Aptima™ HCV Quant Dx Assay

For i*n vitro* diagnostic use.
For US export only.

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 ethylendiaminetetraacetic 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, despite detectable antibody (typically within 5-12 weeks), 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), HCV viral load measurements are taken prior to therapy to establish baseline viral load, during treatment for on-treatment responses and after therapy to evaluate SVR (or relapse). Almost all patients achieve on-treatment virological responses to DAAs defined as below the lower limit of quantitation (<LLoQ) for the assay, followed by greater than 90% SVR rates at 12 weeks after therapy with most regimens.[8,11] HCV RNA detection and quantitation will continue to play a pivotal role in HCV diagnosis and management of patients on antiviral therapy.

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 quantify HCV RNA before therapy 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.

* All references to the Panther system in this document are applicable to the Panther system and the Panther Fusion system. There are no changes to the indications for use, labeling, and principles of operation for the Aptima HCV Quant Dx Assay on the Panther system as a result of the add-on Panther Fusion Module.

Warnings and Precautions

A. To reduce the risk of invalid results, carefully read the entire package insert and the *Panther System Operator's Manual* prior to performing this assay.

Laboratory Related

B. CAUTION: The controls for this assay contain human plasma. The plasma is negative 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.[15,16,17]

C. 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.

D. Use only supplied or specified disposable laboratory ware.

E. 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.

F. 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.

G. Dispose of all materials that have come in contact with specimens and reagents according to local, state, and federal regulations.[15,16,17,18] Thoroughly clean and disinfect all work surfaces.

H. 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.

I. 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

J. Specimens may be infectious. Use Universal Precautions[15,16,17] when performing this assay. Proper handling and disposal methods should be established according to local regulations.[18] Only personnel adequately trained in the use of the Aptima HCV Quant Dx assay and trained in handling infectious materials should perform this procedure.

K. 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.

L. 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

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

N. 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.

O. Avoid microbial and nuclease contamination of reagents.

P. 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.

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

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

Note: *For information on any hazard and precautionary statements that may be associated with reagents, refer to the Safety Data Sheet Library at www.hologic.com/sds.*

HCV VL Kit Controls
Sodium Azide 0.2%
Human Plasma 95-100%

WARNING
EUH032 - Contact with acids liberates very toxic gas
H302 - Harmful if swallowed
H312 - Harmful in contact with skin
H402 - Harmful to aquatic life.
H412 - Harmful to aquatic life with long lasting effects.
P280 - Wear protective gloves/protective clothing/eye protection/face protection
P301 + P312 - IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell
P302 + P352 - IF ON SKIN: Wash with plenty of soap and water
P321 - Specific treatment (see supplemental first aid instructions on this label)
P362 + P364 - Take off contaminated clothing and wash it before reuse
P363 - Wash contaminated clothing before reuse
P501 - Dispose of contents/container in accordance with local/regional/national/international regulation

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 5 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.

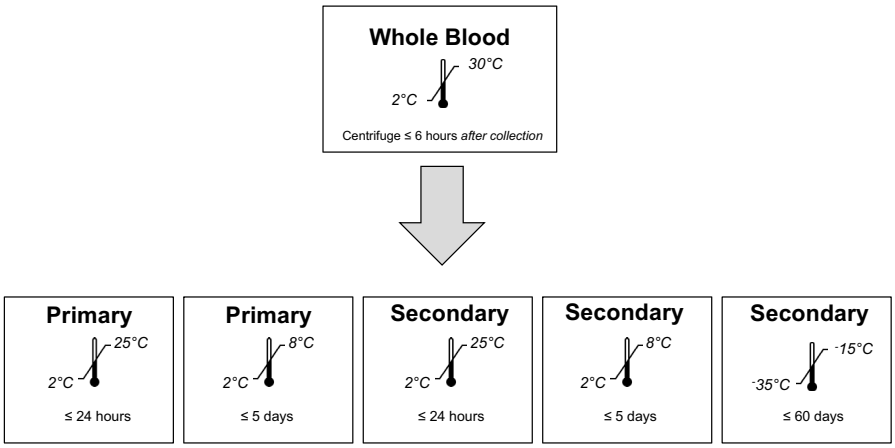

Figure 1. Storage Conditions for EDTA/ACD Tubes

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.

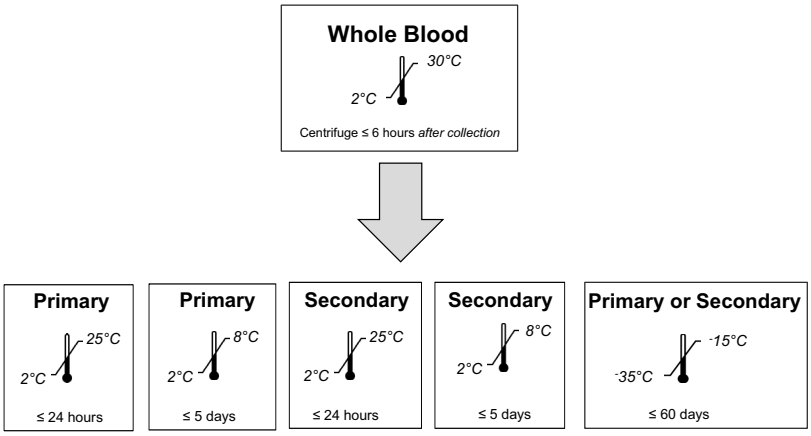

Figure 2. Storage Conditions for PPTs

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.

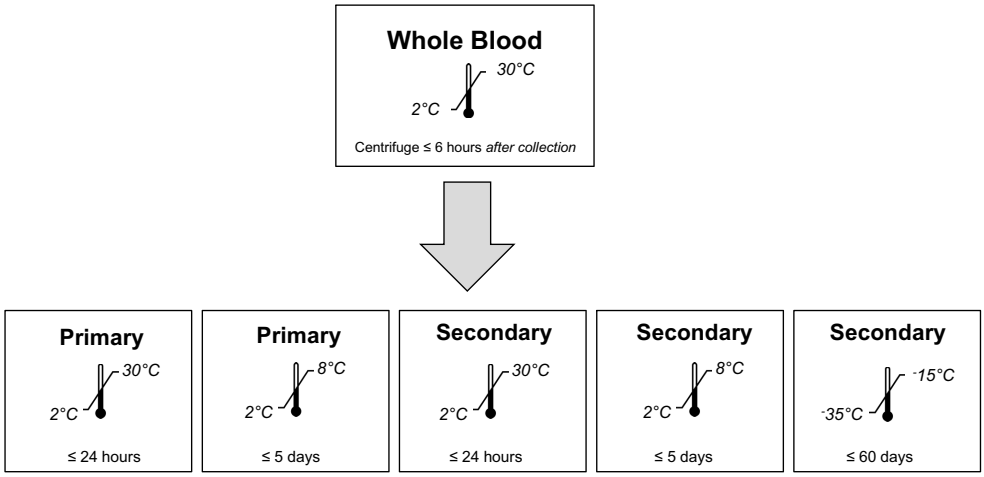

Figure 3. Storage Conditions for Serum Tubes

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.

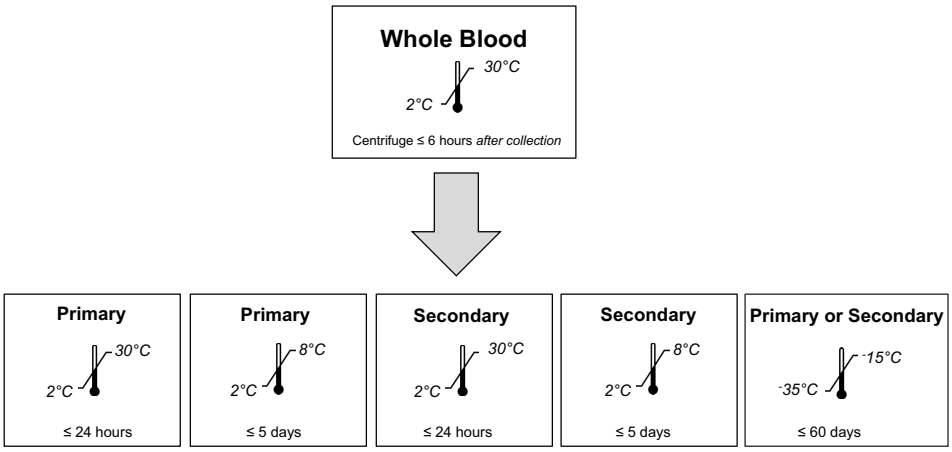

Figure 4. Storage Conditions SSTs

C. Long Term Frozen Storage

Plasma or serum samples may be stored at -65°C to -85°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.

⚠ *Dilution of plasma and serum specimens may only be used for quantitative results. Do not dilute plasma or serum samples for diagnostic results.*

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

Note: *For information on any hazard and precautionary statements that may be associated with reagents, refer to the Safety Data Sheet Library at www.hologic.com/sds.*

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	—
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 conductive, liquid sensing	10612513 (Tecan)
Bleach, 5% to 7% (0.7 M to 1.0 M) sodium hypochlorite solution	—
Disposable, powderless gloves	—
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)	503762
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, for example: *HCV from Quality Control for Molecular Diagnostics (QCMD) or SeraCare ACCURUN HCV Panels*	—
Cotton-tipped swabs	—
Tube rocker	PRD-03488

Panther System Test Procedure

Note: *See the Panther 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.*

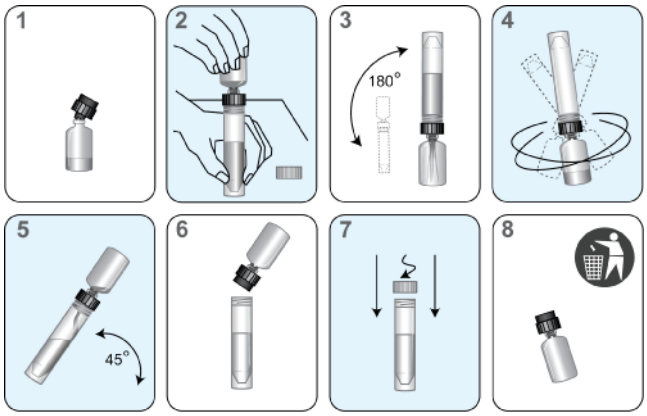

Figure 5. Reagent Reconstitution Process

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.
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 on page 7.
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* on page 7 to identify dead volume requirements for each primary and secondary tube type. If specimen dilution is necessary, see step E.6 below for additional information.
5. 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 can 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.

6. 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.

⚠ Dilution of specimens may only be used for quantitative results. Do not dilute specimens for diagnostic results.

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 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, 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 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.*

F. System Preparation

1. Set up the system according to the instructions in the *Panther 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 is 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. 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 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 concentration for the neat specimen by multiplying the diluted concentration by the dilution factor and diluted samples 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.

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.

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.[19]

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 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 shown in Table 2 are the results from the reagent lot with the highest predicted detection limit. The 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.

Table 2: Limit of Detection Using the WHO 2nd International Standard for HCV

Predicted Detection Limit	Concentration (IU/mL)	
	Plasma	Serum
10%	0.3	0.3
20%	0.4	0.5
30%	0.5	0.6
40%	0.7	0.8
50%	0.9	1.0
60%	1.1	1.2
70%	1.5	1.5
80%	2.0	2.0
90%	3.0	2.9
95%	4.3	3.9

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 a comparator* 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 50% and 95% predicted detection limits. The LoD values shown in Table 3 are the results from the reagent lot with the highest predicted detection limit.

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

Genotype	Predicted Detection Limit	Concentration (IU/mL)	
		Plasma	Serum
1	50%	0.8	1.3
	95%	3.8	5.1
2	50%	1.0	1.1
	95%	2.8	4.0
3	50%	1.1	1.0
	95%	4.3	3.4
4	50%	1.3	0.7
	95%	4.8	2.3
5	50%	0.8	0.9
	95%	2.1	3.2
6	50%	0.6	0.9
	95%	3.9	3.9

Linear Range

The linear range was established by testing panels of HCV Armored RNA diluted in HCV negative human plasma and serum according to CLSI EP06-A.[20] Panels ranged in concentration from 1.0 log IU/mL to 8.2 log 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 IU/mL, as shown in Figure 6.

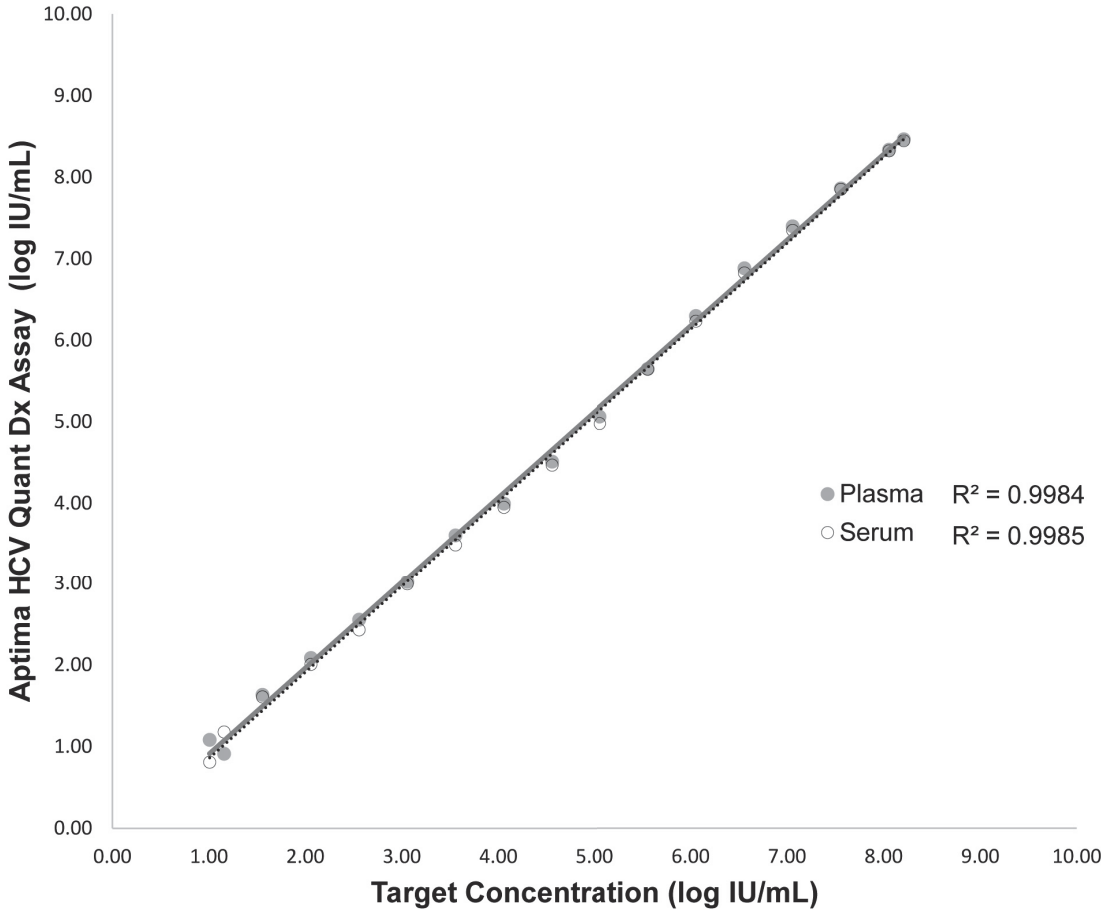

Figure 6. Linearity in Plasma and Serum

Linearity Across HCV Genotypes

The linear response for genotypes 1, 2, 3, 4, 5 and 6 was confirmed by testing panels of HCV transcript diluted in buffer at concentrations ranging from 1.36 log IU/mL to 7.36 log IU/mL. Testing was performed on three Panther systems using three reagent lots. Linearity was demonstrated across the range tested for all genotypes tested as shown in Figure 7.

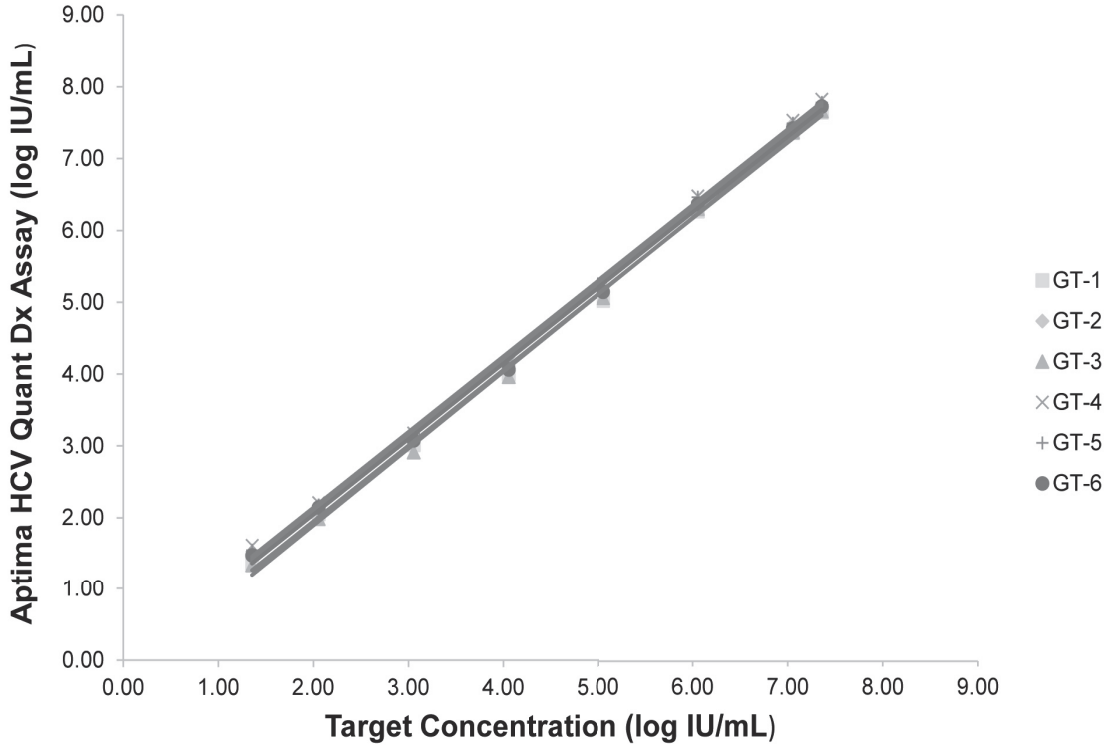

Figure 7. Linearity Across HCV Genotypes 1 to 6

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.[19] 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 IU/mL (i.e., at the LLoQ, the difference between two measurements of more than 1 log 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. A minimum of 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 IU/mL) for plasma and 9 IU/mL (0.93 log IU/mL) for serum.as summarized in Table 6. The LLoQ was established across genotypes (see next section "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	Target Concentration	Target Concentration	Aptima HCV Quant Dx	SD	|Bias|	Calculated TE	Calculated TAE
	(IU/mL)	(log IU/mL)	(log IU/mL)	(log IU/mL)	(log IU/mL)	(log IU/mL)	(log IU/mL)
1	3	0.48	0.53	0.30	0.05	0.85	0.65
	6	0.78	0.73	0.31	-0.04	0.88	0.67
	8	0.90	1.08	0.23	0.18	0.65	0.64
2	3	0.48	0.37	0.32	-0.11	0.92	0.75
	6	0.78	0.82	0.27	0.04	0.78	0.59
	8	0.90	0.96	0.26	0.06	0.74	0.58
3	3	0.48	0.46	0.25	-0.01	0.71	0.52
	6	0.78	0.76	0.34	-0.02	0.95	0.69
	8	0.90	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	Target Concentration	Target Concentration	Aptima HCV Quant Dx	SD	Bias	Calculated TE	Calculated TAE
	(IU/mL)	(log IU/mL)	(log IU/mL)	(log IU/mL)	(log IU/mL)	(log IU/mL)	(log IU/mL)
1	3	0.48	0.65	0.33	0.17	0.94	0.84
	6	0.78	0.93	0.32	0.15	0.90	0.79
	8	0.90	1.08	0.28	0.18	0.80	0.74
2	3	0.48	0.52	0.36	0.04	1.02	0.76
	6	0.78	0.89	0.32	0.11	0.90	0.75
	8	0.90	1.01	0.21	0.11	0.60	0.53
3	3	0.48	0.47	0.39	-0.01	1.11	0.79
	6	0.78	0.71	0.30	-0.06	0.86	0.67
	8	0.90	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	
	(log IU/mL)	(IU/mL)	(log IU/mL)	(IU/mL)
1	0.73	5	0.93	9
2	0.82	7	0.89	8
3	0.76	6	0.71	5

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. A minimum of 36 replicates of each panel member was 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 requirements are shown in Table 7 for plasma and Table 8 for serum. The LLoQ for genotypes 1 to 6 in plasma and serum are summarized in Table 9. This established the overall LLoQ for the assay as 10 IU/mL.

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Table 7: Determination of LLoQ Across Genotypes in Plasma

Genotype	Target Concentration	Target Concentration	Aptima HCV Quant Dx	SD	Bias	Calculated TE	Calculated TAE
	(IU/mL)	(log IU/mL)	(log IU/mL)	(log IU/mL)	(log IU/mL)	(log IU/mL)	(log IU/mL)
1	3	0.48	0.65	0.38	0.17	1.08	0.94
	6	0.78	0.88	0.35	0.11	1.00	0.82
	8	0.90	0.99	0.24	0.09	0.68	0.56
2	3	0.48	0.63	0.40	0.16	1.13	0.95
	4	0.60	0.76	0.29	0.15	0.81	0.73
	6	0.78	1.12	0.30	0.34	0.86	0.94
3	6	0.78	0.52	0.30	-0.26	0.85	0.86
	10	1.00	0.80	0.21	-0.20	0.59	0.62
	12	1.08	0.89	0.26	-0.19	0.74	0.71
4	8	0.90	0.61	0.39	-0.29	1.11	1.07
	10	1.00	0.82	0.31	-0.18	0.87	0.80
	12	1.08	1.01	0.29	-0.07	0.83	0.65
5	3	0.48	0.57	0.37	0.10	1.06	0.85
	6	0.78	0.87	0.31	0.09	0.89	0.72
	10	1.00	1.15	0.16	0.15	0.44	0.46
6	2	0.30	0.66	0.36	0.36	1.02	1.08
	3	0.48	0.79	0.28	0.31	0.80	0.87
	6	0.78	1.14	0.26	0.36	0.74	0.89

SD=standard deviation

Table 8: Determination of LLoQ Across Genotypes in Serum

Genotype	Target Concentration	Target Concentration	Aptima HCV Quant Dx	SD	Bias	Calculated TE	Calculated TAE
	(IU/mL)	(log IU/mL)	(log IU/mL)	(log IU/mL)	(log IU/mL)	(log IU/mL)	(log IU/mL)
1	6	0.78	0.75	0.36	-0.03	1.02	0.75
	8	0.90	0.88	0.32	-0.02	0.89	0.65
	10	1.00	1.04	0.29	0.04	0.81	0.61
2	3	0.48	0.48	0.35	0.00	0.99	0.70
	6	0.78	0.80	0.31	0.02	0.86	0.63
	10	1.00	1.04	0.25	0.04	0.72	0.54
3	6	0.78	0.45	0.25	-0.33	0.72	0.84
	10	1.00	0.67	0.22	-0.33	0.63	0.78
	12	1.08	0.92	0.19	-0.16	0.54	0.54
4	1	0.00	0.19	0.27	0.19	0.77	0.73
	2	0.30	0.65	0.32	0.35	0.91	0.99
	3	0.48	0.65	0.34	0.17	0.96	0.85
5	3	0.46	0.48	0.37	0.02	1.04	0.76
	6	0.76	0.72	0.29	-0.04	0.81	0.61
	9	0.94	1.04	0.27	0.10	0.77	0.65
6	3	0.48	0.58	0.37	0.11	1.04	0.84
	6	0.78	0.99	0.22	0.21	0.61	0.64
	10	1.00	1.25	0.22	0.25	0.63	0.70

SD=standard deviation

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

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

Precision

To assess precision, a 10 member panel was made by diluting HCV positive clinical specimens or spiking armored RNA into HCV negative plasma and serum. The panel was tested by three operators using three reagents lots on three Panther systems over 21 days.

Table 10 shows the precision of assay results (in log IU/mL) between instruments, between operators, between lots, between runs, within runs, and overall. Total variability was ≤13.31% across all panel members, primarily due to within-run variability (i.e., random error).

Table 10: Precision of the Aptima HCV Quant Dx Assay

Matrix	N	Mean Concentration (log IU/mL)	Inter-Instrument		Inter-Operator		Inter-Lot		Inter-Run		Intra-Run		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV(%)	SD	CV (%)	SD	CV(%)
Plasma	98[a]	1.23	0.00	0.00	0.00	0.00	0.04	3.35	0.06	4.54	0.12	9.84	0.14	11.34
Plasma	162	2.06	0.05	2.23	0.00	0.00	0.07	3.20	0.03	1.47	0.10	4.91	0.14	6.88
Plasma	162	3.02	0.03	0.89	0.01	0.24	0.04	1.47	0.01	0.33	0.09	3.08	0.11	3.77
Plasma	162	4.87	0.00	0.00	0.00	0.00	0.03	0.60	0.04	0.86	0.06	1.13	0.10	2.04
Plasma	162	7.16	0.02	0.21	0.01	0.20	0.04	0.52	0.00	0.00	0.05	0.75	0.09	1.27
Serum	132[a]	1.27	0.00	0.00	0.00	0.00	0.07	5.16	0.00	0.00	0.15	12.17	0.17	13.31
Serum	162	2.17	0.02	0.99	0.04	1.71	0.07	3.01	0.05	2.15	0.08	3.53	0.12	5.61
Serum	162	3.09	0.02	0.67	0.00	0.00	0.06	1.79	0.03	0.90	0.07	2.26	0.11	3.44
Serum	161	4.86	0.00	0.00	0.04	0.78	0.08	1.67	0.00	0.00	0.08	1.72	0.13	2.65
Serum	162	7.16	0.00	0.00	0.05	0.70	0.05	0.73	0.02	0.31	0.04	0.55	0.10	1.35

CV=coefficient of variation, 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 and CV are shown as 0.

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 3.3 log 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 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

Specificity

Specificity was determined using 198 fresh and 538 frozen HCV negative clinical specimens. A total of 370 plasma and 366 serum specimens were tested. 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: Specificity in 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 3.3 log 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	PFU/mL[b]
HIV-1	100,000	copies/mL
HIV-2	100,000	PFU/mL
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
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	LD/mL[e]
Yellow fever virus	100,000	cells/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]LD/mL = Lethal dose per mL
[f]CFU/mL = Colony forming units per mL
[g]IFU/mL = Inclusion forming units per 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 3.3 log IU/mL HCV RNA. 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

Repeatability of Clinical Specimens

Repeatability was evaluated by testing three replicates of naturally infected HCV positive plasma and serum clinical specimens. The average concentration and standard deviation for the plasma and serum samples tested are shown in Tables 16 and 17.

Table 16: Repeatability of Clinical Plasma Specimens

Plasma Specimen ID	Average Concentration (log IU/mL)	SD
1	1.68	0.05
2	1.69	0.17
3	1.73	0.06
4	1.73	0.06
5	1.84	0.05
6	2.30	0.10
7	2.71	0.09
8	2.80	0.06
9	3.22	0.04
10	3.80	0.05
11	4.12	0.08
12	4.21	0.11
13	4.90	0.14
14	6.94	0.11
15	7.05	0.04

Table 17: Repeatability of Clinical Serum Specimens

Serum Specimen ID	Average Concentration (log IU/mL)	SD
1	1.67	0.07
2	1.74	0.10
3	1.86	0.15
4	1.67	0.07
5	2.00	0.07
6	2.51	0.08
7	3.12	0.23
8	3.29	0.02
9	3.70	0.18
10	3.99[a]	0.13
11	4.35	0.05
12	5.48	0.12
13	5.74	0.07
14	5.80	0.13
15	7.15	0.10

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

Sample Dilution Using Specimen Diluent

To assess recovery of HCV RNA in samples diluted with Aptima Specimen Diluent, plasma and serum samples that spanned the linear range were diluted 1:3 with Aptima Specimen Diluent. In addition, high-titer naturally infected clinical specimens and Armored RNA spiked samples with concentrations above the ULoQ were diluted 1:100 with the Aptima Specimen Diluent. Each sample was tested neat and diluted (1:3 or 1:100) in triplicate. The differences between the average reported concentration (dilution factor applied to the diluted sample result) and the average neat concentration are shown in Table 18 for plasma and Table 19 for serum. The sample concentrations were accurately recovered in the diluted samples.

Table 18: Sample Dilution With Aptima Specimen Diluent – Plasma

Dilution	Average Neat Concentration (log IU/mL)	Average Reported Concentration[a] (log IU/mL)	Difference
1:3	1.68	1.89	-0.21
	1.69	1.69	0.00
	1.73	2.11	-0.38
	1.73	1.78	-0.05
	1.84	1.80	0.04
	2.30	2.23	0.07
	2.71	2.76	-0.05
	2.80	2.76	0.04
	3.22	3.28	-0.06
	3.80	3.71	0.09
	4.12	4.06	0.06
	4.21	4.07	0.14
	4.90	4.68	0.22
	6.94	6.72	0.22
	7.05	6.91	0.14
1:100	7.05	6.59	0.46
	>8.00 (8.45)[b]	8.38	0.07

[a]Reported concentration is the value calculated after the dilution factor has been applied.
[b]Spiked specimen.

Note: All results > 8.00 log IU/mL were estimated using additional analysis.

Table 19: Sample Dilution With Aptima Specimen Diluent – Serum

Dilution Factor	Average Neat Concentration (log IU/mL)	Average Reported Concentration[a] (log IU/mL)	Difference
1:3	1.67	1.85	-0.18
	1.74	1.72	0.02
	1.86	1.66	0.20
	1.67	1.85	-0.18
	2.00	1.91	0.09
	2.51	2.37	0.14
	3.12	3.01	0.11
	3.29	3.35	-0.06
	3.70	3.53	0.17
	3.99[c]	3.87	0.12
	4.35	4.30	0.05
	5.48	5.25	0.23
	5.74	5.62	0.12
	5.80	5.50	0.30
	7.15	6.86	0.29
1:100	7.15	6.65	0.50
	>8.00 (8.44)[b]	8.46	-0.02

[a]Reported concentration is the value calculated after the dilution factor has been applied.
[b]Spiked specimen.
[c]Result from two out of three replicates tested. One outlier replicate removed.

Note: All results > 8.00 log IU/mL were estimated using additional analysis.

Method Correlation

The performance of the Aptima HCV Quant Dx assay was assessed against a 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 8.

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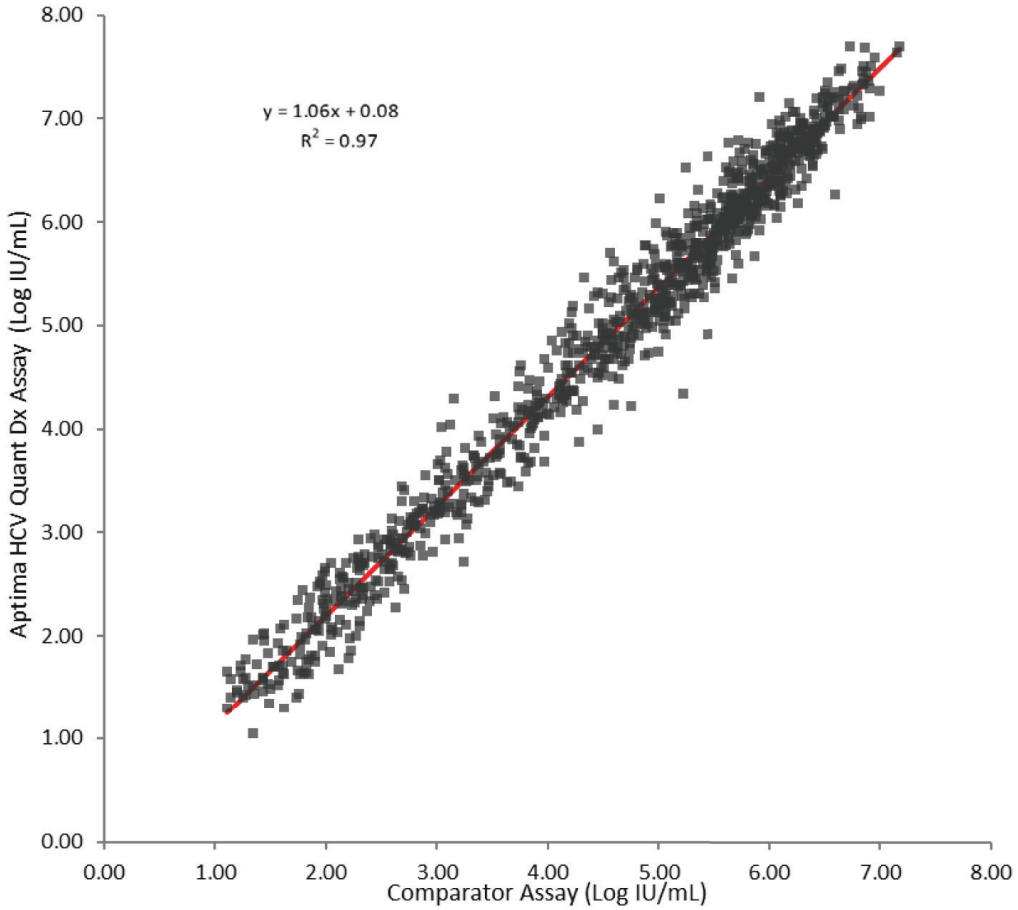

Figure 8. Correlation between the Aptima HCV Quant Dx Assay and Comparator Assay

Diagnostic Agreement

To assess diagnostic agreement, 227 plasma and serum specimens from HCV positive individuals were tested using the Aptima HCV Quant Dx assay and a comparator qualitative assay. Any result giving a quantifiable or detectable result was categorized as “Detected.” Any result of target not detected was categorized as “Target Not Detected.” Diagnostic agreement between assays was 100% as shown in Table 20.

Table 20: Diagnostic Agreement between the Aptima HCV Quant Dx Assay and Comparator Assay

		Aptima HCV Quant Dx Assay	
		Detected	**Target Not Detected**
Comparator Assay	**Detected**	99	0
	Target Not Detected	0	128

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 Armored RNA spiked plasma samples (7 log 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 21. The Aptima HCV Quant Dx assay detected the presence of HCV an average of 20 days earlier than antibody tests.

Table 21: 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
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 Abbot 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.

Clinical Performance

Samples were tested with a comparator qualitative HCV RNA test and the Aptima HCV Quant Dx assay. Agreement between comparator HCV RNA test and Aptima HCV Quant Dx assay qualitative results was calculated using the Aptima HCV Quant Dx assay's limit of detection as the cutoff to determine presence or absence of active infection (e.g., results of "not detected" indicated absence of active infection. See Table 22). Of the 338 HCV antibody positive subjects, 259 had HCV RNA detected by the comparator HCV RNA test, all of which were detected by the Aptima HCV Quant Dx assay (positive agreement=100%).

Table 22: Agreement Between Comparator HCV RNA Test and Aptima HCV Quant Dx Assay Results in Anti-HCV Positive Subjects

	HCV RNA Test Reactive		HCV RNA Test Nonreactive			
n	Aptima Detected	Aptima Not Detected	Aptima Detected	Aptima Not Detected	% Positive Agreement (95% CI)[1]	% Negative Agreement (95% CI)[4a]
338	259	0	3[b]	76	100 (98.5-100)	96.2 (89.4-98.7)

Aptima=Aptima HCV Quant Dx assay, CI = confidence interval

[a] Score CI.

[b] All 3 anti-HCV positive subjects had Aptima HCV Quant Dx assay results of <10 IU/mL.

Reproducibility Study

Reproducibility was evaluated on the Panther system at three external 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 23 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.

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 23: Reproducibility of Aptima HCV Quant Dx Assay HCV RNA Levels (in Log_{10} IU/mL) on the Panther System in Positive Panel Members

GT	Observed Mean		Between Sites	Between Operators	Between Lots	Between Days	Between Runs	Within Runs	Total	Below LLOQ n (%)	Above ULOQ n (%)
	IU/mL	Log_{10} IU/mL	SD (%CV)	SD (%CV)	SD (%CV)	SD (%CV)	SD (%CV)	SD (%CV)	SD (%CV)		
1	10.6	1.0	0.070 (16.22)	0.000 (0.00)	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)
	19.5	1.3 [a]	0.034 (7.77)	0.009 (2.10)	0.035 (7.97)	0.000 (0.00)	0.064 (14.91)	0.169 (40.31)	0.187 (45.12)	9 (8.4)	0 (0.0)
	43.6	1.6	0.000 (0.00)	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)
	602.7	2.8	0.042 (9.58)	0.022 (5.05)	0.035 (8.02)	0.000 (0.00)	0.023 (5.30)	0.075 (17.40)	0.098 (22.81)	0 (0.0)	0 (0.0)
	22710.9	4.3	0.075 (17.42)	0.043 (9.97)	0.045 (10.49)	0.006 (1.46)	0.058 (13.46)	0.071 (16.57)	0.135 (31.74)	0 (0.0)	0 (0.0)
	4195539.0	6.6	0.007 (1.65)	0.000 (0.00)	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)
	58549271.2	7.8	0.010 (2.26)	0.000 (0.00)	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	11.3	1.0	0.062 (14.29)	0.000 (0.00)	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)
	13.9	1.1	0.000 (0.00)	0.128 (30.18)	0.125 (29.36)	0.000 (0.00)	0.068 (15.83)	0.211 (51.69)	0.285 (73.45)	46 (42.6)	0 (0.0)
	62.6	1.8	0.000 (0.00)	0.101 (23.48)	0.044 (10.18)	0.000 (0.00)	0.069 (16.00)	0.124 (29.25)	0.180 (43.24)	0 (0.0)	0 (0.0)
	351.0	2.5	0.022 (5.18)	0.000 (0.00)	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)
	14519.2	4.1	0.000 (0.00)	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)
	5810012.3	6.8	0.000 (0.00)	0.034 (7.82)	0.017 (4.00)	0.024 (5.44)	0.015 (3.53)	0.104 (24.25)	0.114 (26.73)	0 (0.0)	0 (0.0)
	77499195.7	7.9	0.000 (0.00)	0.022 (5.09)	0.018 (4.24)	0.018 (4.14)	0.000 (0.00)	0.077 (17.77)	0.084 (19.46)	0 (0.0)	10 (9.3)

Table 23:Reproducibility of Aptima HCV Quant Dx Assay HCV RNA Levels (in Log_{10} IU/mL) on the Panther System in Positive Panel Members (cont'd)

GT	Observed Mean		Between Sites	Between Operators	Between Lots	Between Days	Between Runs	Within Runs	Total	Below LLOQ n (%)	Above ULOQ n (%)
	IU/mL	Log_{10} IU/mL	SD (%CV)	SD (%CV)	SD (%CV)	SD (%CV)	SD (%CV)	SD (%CV)	SD (%CV)		
3	8.6	0.9 [a]	0.049 (11.32)	0.000 (0.00)	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)
	14.3	1.1	0.157 (37.31)	0.076 (17.53)	0.000 (0.00)	0.050 (11.46)	0.000 (0.00)	0.178 (42.88)	0.254 (63.90)	36 (33.3)	0 (0.0)
	49.9	1.7 [a]	0.119 (27.96)	0.032 (7.35)	0.065 (15.08)	0.034 (7.89)	0.034 (7.81)	0.109 (25.42)	0.183 (44.15)	0 (0.0)	0 (0.0)
	218.3	2.2	0.324 (86.23)	0.132 (31.00)	0.043 (10.01)	0.045 (10.31)	0.063 (14.64)	0.082 (19.09)	0.370 (103.21)	0 (0.0)	0 (0.0)
	8541.1	3.9 [a]	0.248 (61.97)	0.149 (35.41)	0.056 (12.90)	0.021 (4.82)	0.048 (10.99)	0.078 (18.19)	0.309 (81.20)	0 (0.0)	0 (0.0)
	4128761.4	6.6 [a]	0.044 (10.17)	0.000 (0.00)	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)
	63813728.0	7.8	0.012 (2.84)	0.000 (0.00)	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] N=107 for these panel members. One replicate had an invalid result.

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