapies are used.

Definitions:

Week 1 viral load (VL) = HCV RNA < LLoQ = 10 IU/mL at Week 1 of antiviral therapy

Week 2 VL = HCV RNA < LLoQ = 10 IU/mL at Week 2 of antiviral therapy

Week 4 VL = HCV RNA < LLoQ = 10 IU/mL at Week 4 of antiviral therapy

Week 8 VL = HCV RNA < LLoQ = 10 IU/mL at Week 8 of antiviral therapy

Week 8 VL (EOT): HCV RNA < LLoQ at Week 8 of antiviral therapy

Week 12 VL = HCV RNA < LLoQ = 10 IU/mL at Week 12 of antiviral therapy

Week 12 VL (EOT): HCV RNA < LLoQ at Week 12 of antiviral therapy

Week 24 VL (EOT): HCV RNA < LLoQ at Week 24 of antiviral therapy

Sustained Virologic Response (SVR)12: HCV RNA < LLoQ at Week 12 after completion of antiviral therapy, measured with an independent HCV RNA test.

Table 28 and Table 29 show the frequency of SVR response and non-response, respectively, by genotype and drug regimen overall, and for cirrhotic, non-cirrhotic, treatment-naive, and treatment-experienced subjects. Most subjects achieved SVR across genotypes: 92.4% genotype 1, 87.9% genotype 2, and 88.0% genotype 3. Across genotypes, SVR rates generally were higher in non-cirrhotic subjects than in cirrhotic subjects, and similar between treatment-naive and treatment-experienced subjects.

Table 28: Frequency of SVR Response by Genotype and Drug Regimen

GT	DRA	SVR Responsive Subjects n (%)				
		Overall	Cirrhotic	Non-cirrhotic	Treatment-naive	Treatment-experienced
1	Ledipasvir and Sofosbuvir for 8 weeks	13/14 (92.9)	N/A	13/14 (92.9)	13/14 (92.9)	N/A
	Ledipasvir and Sofosbuvir for 12 weeks	39/39 (100)	9/9 (100)	30/30 (100)	34/34 (100)	5/5 (100)
	Ledipasvir and Sofosbuvir for 24 weeks	6/7 (85.7)	6/7 (85.7)	N/A	N/A	6/7 (85.7)
	Sofosbuvir and Peginterferon and Ribavirin for 12 weeks	24/25 96.0)	7/7 (100)	17/18 (94.4)	22/23 (95.7)	2/2 (100)
	Sofosbuvir and Ribavirin for 24 weeks	6/6 (100)	1/1 (100)	5/5 (100)	5/5 (100)	1/1 (100)
	Sofosbuvir and Simeprevir and/or Ribavirin for 12 weeks	54/63 (85.7)	21/29 (72.4)	33/34 (97.1)	45/54 (83.3)	9/9 (100)
	Sofosbuvir and Simeprevir and/or Ribavirin for 24 weeks	4/4 (100)	4/4 (100)	N/A	2/2 (100)	2/2 (100)
2	Sofosbuvir and Ribavirin for 12 weeks	58/66 (87.9)	16/22 (72.7)	42/44 (95.5)	53/60 (88.3)	5/6 (83.3)
3	Sofosbuvir and Peginterferon and Ribavirin for 12 weeks	3/3 (100)	3/3 (100)	N/A	1/1 (100)	2/2 (100)
	Sofosbuvir and Ribavirin for 24 weeks	41/47 (87.2)	12/16 (75.0)	29/31 (93.5)	35/39 (89.7)	6/8 (75.0)

DRA=drug regimen assignment, GT=genotype, N/A=drug regimen is not applicable for this patient group

Table 29: Frequency of SVR Non-response by Genotype and Drug Regimen

GT	DRA	SVR Non-responsive Subjects n (%)				
		Overall	Cirrhotic	Non-cirrhotic	Treatment-naive	Treatment-experienced
1	Ledipasvir and Sofosbuvir for 8 weeks	1/14 (7.1)	N/A	1/14 (7.1)	1/14 (7.1)	N/A
	Ledipasvir and Sofosbuvir for 12 weeks	0/39 (0.0)	0/9 (0.0)	0/30 (0.0)	0/34 (0.0)	0/5 (0.0)
	Ledipasvir and Sofosbuvir for 24 weeks	1/7 (14.3)	1/7 (14.3)	N/A	N/A	1/7 (14.3)
	Sofosbuvir and Peginterferon and Ribavirin for 12 weeks	1/25 (4.0)	0/7 (0.0)	1/18 (5.6)	1/23 (4.3)	0/2 (0.0)
	Sofosbuvir and Ribavirin for 24 weeks	0/6 (0.0)	0/1 (0.0)	0/5 (0.0)	0/5 (0.0)	0/1 (0.0)
	Sofosbuvir and Simeprevir and/or Ribavirin for 12 weeks	9/63 (14.3)	8/29 (27.6)	1/34 (2.9)	9/54 (16.7)	0/9 (0.0)
	Sofosbuvir and Simeprevir and/or Ribavirin for 24 weeks	0/4 (0.0)	0/4 (0.0)	N/A	0/2 (0.0)	0/2 (0.0)
2	Sofosbuvir and Ribavirin for 12 weeks	8/66 (12.1)	6/22 (27.3)	2/44 (4.5)	7/60 (11.7)	1/6 (16.7)
3	Sofosbuvir and Peginterferon and Ribavirin for 12 weeks	0/3 (0.0)	0/3 (0.0)	N/A	0/1 (0.0)	0/2 (0.0)
	Sofosbuvir and Ribavirin for 24 weeks	6/47 (12.8)	4/16 (25.0)	2/31 (6.5)	4/39 (10.3)	2/8 (25.0)

DRA=drug regimen assignment, GT=genotype, N/A=drug regimen is not applicable for this patient group

Table 30 shows the percent of subjects with virologic response (defined as Aptima HCV Quant Dx assay results < LLoQ) for each genotype and drug regimen assignment at each on-treatment and post-treatment week visit. Most subjects achieved SVR by week 4 across the genotypes, and all subjects achieved SVR by week 8.

Table 30: Probability of Achieving SVR Across On-treatment and Post-treatment Weeks

GT	DRA	N	SVR Responsive % (n)	SVR Not Responsive % (n)	Percent of Subjects With Virologic Response By Visit[a] n (%)						
					Weeks					EOT	PT04
					1	2	4	8	12		
1	LDV + SOF (8 weeks)	14	92.9 (13/14)	7.1 (1/14)	42.9 (6/14)	76.9 (10/13)	100 (14/14)	N/A	N/A	100 (14/14)	90 (9/10)
	LDV + SOF (12 weeks)	39	100 (39/39)	0.0 (0/39)	11.1 (4/36)	52.6% (20/38)	92.1 (35/38)	100 (36/36)	N/A	100 (39/39)	100 (33/33)
	LDV + SOF (24 weeks)	7	85.7 (6/7)	14.3 (1/7)	0.0 (0/6)	42.9 (3/7)	71.4 (5/7)	100 (7/7)	100 (7/7)	100 (6/6)	80 (4/5)
	SOF+PEG+RBV (12 weeks)	25	96.0 (24/25)	4.0 (1/25)	40.0 (10/25)	80.0 (20/25)	100 (25/25)	100 (25/25)	N/A	100 (25/25)	100 (25/25)
	SOF+RBV (24 weeks)	6	100 (6/6)	0.0 (0/6)	16.7 (1/6)	66.7 (4/6)	83.3 (5/6)	100 (5/5)	100 (6/6)	100 (5/5)	100 (6/6)
	SOF+SIM+RBV (12 weeks)	63	85.7 (54/63)	14.3 (9/63)	20.7 (12/58)	67.9 (38/56)	98.4 (62/63)	100 (57/57)	N/A	96.6 (57/59)	90.0 (54/60)
	SOF+SIM+RBV (24 weeks)	4	100 (4/4)	0.0 (0/4)	66.7 (2/3)	66.7 (2/3)	100 (4/4)	100 (3/3)	100 (3/3)	100 (4/4)	100 (4/4)
2	SOF+RBV (12 weeks)	66	87.9 (58/66)	12.1 (8/66)	22.2 (12/54)	69.4 (43/62)	95.4 (62/65)	100 (64/64)	N/A	98.5 (64/65)	93.5 (58/62)
3	SOF+PEG+RBV (12 weeks)	3	100 (3/3)	0.0 (0/3)	33.3 (1/3)	100 (2/2)	100 (3/3)	100 (2/2)	N/A	100 (3/3)	100 (3/3)
	SOF+RBV (24 weeks)	47	87.2 (41/47)	12.8 (6/47)	20.9 (9/43)	70.5 (31/44)	100 (46/46)	100 (46/46)	100 (47/47)	100 (42/42)	89.1 (41/46)

DRA= drug regimen assignment, EOT=end of treatment, N/A=not applicable because visit corresponds to EOT for given treatment or is beyond treatment window, PT04=post-treatment week 4

[a]On-treatment and post-treatment viral response defined as < 10 IU/mL.

Note: EOT is not included in treatment week

For subjects with genotype 1, 95.5% (150/157) of the evaluated subjects had on-treatment viral responses by week 4. The 7 genotype 1 subjects who had not responded by week 4 (3 subjects treated with LDV + SOF [12 weeks], 2 subjects treated with LDV + SOF [24 weeks], 1 subject each treated with SOF + RBV [24 weeks] or SOF + SIM ± RBV [12 weeks]) had on treatment viral responses by week 8. Two (2) subjects treated with SOF + SIM ± RBV (12 weeks) had relapsed by end-of treatment, and 6 (1 subject treated with LDF + SOF [8 weeks], 1 subject treated with LDF + SOF [24 weeks], and 4 subjects treated with SOF + SIM ± RBV [12 weeks]), had relapsed by post-treatment week 4.

For subjects with genotype 2, 95.4% (62/65) of the evaluated subjects had on-treatment viral responses by week 4. The 3 genotype 2 subjects who had not responded by week 4 had on treatment viral responses by week 8. One (1) subject treated with SOF + RBV (12 weeks) had relapsed by end-of treatment, and 4 subjects with the same drug regimen assignment had relapsed by post-treatment week 4.

For subjects with genotype 3, 100% (49/49) of the evaluated subjects had on-treatment viral responses by week 4. No (0) subjects had relapsed by end-of treatment, and 5 subjects treated with SOF + RBV (24 weeks) had relapsed by post-treatment week 4.

Based on the results of this study, the Aptima HCV Quant Dx assay can be used as an aid in the management of HCV-infected patients undergoing antiviral sofosbuvir-based therapy and can be used to determine SVR response and non-response. Results from the assay can be used to quantitatively measure HCV RNA levels in human plasma at baseline and during treatment.

Diagnostic Utility Study

Sensitivity was evaluated in 338 HCV antibody positive subjects from a high-risk population. Table 31 shows subject demographic and clinical characteristics.

Table 31: Demographic and Clinical Characteristics in HCV Antibody Positive Subjects

Characteristics		
Total	*N*	338
Sex	Female, n (%)	80 (23.7)
	Male, n (%)	258 (76.3)
Age (years)	Mean	48.2
	Median	51.0
	SD	11.01
	<21, n (%)	2 (0.6)
	21-49, n (%)	152 (45.0)
	50-70, n (%)	182 (53.8)
	>70, n (%)	2 (0.6)
Race/Ethnicity[a]	White, n (%)	86 (25.4)
	Black, n (%)	118 (34.9)
	Asian, n (%)	1 (0.3)
	Hispanic, n (%)	123 (36.4)
	Mixed ethnicities, n (%)	4 (1.2)
	Other, n (%)	10 (3.0)
	Unknown, n (%)	0 (0.0)
Risk Factors[b]	Injection drug use, n (%)	294 (87.0)
	Recipients of clotting factors made before 1990, n (%)	0 (0.0)
	Diagnosed/treated for hepatitis, n (%)	172 (50.9)
	Diagnosed/treated for HIV, n (%)	13 (3.8)
	History of incarceration, n (%)	311 (92.0)
	Receiving a transfusion of blood or blood components, n (%)	39 (11.5)
	Receiving a transplant, n (%)	0 (0.0)
	Hemodialysis, n (%)	3 (0.9)
	Persistently elevated ALT levels, n (%)	2 (0.6)
	Born between 1945 and 1965, n (%)	192 (56.8)
HCV Infection Status	HCV RNA positive, n (%)	259 (76.6)
	HCV RNA negative, n (%)	79 (23.4)

ALT=alanine aminotransferase, HIV=human immunodeficiency virus
[a]May report multiple races.
[b]May report multiple risk factors.

Specimens were tested with an approved qualitative HCV RNA test, an approved HCV antibody test and the Aptima HCV Quant Dx assay. The study included 338 HCV antibody reactive subjects. According to current AASLD guidelines, an FDA-approved NAAT with a detection level of 25 IU/mL or lower[20] should be used to confirm a positive HCV infection status following a reactive HCV antibody test result. The agreement between the patient infected status and Aptima HCV Quant Dx assay results was determined using a 25 IU/mL cutoff. Specimens < 25 IU/mL were considered "not detected" and specimens with results ≥ 25 IU/mL were considered "detected." See Table 32.

Aptima HCV Quant Dx assay results were compared to a patient infection status based on approved HCV antibody test results and approved qualitative RNA test results. The patient infection status was considered HCV positive if both the approved HCV antibody test and approved qualitative test results had reactive results. The patient infection status was considered HCV negative if the approved HCV antibody test had a nonreactive result.

Of the 338 subjects with HCV antibody reactive results, 256 were detected by the Aptima HCV Quant Dx assay (% positive agreement = 98.8%). Of the three subjects who had HCV RNA quantified below 25 IU/mL or not detected, two subjects had a detected but not quantified result (HCV RNA < 10 IU/mL) and one subject had a quantitative HCV RNA result of 12 IU/mL.

Table 32: Agreement Between Aptima HCV Quant Dx Assay Results With the Patient Infection Status Using a Cutoff of 25 IU/mL

Aptima HCV Quant Dx Assay Result	Patient Infected Status		Total
	HCV Positive	HCV Negative	
HCV RNA Quantitated at or above 25 IU/mL	256	0	256
HCV RNA Quantitated or Detected Below 25 IU/mL or Not Detected	3[a]	79	82
Total	259	79	338
% Positive Agreement (95% Score CI)	98.8 (96.7-99.6)	N/A	N/A
% Negative Agreement (95% Score CI)	N/A	100 (95.4-100)	N/A

CI = confidence interval, N/A = not applicable

[a] The Aptima HCV Quant Dx assay detected but did not quantitate HCV RNA (<10 IU/mL) for two subjects, and the third subject had HCV RNA quantitated at 12 IU/mL.

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

Cross-Reactivity in Subjects With Non-HCV Related Liver Disease

Serum and plasma samples (n=214) from individuals with other liver diseases (where active HCV infection was not indicated as the underlying cause) were obtained. Samples were tested with an approved anti-HCV test and an approved qualitative HCV RNA test to confirm the samples were HCV negative. Seventeen (17) HCV-positive samples (with anti-HCV positive results and/or reactive qualitative HCV RNA test results) and three ineligible samples were excluded. Aptima HCV Quant Dx assay testing was performed in-house with three reagent kit lots. Table 33 shows subject demographics and liver disease status for the 194 evaluable subjects.

Table 33: Subject Demographic Characteristics and Liver Disease Status

Characteristics		All
Total	N	194
Sex	n	193
	Female, n (%)	116 (60.1)
	Male, n (%)	77 (39.9)
Age (years)	n	194
	Mean	55.2
	Median	56.0
	SD	12.19
	Minimum	25
	Maximum	82
	21-49, n (%)	60 (30.9)
	50-70, n (%)	114 (58.8)
	>70, n (%)	20 (10.3)
Ethnicity	n	194
	Hispanic or Latino, n (%)	9 (4.6)
	Not Hispanic or Latino, n (%)	147 (75.8)
	Unknown, n (%)	38 (19.6)
	Refused, n (%)	0 (0.0)
Race[a]	n	194
	White, n (%)	122 (62.9)
	Black or African American, n (%)	20 (10.3)
	Asian, n (%)	17 (8.8)
	American Indian/Alaska Native, n (%)	0 (0.0)
	Native Hawaiian/Pacific Islander, n (%)	1 (0.5)
	Other, n (%)	34 (17.5)
	Unknown, n (%)	2 (1.0)
	Refused, n (%)	0 (0.0)
Liver Disease[b]	n	194
	Autoimmune hepatitis, n (%)	31 (16.0)
	Alcoholic liver disease, n (%)	51 (26.3)
	Chronic HBV, n (%)	34 (17.5)
	Primary biliary cirrhosis, n (%)	37 (19.1)
	NASH, n (%)	39 (20.1)
	Multiple Liver Diseases Reported, n (%)	2 (1.0)

HBV = hepatitis B virus, NASH= non-alcoholic steatohepatitis

[a] Subjects could report multiple races.

[b] Subjects with multiple liver diseases reported both autoimmune hepatitis and primary biliary cirrhosis.

Table 34 shows Aptima HCV Quant Dx assay specificity by liver disease and the distribution of Aptima HCV Quant Dx assay results across viral load categories. 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 in 1 subject with autoimmune hepatitis (< 10 IU/mL) and 4 subjects with alcoholic liver disease (< 10 IU/mL for 3 subjects and 16 IU/mL for 1 subject). One of the 5 subjects had HCV RNA detected by an approved HCV quantitative assay.

Table 34: Specificity of the Aptima HCV Quant Dx Assay and Distribution Results in HCV Antibody and RNA Negative Subjects With Non-HCV Related Liver Diseases

Liver Disease	Specificity (%) 95% CI[a]	Not Detected	<10 IU/mL	10 to <25 IU/mL	25 to 100,000,000 IU/mL	>100,000,000 IU/mL	Total
Autoimmune hepatitis	96.8 (83.8-99.4)	30 (96.8)	1 (3.2)	0 (0.0)	0 (0.0)	0 (0.0)	31
Alcoholic liver disease	92.2 (81.5-96.9)	47 (92.2)	3 (5.9)[b]	1 (2.0)	0 (0.0)	0 (0.0)	51
Chronic HBV	100 (89.8-100)	34 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	34
Primary biliary cirrhosis	100 (90.6-100)	37 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	37
NASH	100 (91.0-100)	39 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	39
Multiple Liver Diseases[c]	100 (34.2-100)	2 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2
Total	97.4 (94.1-98.9)	189 (97.4)	4 (2.1)	1 (0.5)	0 (0.0)	0 (0.0)	194

HBV = hepatitis B virus, NASH= non-alcoholic steatohepatitis
[a]Score CI.
[b]One subject had HCV RNA detected by an FDA-approved HCV quantitative assay.
[c]Subjects had both autoimmune hepatitis and primary biliary cirrhosis.

Bibliography

1. Averhoff FM, Glass N and Holtzman D. Global Burden of Hepatitis C: Considerations for Healthcare Providers in the United States. Clinical Infectious Diseases 2012; 55 (S1): S10-15.
2. Zalesak M, Francis K, Gedeon A, et al. Current and Future Disease Progression of the Chronic HCV Population in the United States. PLoS One. 2013 May 21;8(5):e63959
3. Engle RE, Bukh J, Alter HJ et al., Transfusion-associated hepatitis before the screening of blood for hepatitis risk factors.Transfusion. 2014 May 5.
4. Lee M-H, Yang, H-I, Yuan Y et al., Epidemiology and natural history of hepatitis C virus infection. World J Gasteroenterology 2014: 20 (28): 9270-9280.
5. Tan SL, editor. Hepatitis C Viruses: Genome and Molecular Biology. Norfolk (UK): Horizon Bioscience; 2006.
6. Smith DB, Bukh J, Kuiken C, et al, P. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource.Hepatology. 2014 Jan;59(1):318-27.
7. EASL Recommendations on treatment of Hepatitis C 2014: www.easl.eu/_clinical-practice-guideline.
8. AASLD and the Infectious Diseases Society of America (IDSA), in collaboration with the International Antiviral Society-USA (IAS-USA). HCV Guidance: Recommendations for Testing, Managing, and Treating Hepatitis C. 2014: www.hcvguidelines.org.
9. CDC. Testing for HCV infection: An update for clinicians and laboratories. MMWR 2013; 62 (18).
10. Sidharthan, S., Kohli, A., Sims, Z., Nelson, A., Osinusi, A., Masur, H., Kottilil, S. Utility of Hepatitis C Viral Load Monitoring on Directly Acting Antiviral Therapy. Clinical Infectious Diseases, 2015.
11. Kohli, A., Shaffer, A., Sherman, A., Kottilil, S. Treatment of hepatitis C: a systematic review. JAMA, 2014.
12. AASLD. Recommendations for Testing, Managing, and Treating Hepatitis C. 2015. Published on hcvguidelines.org, accessed Feb 11, 2016.
13. Simmons, B., Saleem, J., Heath, K., Cooke, G., Hill, A. Long-term treatment outcomes of patients infected with Hepatitis C virus: a systematic review and meta-analysis of the survival benefit of achieving a Sustained Virological Response. Clinical Infectious. 2015 Sep 1;61(5):730-40.
14. **Clinical and Laboratory Standards Institute.** 2005. Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline. CLSI Document MM13-A. Wayne, PA.
15. **29 CFR Part 1910.1030.** Occupational Exposure to Bloodborne Pathogens; current version.
16. **Centers for Disease Control and Prevention/National Institutes of Health.** Biosafety in Microbiological and Biomedical Laboratories (BMBL); current version.
17. **Clinical and Laboratory Standards Institute.** 2002. Clinical Laboratory Waste Management. CLSI Document GP5-A2. Villanova, PA.
18. **Clinical and Laboratory Standards Institute (CLSI)**. 2012. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition. CLSI Document EP17-A2. Clinical and Laboratory Standards Institute, Wayne, PA.
19. **Clinical and Laboratory Standards Institute (CLSI)**. 2003. Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. CLSI document EP06-A. Clinical and Laboratory Standards Institute, Wayne, PA.
20. AASLD/IDSA. Recommendations for testing, managing, and treating hepatitis C. AASLD and IDSA. http://www.hcvguidelines.org/fullreport. Updated 21 March 2014. Accessed 01 March 2016.
21. AASLD/IDSA HCV Guidance Panel. Hepatitis C Guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus. Hepatology. 2015;62(3):932-954.

Contact Information and Revision History



Hologic, Inc.
10210 Genetic Center Drive
San Diego, CA 92121 USA

IVD

CE 2797

EC REP
Hologic BV
Da Vincilaan 5
1930 Zaventem
Belgium

Australian Sponsor
Hologic (Australia & New Zealand) Pty Ltd.
Macquarie Park NSW 2113

For country-specific Technical Support and Customer Service email address and telephone number, visit www.hologic.com/support.

Serious incidents occurring in relation to the device in the European Union should be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Hologic, Aptima, Panther, Panther Fusion, and associated logos are trademarks or registered trademarks of Hologic, Inc. and/or its subsidiaries in the United States and/or other countries.

Armored RNA is a trademark of Asuragen, Inc.

All other trademarks that may appear in this package insert are the property of their respective owners.

This product may be covered by one or more U.S. patents identified at www.hologic.com/patents.

©2016–2025 Hologic, Inc. All rights reserved.

AW-28216-001 Rev. 001

2025-12

Revision History	Date	Description
AW-28216 Rev. 001	December 2025	• Initial release.