Aptima™ HIV-1 Quant Assay

For *in vitro* diagnostic use

For US export only

General Information

Intended Use

The Aptima HIV-1 Quant assay is an *in vitro* nucleic acid amplification test (NAAT) for the quantitation of human immunodeficiency virus type 1 (HIV-1) RNA in human plasma from HIV-1 infected individuals on the fully automated Panther™ system. The Aptima HIV-1 Quant assay quantitates HIV-1 RNA groups M, N, and O over the range of 30 to 10,000,000 copies/mL. One international unit is equivalent to 0.35 copies of HIV-1 RNA for the 3rd HIV-1 WHO International Standard (subtype B, NIBSC code: 10/152).

The Aptima HIV-1 Quant assay is intended for use in conjunction with clinical presentation and other laboratory markers for disease prognosis and for use as an aid in monitoring the effects of antiretroviral treatment, as measured by changes in plasma HIV-1 RNA levels.

This assay is not intended to be used as a donor screening test for HIV-1 or as a diagnostic test to confirm the presence of HIV-1 infection.

Summary and Explanation of the Test

Epidemiological studies identified human immunodeficiency virus type 1 (HIV-1) as the etiological agent of acquired immunodeficiency syndrome (AIDS) (1-7). HIV can be transmitted by sexual contact, exposure to infected blood or blood products, or through mother-to-child transmission (8). Within 3 to 6 weeks of exposure to HIV, infected individuals generally develop a brief, acute syndrome characterized by flu-like symptoms, and is associated with high levels of viremia in the peripheral blood (9-12). In most infected individuals, this early phase is followed by an HIV-specific immune response and a decline of plasma viremia, usually within 4 to 6 weeks of the onset of symptoms (13-14). After seroconversion, infected individuals typically enter a clinically stable, asymptomatic phase that can last for years (15-17). The asymptomatic period is characterized by persistent, low-level plasma viremia (18) and a gradual depletion of CD4+ T lymphocytes. This depletion leads to severe immunodeficiency, multiple opportunistic infections, malignancies, and death (19). Although levels of virus in the peripheral blood are relatively low during the asymptomatic phase of the infection, virus replication and clearance appear to be dynamic processes in which high rates of virus production and infection of CD4+ cells are balanced by equally high rates of virus clearance, death of infected cells, and replenishment of CD4+ cells, resulting in relatively stable levels of both plasma viremia and CD4+ cells (20-22).

Quantitative measurements of HIV in the peripheral blood have shown that higher virus levels may be correlated with increased risk of clinical progression of HIV-associated disease, and shown that reductions in plasma virus levels may be associated with decreased risk of clinical progression (23-25). Virus levels in the peripheral blood can be quantitated by measurement of the HIV p24 antigen in serum, by quantitative culture of HIV from plasma, or by direct measurement of viral RNA in plasma using nucleic acid amplification or signal amplification technologies (26-30).

Molecular techniques such as transcription mediated amplification (TMA) have been widely used to amplify nucleic acids (31). TMA uses specific target capture and isothermal amplification to detect nucleic acids in multiple infectious diseases, including CT, NG, HPV, Trich, and HIV/HCV/HBV for blood donor testing (32).

The Aptima HIV-1 Quant assay, through TMA, utilizes multiple, long primers that target several regions of the HIV-1 genome in order to compensate for the high mutation rate of HIV-1.

Principles of the Procedure

The Aptima HIV-1 Quant assay involves three main steps, which all take place in a single tube on the Panther system: target capture, target amplification by transcription-mediated amplification (TMA), and detection of the amplification products (amplicon) by the fluorescent labeled probes (torches).

During target capture, viral nucleic acids are 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 the HIV-1 genome, 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, MMLV (Moloney murine leukemia virus) 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 HIV-1 Quant assay utilizes the TMA method to amplify two regions of HIV-1 RNA (pol and LTR). Amplification of these specific regions is achieved using specific primers which are designed to amplify HIV-1 groups M, N, and O. The primer design and the dual target approach ensure accurate detection and quantitation of HIV-1.

Detection is achieved using single-stranded nucleic acid torches that are present during the amplification of the target and that hybridize specifically to the amplicons 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 HIV-1 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 HIV-1 and IC signals for each reaction and comparing them to calibration information.

Warnings and Precautions

A. For *in vitro* diagnostic use.

B. 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

C. 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 (33-35).

D. Only personnel adequately trained in the use of the Aptima HIV-1 Quant assay and in handling potentially infectious materials should perform this procedure. If a spill occurs, immediately disinfect following appropriate site procedures.

E. Use only supplied or specified disposable laboratory ware.

F. 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.

G. 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.

H. Dispose of all materials that have come in contact with specimens and reagents according to local, state, and federal regulations (33-36). Thoroughly clean and disinfect all work surfaces.

I. 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.

J. 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

K. Specimens may be infectious. Use Universal Precautions (33-35) when performing this assay. Proper handling and disposal methods should be established according to local regulations (36). Only personnel adequately trained in the use of the Aptima HIV-1 Quant assay and trained in handling infectious materials should perform this procedure.

L. 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.

M. 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

N. Quantitative results of the Aptima HIV-1 Quant assay have been evaluated with plasma.

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

P. 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.

Q. Avoid microbial and nuclease contamination of reagents.

R. 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.

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

Controls Kit

Sodium Azide 0.2%

Human Plasma 95-100%

WARNING

EUH032 - Contact with acids liberates very toxic gas
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
P363 - Wash contaminated clothing before reuse
P501 - Dispose of contents/container in accordance with local/regional/national/international regulation

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.*

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
qHIV-1 Amplification Reagent	2°C to 8°C		
qHIV-1 Amplification Reconstitution Solution	2°C to 8°C	2°C to 8°C	30 days[a]
qHIV-1 Enzyme Reagent	2°C to 8°C		
qHIV-1 Enzyme Reconstitution Solution	2°C to 8°C	2°C to 8°C	30 days[a]
qHIV-1 Promoter Reagent	2°C to 8°C		
qHIV-1 Promoter Reconstitution Solution	2°C to 8°C	2°C to 8°C	30 days[a]
qHIV-1 Target Capture Reagent	2°C to 8°C	2°C to 8°C	30 days[a]
qHIV-1 NC CONTROL – (Negative Control)	-15°C to -35°C	15°C to 30°C	Single use vial Use within 20 hours
qHIV-1 LPC CONTROL + (Low Positive Control)	-15°C to -35°C	15°C to 30°C	Single use vial Use within 20 hours
qHIV-1 HPC CONTROL + (High Positive Control)	-15°C to -35°C	15°C to 30°C	Single use vial Use within 20 hours
qHIV-1 PCAL (Positive Calibrator)	-15°C to -35°C	15°C to 30°C	Single use vial Use within 20 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.*

The following glass or plastic primary collection tubes may be used for collecting whole blood specimens:

- Tubes containing EDTA or Acid Citrate Dextrose (ACD) anticoagulants or
- Plasma Preparation Tubes (PPTs).

A. Specimen Collection

Whole blood can be stored at 2°C to 30°C and must be centrifuged within 24 hours of specimen collection. Separate the plasma from the pelleted red blood cells following the manufacturer's instructions for the tube used. Plasma can be tested on the Panther system in the primary tube or transferred to the secondary Aptima Specimen Aliquot Tube (SAT) and can be tested on the Panther system. The minimum volume of plasma for primary collection tubes is 1200 µL of plasma and for SATs, the minimum volume is 700 µL to obtain the 500 µL reaction volume.

If not tested immediately, plasma can be stored in accordance with the specifications below. If transferred to the SAT, plasma may be frozen at -20°C or -70°C. Do not exceed three freeze–thaw cycles to avoid affecting the result. Do not freeze specimens in EDTA or ACD primary collection tubes.

B. Specimen Storage Conditions

1. EDTA and ACD Plasma Specimens

For up to 24 hours after specimen collection, primary collection tubes containing centrifuged plasma may be stored at 2°C to 30°C (Figure 1, upper box). After 24 hours, plasma may be stored for a longer period of time under one of the following conditions (Figure 1, lower boxes):

- In the primary collection tube at 2°C to 8°C for up to 3 days,
- In the SAT at 2°C to 8°C for up to 5 days, or
- In the SAT at -20°C or -70°C for up to 90 days.

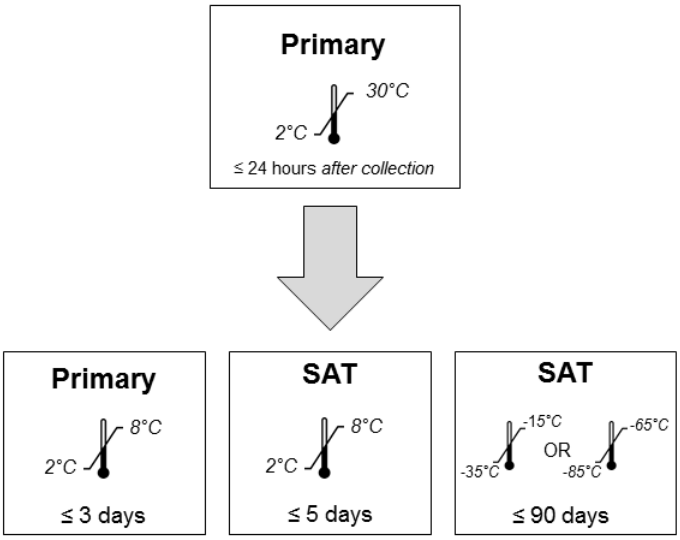

Figure 1. Storage Conditions for EDTA/ACD Tubes

2. PPT Specimens

   For up to 24 hours after specimen collection, PPTs containing centrifuged plasma may be stored at 2°C to 30°C (Figure 2, upper box). After 24 hours, plasma may be stored for a longer period of time under one of the following conditions (Figure 2, lower boxes):

   - In the PPT at 2°C to 8°C for up to 3 days,
   - In the SAT at 2°C to 8°C for up to 5 days, or
   - In the PPT or SAT at -20°C or -70°C for up to 90 days.

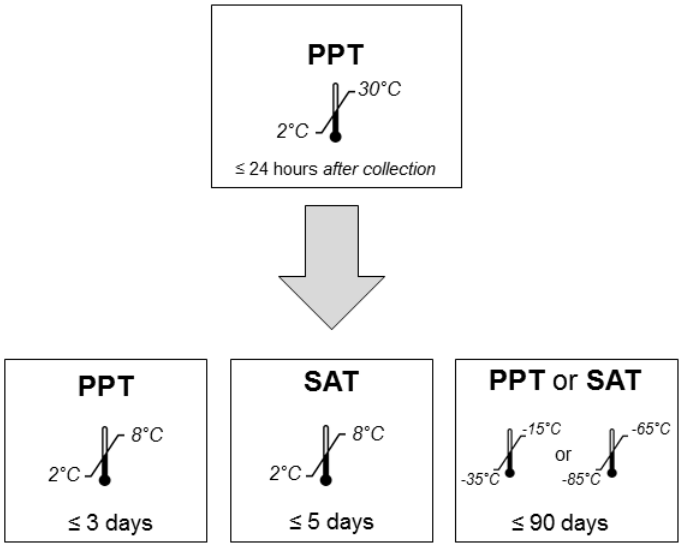

Figure 2. Storage Conditions for PPTs

C. Dilution of Plasma Specimens

A plasma specimen may be diluted in the SAT for testing on the Panther system. See *Panther System Test Procedure*, step E.6 below for more information.

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 a total of 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 HIV-1 Quant 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 HIV-1 Quant Assay Kit, 100 tests, Cat. No. PRD-03565 (1 assay box, 1 calibrator kit, and 1 controls kit)

Additional calibrators and controls may be ordered separately. See respective catalog numbers below.

Aptima HIV-1 Quant Assay Box
(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity
A	**qHIV-1 Amplification Reagent** *Non-infectious nucleic acids dried in buffered solution.*	1 vial
E	**qHIV-1 Enzyme Reagent** *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution.*	1 vial
PRO	**qHIV-1 Promoter Reagent** *Non-infectious nucleic acids dried in buffered solution.*	1 vial
AR	**qHIV-1 Amplification Reconstitution Solution** *Aqueous solution containing glycerol and preservatives.*	1 x 7.2 mL
ER	**qHIV-1 Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 x 5.8 mL
PROR	**qHIV-1 Promoter Reconstitution Solution** *Aqueous solution containing glycerol and preservatives.*	1 x 4.5 mL
TCR	**qHIV-1 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 HIV-1 Quant Calibrator Kit (Cat. No. PRD-03566)
(store at -15°C to -35°C upon receipt)

Symbol	Component	Quantity
PCAL	**qHIV-1 Positive Calibrator** *Transcript in buffered solution.*	5 x 2.5 mL
	Calibrator Barcode Label	—

Aptima HIV-1 Quant Controls Kit (Cat. No. PRD-03567)
(store at -15°C to -35°C upon receipt)

Symbol	Component	Quantity
NC	**qHIV-1 Negative Control** *HIV-1 negative defibrinated human plasma containing gentamicin and 0.2% sodium azide as preservatives.*	5 x 1.5 mL
LPC	**qHIV-1 Low Positive Control** *Non-infectious HIV-1 Armored RNA in defibrinated human plasma containing gentamicin and 0.2% sodium azide as preservatives.*	5 x 1.5 mL
HPC	**qHIV-1 High Positive Control** *Non-infectious HIV-1 Armored RNA in defibrinated human plasma containing gentamicin and 0.2% sodium azide as preservatives.*	5 x 1.5 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	—

Material	Cat. No.
Primary collection tubes (EDTA, ACD, PPT) of the following dimensions may be used: *13 mm x 100 mm* *13 mm x 75 mm* *16 mm x 100 mm*	—
Centrifuge	—
Vortex mixer	—

Optional Materials

Material	Cat. No.
Aptima Specimen Aliquot Tubes (SATs) (100 pack)	503762
Transport Tube Cap (100 pack) *cap for SAT*	504415
Aptima Specimen Diluent	PRD-03503
Aptima Specimen Diluent Kit *contains specimen diluent, 100 SATs, and 100 caps*	PRD-03654
Transfer pipets	—
Commercially available panels, for example: *HIV-1 from Quality Control for Molecular Diagnostics (QCMD) or College of American Pathologists (CAP) HIV viral load survey panel or SeraCare ACCURUN HIV Panels*	—
Cotton-tipped swabs	—
Tube rocker	—

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 on which the reagents and samples will be prepared 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 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 3, 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 3, Step 2).
      v. Slowly invert the assembled bottles (vial attached to solution bottle) to allow the solution to drain into the glass vial (Figure 3, Step 3).

vi. Pick up the assembled bottles, and swirl the assembled bottles for at least 10 seconds (Figure 3, 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 3, Step 5).

d. Carefully remove the reconstitution collar and glass vial (Figure 3, Step 6).

e. Recap the bottle. Record operator initials and reconstitution date on the label (Figure 3, Step 7).

f. Discard the reconstitution collar and glass vial (Figure 3, Step 8).

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

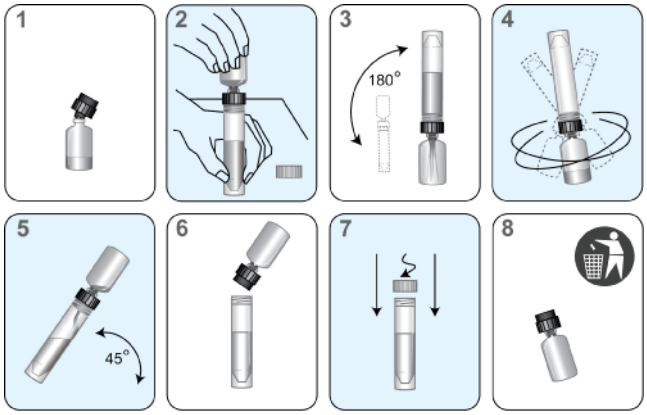

Figure 3. Reagent Reconstitution Process

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 frozen specimens are thoroughly thawed. Vortex the thawed specimens for 3 to 5 seconds to mix thoroughly.
2. Allow the specimens to reach 15°C to 30°C prior to processing. See *Samples Onboard the Panther System* for additional onboard information.

3. Ensure that each primary collection tube contains at least 1200 µL of plasma. Ensure that each Aptima Specimen Aliquot Tube (SAT) contains at least 700 µL of specimen. If specimen dilution is necessary, see step E.6 below for additional information.
4. Vortex specimens in SATs for 3 to 5 seconds to mix thoroughly.
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 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. Diluting a plasma specimen in the SAT

   A plasma specimen may be diluted in the SAT for testing on the Panther system.

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

   a. Dilution of low-volume specimens

      The volume of plasma specimens may be increased to the minimum volume required (700 µL) using Aptima Specimen Diluent. Specimens with at least 240 µL of plasma 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 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.

      ii. Add 2970 µL of 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 a 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 the Sample Bay.*

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

Procedural Notes

A. Calibrator and Controls

1. The qHIV-1 positive calibrator, qHIV-1 low positive control, qHIV-1 high positive control, and qHIV-1 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 HIV-1 Quant assay reagent kit, specimens can be tested with the associated, reconstituted kit for up to 24 hours **unless:**
   a. The calibrator 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 they 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, of the low positive control, and of 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 Target: ~ 2.9 Log_{10} c/mL, HPC Target: ~ 5.0 Log_{10} c/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 System Operator's Manual*.

Interpretation of Results

The Panther system automatically determines the concentration of HIV-1 RNA for specimens and controls by comparing the results to a calibration curve. HIV-1 RNA concentrations are reported in copies/mL and $\log_{10}$ copies/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 HIV-1 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 "<30 detected" may be generated by diluting a specimen with a concentration above, but close to the LoD (limit of detection) or LLoQ (lower limit of quantitation). It is recommended to collect and test another neat specimen if a quantitative result is not obtained.*

Table 1: Result Interpretation

Reported Aptima HIV-1 Quant Assay Result		HIV-1 RNA Concentration Interpretation
Copies /mL[a]	**$\log_{10}$ Value[b]**	
Not Detected	Not Detected	HIV-1 RNA not detected.
<30 detected	<1.47	HIV-1 RNA is detected but at a level below the LLoQ.
30 to 10,000,000	1.47 to 7.00	HIV-1 RNA concentration is within the linear range of 30 to 10,000,000 copies/mL.
>10,000,000	>7.00	HIV-1 RNA concentration is above the upper limit of quantitation (ULoQ).
Invalid[c]	Invalid[c]	There was an error in the generation of the result. Specimen should be retested.

[a] The conversion factor for copies to International Unit (IU) for the 3rd International Standard for HIV-1 RNA (10/152) is 0.35 copies/IU.
[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.

C. Though rare, mutations within the highly conserved regions of the viral genome covered by the primers and/or probes in the Aptima HIV-1 Quant assay may result in underquantification of or failure to detect the virus.

Nonclinical Performance

Limit of Detection (LoD) Using the 3rd HIV-1 WHO International Standard

The limit of detection (LoD) is defined as the concentration of HIV-1 RNA that is detected at 95% or greater probability according to CLSI EP17-A2 (37). The LoD was determined by testing panels that consisted of dilutions of the 3rd HIV-1 WHO International Standard (subtype B, NIBSC code: 10/152) in HIV-1 negative plasma. Thirty replicates of each dilution were run on three Panther systems using three reagent lots for a total of 90 replicates for each dilution. Per CLSI EP17-A2, the LoDs were defined using the results from the reagent lot with the highest concentration for the predicted detection limit LoD and are shown in Table 2. Through Probit analysis, the 95% predicted detection limit for LoD for the Aptima HIV-1 Quant assay is 12 copies/mL (35 IU/mL; 0.35 copies = 1 IU).

Table 2: Limit of Detection of the Aptima HIV-1 Quant Assay Using the 3rd HIV-1 WHO International Standard

Predicted Detection Limit	Concentration (copies/mL)
10%	1.2
20%	1.6
30%	2.0
40%	2.5
50%	3.1
60%	3.8
70%	4.8
80%	6.2
90%	9.0
95%	12.1

Limit of Detection Across HIV-1 Subtypes and Groups

For HIV-1 group M (subtypes A, C, D, F, G, CRF01_AE, CRF02_AG) and groups N and O, seven panels were created by spiking either cultured HIV-1 virus or positive clinical specimens into HIV-1 negative human plasma (0 to 40 copies/mL). Each panel member was tested in 30 replicates with two reagent lots for a total of 60 replicates per panel member. Assignment of the concentration for clinical specimens or cultured virus stocks was determined using a comparator assay. Probit analysis was performed to generate 50% and 95% predicted detection limits. Per CLSI EP17-A2 (37), the LoDs were defined using the results from the reagent lot with the highest concentration for the predicted detection limit are defined as LoD and are shown in Table 3.

Table 3: Limit of Detection Across HIV-1 Subtypes and Groups

Subtype/Group	Predicted Detection Limit	Concentration (copies/mL)
A	50%	3.0
	95%	12.3
CRF01_AE	50%	1.8
	95%	6.2
CRF02_AG	50%	3.4
	95%	15.4
C	50%	2.0
	95%	10.7
D	50%	3.7
	95%	14.0
F	50%	2.1
	95%	8.3
G	50%	3.1
	95%	17.5
N	50%	1.2
	95%	7.8
O	50%	1.8
	95%	8.0

Linear Range

The linear range of the Aptima HIV-1 Quant assay was established by testing panels that consisted of cultured HIV-1 subtype B virus diluted in HIV-1 negative human plasma according to CLSI EP06-A (38). Panels ranged in concentration from 1.30 to 7.30 $\log_{10}$ copies/mL. Testing was performed on seven Panther systems with two reagent lots. As shown in Figure 4, the Aptima HIV-1 Quant assay demonstrated linearity across the range tested.

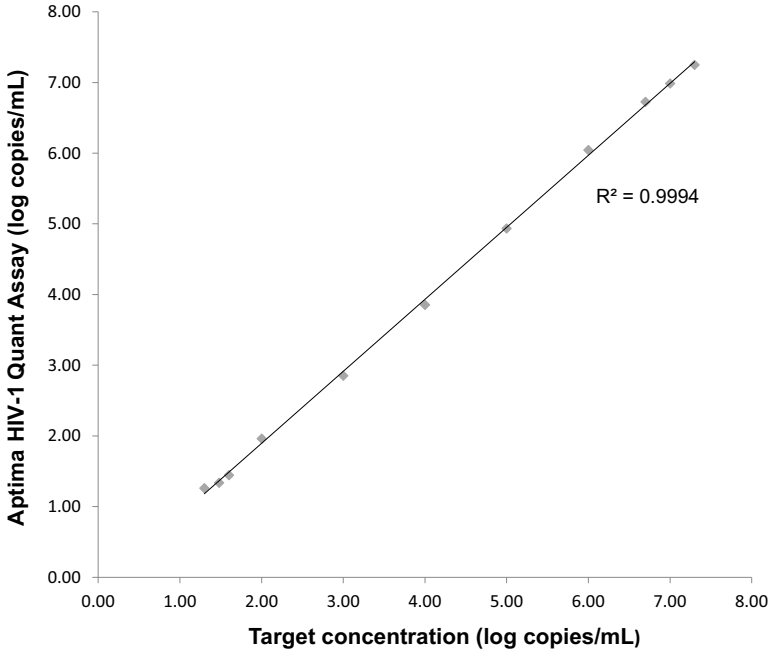

Figure 4. Linearity of the Aptima HIV-1 Quant Assay

Linearity across HIV-1 Subtypes and Groups

The linear response of the Aptima HIV-1 Quant assay across group M (subtypes A, B, C, D, F, G, H, CRF01_AE) and groups N and O was confirmed by testing panels that consisted of HIV-1 transcript diluted in buffer at concentrations ranging from 2.00 to 6.70 $\log_{10}$ copies/mL. Testing was performed on four Panther systems and across six runs. Linearity was demonstrated across the range tested (Figure 5).

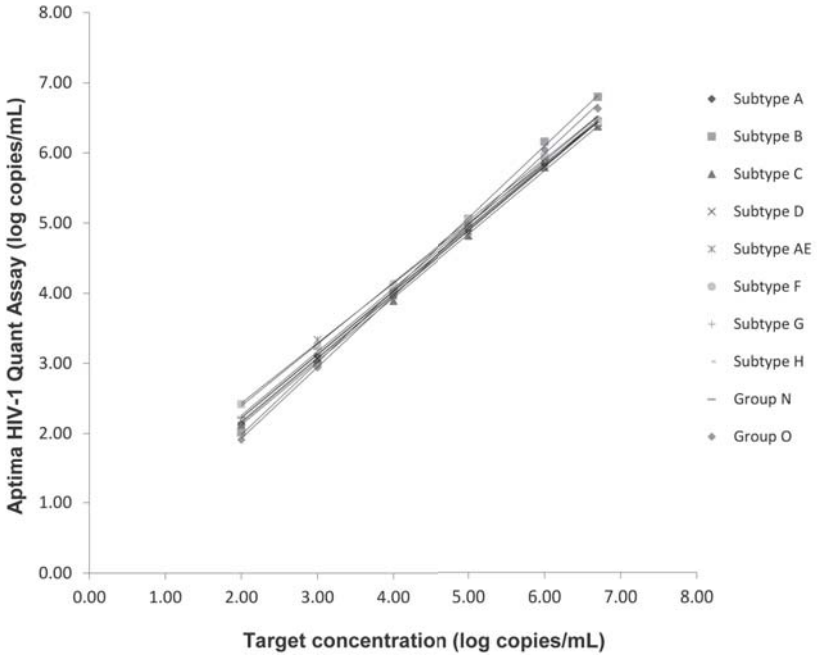

Figure 5. Linearity across Group M (Subtypes A, B, C, D, F, G, H, CRF01_AE) and Groups N and O

Determination of the Lower Limit of Quantitation Using the 3rd HIV-1 WHO International Standard

The lower limit of quantitation (LLoQ) is defined as the lowest concentration at which HIV-1 RNA is reliably quantitated within a total error (TE), according to CLSI EP17-A2 (37). TE was calculated using the Westgard model (TE = |bias| + 2SD). To ensure accuracy and precision of measurements, the TE of the Aptima HIV-1 Quant assay was set at 1 log copies/mL (i.e., at LLoQ, the difference between two measurements of more than 1 log copies/mL is statistically significant).

LLoQ was determined by testing panels that consisted of dilutions of the 3rd HIV-1 WHO International Standard (subtype B, NIBSC code: 10/152) in HIV-1 negative human plasma. Per CLSI EP17-A2, panels were tested with three reagent lots in replicates of 30 for each lot from 23 runs. The results are shown in Table 4. The highest LLoQ across the three lots tested on the Aptima HIV-1 Quant assay using the 3rd HIV-1 WHO International Standard is 15 copies/mL (1.17 $\log_{10}$ copies/mL) (Table 5).

Table 4: Determination of LLoQ of the Aptima HIV-1 Quant Assay Using the 3rd HIV-1 WHO International Standard

Reagent Lot	Target Concentration ($\log_{10}$ copies/mL)	Aptima HIV-1 Quant ($\log_{10}$ copies/mL)	SD ($\log_{10}$ copies/mL)	|Bias| ($\log_{10}$ copies/mL)	Calculated TE ($\log_{10}$ copies/mL)
1	1.15	1.05	0.37	0.10	0.84
	1.24	0.94	0.35	0.30	1.00
	1.42	1.37	0.33	0.05	0.71
	1.54	1.47	0.22	0.07	0.50
	1.94	1.98	0.13	0.04	0.30
	2.42	2.45	0.07	0.03	0.17
2	1.15	0.50	0.33	0.65	1.31
	1.24	0.80	0.44	0.45	1.33
	1.42	0.93	0.37	0.49	1.24
	1.54	1.17	0.31	0.38	0.99
	1.94	1.75	0.21	0.19	0.62
	2.42	2.28	0.21	0.14	0.55
3	1.15	0.88	0.41	0.26	1.09
	1.24	0.98	0.35	0.27	0.97
	1.42	1.15	0.34	0.27	0.96
	1.54	1.35	0.37	0.20	0.93
	1.94	1.84	0.17	0.11	0.44
	2.42	2.37	0.11	0.05	0.27

SD=standard deviation

Table 5: Summary of LLoQ Using the 3rd HIV-1 WHO International Standard (3 Reagent Lots)

Reagent Lot	LLoQ (log_{10} copies/mL)	LLoQ (copies/mL)
1	0.94	8.7
2	1.17	15
3	0.98	9.5

Verification of LLoQ across HIV-1 Subtypes and Groups

LLoQ across HIV-1 subtypes and groups was verified following CLSI EP17-A2 (37). Panels were made for each HIV-1 group M (subtypes A, B, C, D, F, G, CRF01_AE, CRF02_AG), and groups N and O by spiking pooled HIV-1 negative human plasma with either naturally infected clinical samples or clinical isolates. Testing consisted of a total 30 replicates per panel member. The data in Table 6 shows the lowest concentration for each subtype or group at which TE was less than 1 log_{10} copies/mL. The highest LLoQ for all subtypes and groups tested was 30 copies/mL; this higher value, therefore, was selected as the LLoQ for the Aptima HIV-1 Quant assay.

Table 6: Verification of LLoQ by HIV-1 Subtype or Group

Panel	LLoQ (copies/mL)
Subtype A	30
Subtype CRF01_AE	10
Subtype CRF02_AG	30
Subtype B	10
Subtype C	30
Subtype D	15
Subtype F	15
Subtype G	30
Group N	10
Group O	15

Precision

To assess precision of the Aptima HIV-1 Quant assay, a panel that was made by spiking cultured HIV-1 subtype B virus into HIV-1 negative plasma was tested by three operators using three reagents lots on three Panther systems over 20 days (Table 7). The panel consisted of one HIV-1 negative panel member and eight HIV-1 positive panel members. Assignment of the concentration for clinical specimens or cultured virus stocks was determined using a comparator assay.

Table 7: Precision of the Aptima HIV-1 Quant Assay

Number of Valid Replicates	Mean Concentration (log copies/mL)	Inter-Instrument		Inter-Operator		Inter-Lot		Inter-Run		Intra-Run		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
137	1.80	0.00	0.00	0.03	1.72	0.00	0.00	0.00	0.00	0.16	8.93	0.16	9.10
157	2.37	0.00	0.00	0.05	2.08	0.01	0.36	0.08	3.33	0.15	6.19	0.17	7.34
160	2.47[a]	0.00	0.00	0.03	1.37	0.03	1.35	0.07	2.97	0.12	5.03	0.15	6.15
162	2.95	0.00	0.00	0.08	2.57	0.02	0.61	0.10	3.29	0.09	3.04	0.15	5.20
162	3.80	0.01	0.32	0.03	0.80	0.02	0.48	0.06	1.49	0.07	1.80	0.10	2.53
159	4.93	0.00	0.00	0.02	0.37	0.04	0.77	0.05	1.10	0.04	0.71	0.08	1.56
162	5.69	0.00	0.00	0.02	0.27	0.04	0.66	0.03	0.58	0.07	1.29	0.09	1.58
162	6.71	0.00	0.00	0.01	0.22	0.04	0.52	0.04	0.60	0.05	0.78	0.08	1.13

CV=coefficient of variation, SD=standard deviation

[a]This panel member was diluted 1:3 with specimen diluent and tested to evaluate the precision of the diluted sample.

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=0 and CV=0%. The total number of replicates tested was 162 for each panel; only replicates with a numerical value were analyzed.

Potentially Interfering Substances

The susceptibility of the Aptima HIV-1 Quant assay to interference by elevated levels of endogenous substances and by drugs commonly prescribed to HIV-1 infected individuals was evaluated. HIV-1 negative human plasma samples and samples spiked with HIV-1 to a concentration of 3 $\log_{10}$ copies/mL of HIV-1 RNA were tested.

No interference in performance of the Aptima HIV-1 Quant 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).

No interference in performance of the Aptima HIV-1 Quant assay was observed in the presence of the exogenous substances listed in Table 8 at concentrations at least three times the C_{max} (human plasma).

Table 8: Exogenous Substances

Exogenous Substance Pool	Exogenous Substances Tested
1	Lopinavir, indinavir, saquinavir, ritonavir, nelfinavir mesylate, darunavir, amprenavir, atazanavir
2	Nevirapine, efavirenz, rilpivirine, clarithromycin, amphotericin B
3	Tenofovir disoproxil fumarate, adefovir dipivoxil, ribavirin, enfuvirtide, maraviroc, raltegravir, dolutegravir
4	Abacavir sulfate, didanosine, zidovudine, lamivudine, stavudine, entecavir, telbivudine, emtricitabine
5	Paroxetine HCl, fluoxetine, sertraline
6	Ganciclovir, valacyclovir, acyclovir, rifampin/rifampicin, ethambutol
7	Ciprofloxacin, azithromycin, amoxicillin, cephalexin, ampicillin, trimethoprim
8	Valganciclovir hydrochloride, boceprevir, telaprevir, simeprevir, sofosbuvir
9	Pegylated interferon alpha -2b, interferon alpha -2a, interferon alpha -2b
10	Heparin, EDTA, sodium citrate
11	Tipranavir
12	Isoniazid

Clinical plasma specimens listed in Table 9 from patients with elevated levels of defined substances or from patients with the diseases listed were tested with the Aptima HIV-1 Quant assay with and without the presence of 3 $\log_{10}$ copies of HIV-1 RNA. No interference in performance was observed.

Table 9: 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	Alcoholic cirrhosis (AC)
10	Multiple myeloma (MM)
11	Lipemic (elevated lipid)
12	Icteric (elevated bilirubin)
13	Hemolyzed (elevated hemoglobin)
14	Elevated protein albumin
15	HCV antibodies
16	HBV antibodies
17	HIV-2 antibodies

Specificity

Specificity of the Aptima HIV-1 Quant assay was determined using 120 fresh and 510 frozen HIV-1 negative plasma specimens. HIV-1 RNA was not detected in all 630 samples. (specificity of 100%; 95% CI: 99.4-100%).

Table 10: Specificity in Plasma Specimens

	Fresh Plasma	Frozen Plasma	All
Valid replicates (n)	120	510	630
Non-Reactive	120	510	630
Specificity (95% CI)	100% (97.0-100)	100% (99.3-100)	100% (99.4-100)

CI=confidence interval

Cross-Reactivity

Potential cross-reactivity to pathogens (Table 11) was evaluated in the Aptima HIV-1 Quant assay in the presence or absence of 3 $\log_{10}$ copies/mL HIV-1 RNA in HIV-1 negative human plasma. No interference in the performance of the assay was observed in the presence of the pathogens.

Table 11: Pathogens Tested for Cross-Reactivity

Pathogen	Concentration
Hepatitis A virus	100,000 PFU/mL[a]
Hepatitis B virus	100,000 IU/mL[b]
Hepatitis C virus	100,000 IU/mL
Hepatitis G virus	100,000 copies/mL
Herpes simplex virus 1 (HSV-1)	100,000 PFU/mL
Herpes simplex virus 2 (HSV-2)	75,000 PFU/mL
Human herpes virus 6	100,000 copies/mL
Human herpes virus 8	42,000 PFU/mL
HIV-2	5,500 PFU/mL
Human T-cell lymphotropic virus (HTLV)	100,000 vp/mL[c]
West Nile virus	100,000 copies/mL
Parvovirus B19	100,000 IU/mL
Cytomegalovirus	100,000 copies/mL
Epstein-Barr virus	100,000 copies/mL
Adenovirus type 5	100,000 PFU/mL
Dengue virus	100,000 copies/mL
Influenza A virus	100,000 PFU/mL
Staphylococcus aureus	1,000,000 CFU/mL[d]
Propionibacterium acnes	1,000,000 CFU/mL
Staphylococcus epidermidis	1,000,000 CFU/mL
Neisseria gonorrhoeae	1,000,000 CFU/mL
Chlamydia trachomatis	300,000 IFU/mL[e]
Candida albicans	1,000,000 CFU/mL

[a]PFU/mL = Plaque forming units per mL.
[b]IU/mL = International units per mL.
[c]vp/mL = Viral particles per mL.
[d]CFU/mL = Colony forming units per mL.
[e]IFU/mL = Inclusion forming units per mL.

Repeatability of Clinical Specimens

Ten clinical plasma samples were tested in three replicates using the Aptima HIV-1 Quant assay. The average concentration and standard deviation is shown in Table 12.

Table 12: Repeatability of Clinical Specimens

Specimen	Average Concentration (log_{10} copies/mL)	SD
1	2.57	0.06
2	3.20	0.03
3	3.24	0.06
4	3.97	0.02
5	4.20	0.05
6	4.85	0.01
7	5.17	0.04
8	5.51	0.06
9	5.84	0.02
10	6.64	0.00

Sample Dilution Using Specimen Diluent

To evaluate sample dilution, a panel that consisted of 11 samples with concentrations that spanned the linear range of the Aptima HIV-1 Quant assay and that consisted of two samples above the ULoQ of the assay were tested neat and diluted (1:3 or 1:100 in specimen diluent) in triplicate (Table 13).

Table 13: Sample Dilution

Dilution	Average Neat Concentration (log copies/mL)	Average Reported Concentration[a] (log copies/mL)	Difference
	2.57	2.72	0.15
	3.20	3.33	0.13
	3.24	3.55	0.30
	3.97	4.05	0.07
	4.20	4.24	0.04
1:3	4.85	4.81	-0.04
	5.17	5.08	-0.08
	5.51	5.32	-0.19
	5.84	5.94	0.10
	6.64	6.66	0.02
	2.46[b]	2.19	-0.27
1:100	>7.00 (7.16[c])	7.48	0.32
1:100	>7.00 (7.40[c])[b]	7.39	-0.01

[a]Reported concentration is the value reported by the Panther system after the dilution factor has been applied
[b]Spiked specimen
[c] All results > 7.00 log copies/mL were estimated using additional analysis

Carryover

To establish that the Panther system minimizes the risk of false positive results arising from carryover contamination, a multi-run analytical study was conducted using spiked panels on two Panther systems. Carryover was assessed using high titer HIV-1 spiked samples (7 $\log_{10}$ copies/mL) interspersed between HIV-1 negative samples in a checkerboard pattern. Testing was carried out over five runs. The overall carryover rate was 0% (n=469).

Clinical Performance

Method Comparison Study

Quantitation of HIV-1 RNA was compared between the Aptima HIV-1 Quant assay and an FDA-approved comparator assay. The study included testing of clinical plasma samples (stored fresh or frozen) and contrived samples (cultured virus spiked into negative clinical plasma samples). Each sample was tested in duplicate with the Aptima HIV-1 Quant assay and the comparator assay. Aptima HIV-1 Quant assay testing was performed at 3 external sites, with each site using 3 reagent kit lots; comparator assay testing was performed at 1 external laboratory.

Results from 628 samples (within the linear range of both assays) were analyzed using Deming regression. Of these samples, 82 were clinical samples stored fresh (never frozen) before testing with the Aptima HIV-1 Quant assay and comparator assay. Figure 6 shows the results of the Deming regression analysis (y = -0.03 + 1.04x).

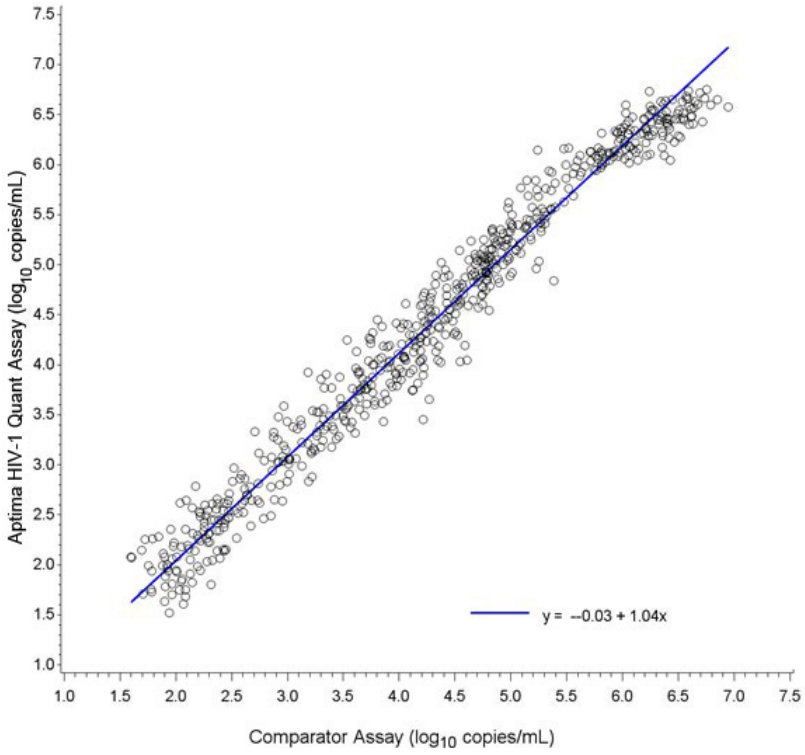

Figure 6. Correlation Between the Aptima HIV-1 Quant Assay and Comparator Assay

Clinical Specificity Study

To assess specificity, previously frozen HIV-1 negative plasma samples obtained from volunteer whole blood donors were tested with the Aptima HIV-1 Quant assay. Testing was performed at 3 external sites with 3 reagent kit lots. Clinical specificity was calculated as the percentage of HIV-1 negative samples with results of "Not Detected." Six hundred (600) HIV-1 negative plasma samples were tested and HIV-1 RNA was not detected in all 600 samples. Specificity was 100% (600/600, 95% Score CI: 99.4% to 100%).

Reproducibility Study

Reproducibility of the Aptima HIV-1 Quant assay was evaluated on the Panther system at 3 external sites. Two operators performed testing at each site. Each operator performed 2 runs per day over 3 days, using 3 reagent lots over the course of testing. Each run had 3 replicates of each panel member.

Reproducibility was tested using panel members that consisted of HIV-1 negative plasma. The positive panel members were created by spiking the negative plasma with cultured virus (HIV-1 subtype B) in concentrations that spanned the linear range of the Aptima HIV-1 Quant assay.

Table 14 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. When only samples with results above the lower limit of quantitation (LLoQ) were included (samples with results below the LLoQ were excluded) total standard deviation (SD) was ≤ 0.2 log copies/mL for all panel members. When all samples with detectable HIV-1 RNA were included, total SD values remained unchanged except for panel member 1, which had a total SD of 0.3 log copies/mL.

For the HIV-1 negative panel member, 108 replicates were tested and HIV-1 RNA was not detected in all 108 replicates (negative agreement=100%, 95% Score CI: 96.6% to 100%).

Table 14: Reproducibility and Precision of Aptima HIV-1 Quant Assay on the Panther System

Panel	N[a]	Mean Log_{10} Copies/mL	Between Sites		Between Operators		Between Lots		Between Days		Between Runs		Within Runs		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	107	1.7[b]	0.0	2.7	0.1	4.1	0.1	4.1	0.0	0.0	0.2	9.9	0.2	14.6	0.3	18.8
	85[c]	1.8	0.1	3.5	0.0	0.0	0.0	1.0	0.0	0.0	0.1	3.1	0.2	9.0	0.2	10.2
2	108	2.9	0.1	2.7	0.0	0.0	0.0	0.0	0.0	1.5	0.0	0.0	0.1	3.7	0.1	4.8
3	108	3.8	0.1	1.8	0.0	0.0	0.0	0.0	0.0	1.0	0.0	1.0	0.1	1.6	0.1	2.7
4	108	4.9	0.1	1.5	0.0	0.0	0.0	0.6	0.0	0.5	0.0	1.0	0.1	1.2	0.1	2.3
5	108	5.7	0.1	1.3	0.0	0.0	0.0	0.2	0.0	0.9	0.0	0.8	0.1	1.2	0.1	2.1
6	108[d]	6.7	0.1	0.8	0.0	0.0	0.0	0.3	0.0	0.4	0.1	1.0	0.1	0.8	0.1	1.6

CV=coefficient of variation, SD=standard deviation

[a]Number of valid test results with detectable HIV-1 RNA.

[b] Includes 22 replicates reported as <1.47 log_{10} copies/mL. These samples had assigned values of 1.176 log_{10} copies/mL.

[c] Number of valid results within the assay's linear range.

[d] Includes 1 replicate reported as >7 log_{10} copies/mL. This sample had an assigned value of 7.18 log_{10} copies/mL.

Note: *Variability from some factors may be numerically negative.This can occur if the variability due to those factors is very small (less than 0.01). In these cases, SD and CV are shown as 0.*

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