

Aptima® HIV-1 Quant Dx Assay

For *in vitro* diagnostic use.

For US export only.

General Information	2
Intended Use	2
Summary and Explanation of the Test	2
Principles of the Procedure	3
Warnings and Precautions	4
Reagent Storage and Handling Requirements	6
Specimen Collection and Storage	7
Samples Onboard the Panther System	10
Specimen Transport	10
Panther System	11
Reagents and Materials Provided	11
Materials Required But Available Separately	12
Optional Materials	13
Panther System Test Procedure	14
Procedural Notes	18
Quality Control	19
Assay Calibration	19
Negative and Positive Controls	19
Internal Calibrator/Internal Control	19
Test Interpretation	20
Results	20
Limitations	21
Performance	22
Limit of Detection	22
Limit of Detection across HIV-1 Subtypes and Groups	22
Linear Range	23
Linearity across HIV-1 Subtypes and Groups	24
Determination of the Lower Limit of Quantitation Using the 3rd WHO International Standard	25
Verification of LLOQ across HIV-1 Subtypes and Groups	26
Precision	26
Potentially Interfering Substances	27
Specificity	29
Analytical Specificity	30
Repeatability of Clinical Specimens	31
Sample Dilution Using Specimen Diluent	31
Method Correlation	32
Diagnostic Agreement	33
Carryover	33
Seroconversion Panel	33
Serum, Plasma Equivalency Study	34
Bibliography	35

General Information

Intended Use

The Aptima® HIV-1 Quant Dx assay is an *in vitro* nucleic acid amplification test for the detection and quantitation of human immunodeficiency virus type 1 (HIV-1) RNA on the fully automated Panther® system. It is intended for use as an aid in the diagnosis of HIV-1 infection, as a confirmation of HIV-1 infection, and as an aid in clinical management of patients infected with HIV-1.

The Aptima HIV-1 Quant Dx assay may be used as an aid in the diagnosis of HIV-1 infection, including acute or primary infection. Presence of HIV-1 RNA in the plasma or serum of patients without antibodies to HIV-1 is indicative of acute or primary HIV-1 infection. The Aptima HIV-1 Quant Dx assay may be used as a supplemental test for specimens that have repeat reactive results with approved HIV immunoassays. If the specimen is reactive in the Aptima HIV-1 Quant Dx assay, HIV-1 infection is confirmed.

The Aptima HIV-1 Quant Dx assay may also be used in conjunction with clinical presentation and other laboratory markers for disease prognosis in HIV-1 infected individuals. The Aptima HIV-1 Quant Dx assay may be used as an aid in monitoring the effect of antiretroviral treatment by measuring changes in the concentration of HIV-1 RNA in plasma.

When the Aptima HIV-1 Quant Dx assay is used as an aid in the diagnosis of HIV-1 infection, performance for qualitative results is established with both plasma and serum specimens. When used as an aid in monitoring the effect of antiretroviral therapy, performance for quantitative results is established with plasma specimens only. Serum specimens may not be used for quantitative results.

This assay is not intended for use in screening blood or plasma donors.

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 by an HIV-positive mother to the fetus (8). Within 3 to 6 weeks of exposure to HIV, infected individuals generally develop a brief, acute syndrome characterized by flu-like symptoms and 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).

Current detection of HIV-1 infection is primarily based on serologic testing for antibodies and/or p24 antigen by an immunoassay. The US Centers for Disease Control recommends the use of an antibody and RNA test to diagnose acute HIV infections (31). Although sensitivity of HIV-1 antibody and p24 antigen detection has improved, there still exists a window period between the time of infection and the time of detection by serological markers. This window period is dependent on the sensitivity of the serological test used. One estimate (32) suggests that 4th generation p24 antigen/antibody assays may detect infection when the HIV-1 RNA concentration reaches 14,000 copies/mL. The limit of detection of the Aptima HIV-1 Quant Dx assay is significantly lower than 14,000 copies/mL and may detect the presence of HIV-1 earlier than HIV immunoassays.

Principles of the Procedure

The Aptima HIV-1 Quant Dx 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 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 HIV-1 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-based 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 Dx 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 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 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 Dx 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 a 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. Express 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 Dx 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 Dx assay have been evaluated with plasma. Serum may not be used to obtain quantitative results. Qualitative results have been evaluated with both plasma and serum.
- 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.

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 CONTROL – (Negative Control)	-15°C to -35°C	15°C to 30°C	Single use vial Use within 20 hours
qHIV-1 CONTROL + L (Low Positive Control)	-15°C to -35°C	15°C to 30°C	Single use vial Use within 20 hours
qHIV-1 CONTROL + H (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.

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

For quantitative measurements:

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

For qualitative determination:

- Tubes containing EDTA or ACD anticoagulants, or
- PPTs, or
- Serum tubes, or
- 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 24 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 the primary tube or transferred to a secondary, specimen aliquot tube (SAT) and can be tested on the Panther system. The minimum volume for primary collection tubes is 1200 µL and for SATs the minimum volume is 700 µL.

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

B. Specimen Storage Conditions

1. EDTA and ACD Plasma Specimens

For up to 24 hours after specimen collection, primary 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, or
- 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.

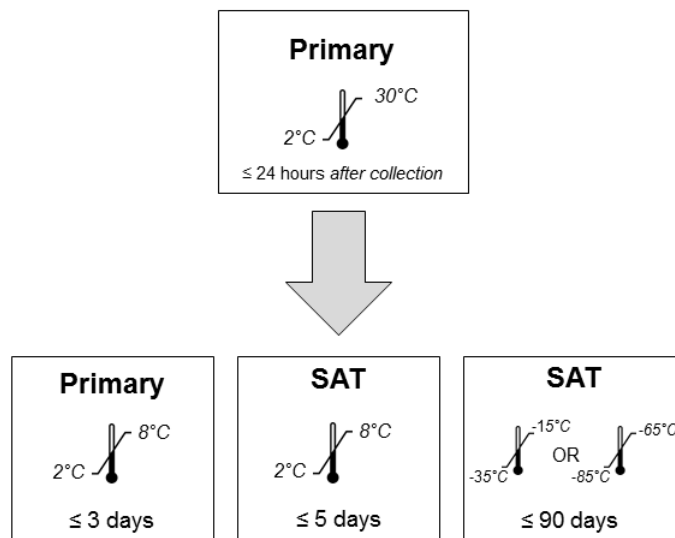


Figure 1. Storage Conditions for EDTA/ACD Primary Tubes

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.

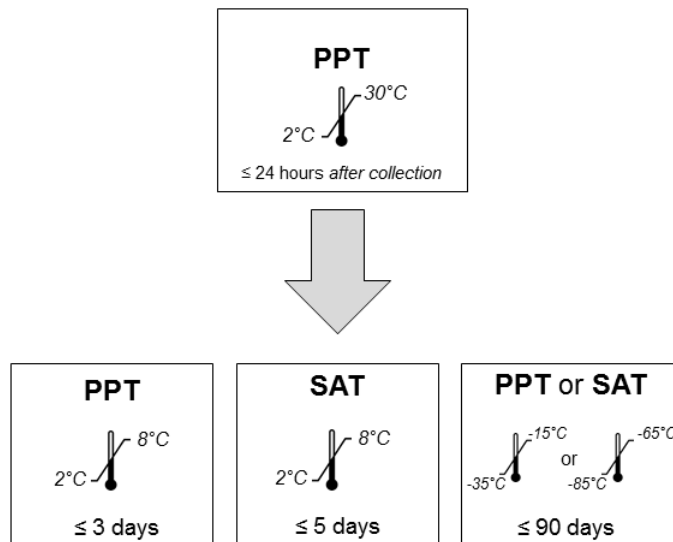


Figure 2. Storage Conditions for PPTs

3. Serum Tube Specimens

For up to 24 hours after specimen collection, serum tubes containing centrifuged serum may be stored at 2°C to 30°C (Figure 3, upper box). After 24 hours, serum may be stored for a longer period of time under one of the following conditions (Figure 3, lower boxes):

- In the serum tube at 2°C to 8°C for up to 5 days,
- In the SAT at 2°C to 8°C for up to 5 days, or
- In the SAT at -20°C for up to 7 days.

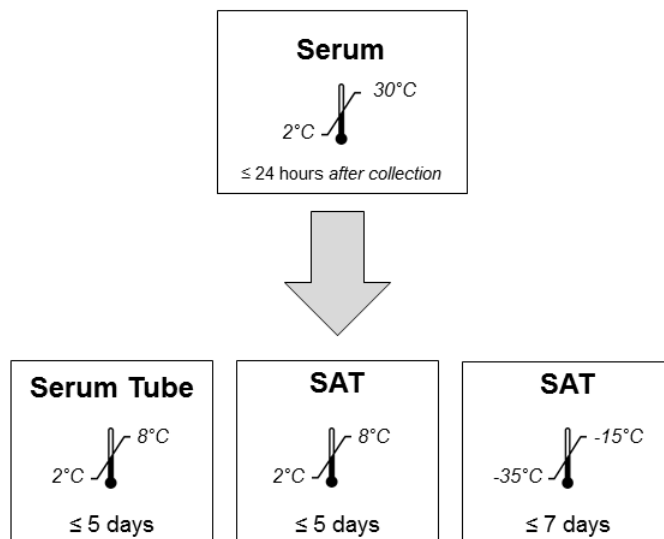


Figure 3. Storage Conditions for Serum Tubes

4. SST Specimens

For up to 24 hours after specimen collection, SSTs containing centrifuged serum may be stored at 2°C to 30°C (Figure 4, upper box). After 24 hours, serum may be stored for a longer period of time under one of the following conditions (Figure 4, lower boxes):

- In the SST at 2°C to 8°C for up to 5 days,
- In the SAT at 2°C to 8°C for up to 5 days, or
- In the SAT at -20°C for up to 7 days.

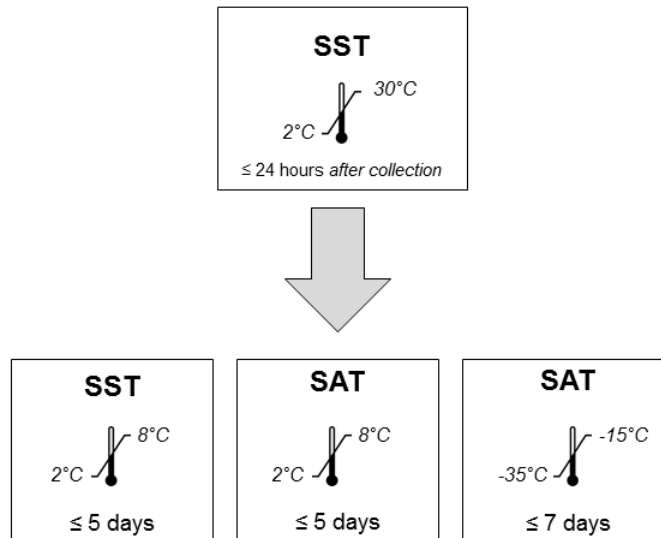


Figure 4. Storage Conditions SSTs

C. Dilution of Plasma Specimens

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

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 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 HIV-1 Quant Dx Assay Kit, 100 tests, Cat. No. PRD-03000 (1 assay box, 1 Calibrator kit, and 1 Controls kit)

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

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

Aptima HIV-1 Quant Dx Controls Kit (Cat. No. PRD-03002)
(store at -15°C to -35°C upon receipt)

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

Aptima HIV-1 Quant Dx Calibrator Kit (Cat. No. PRD-03001)
(store at -15°C to -35°C upon receipt)

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

Materials Required But Available Separately

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

Material	Cat. No.
Panther System	303095
Aptima Assay Fluids Kit <i>contains Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent</i>	303014 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther Waste Bag Kit	902731
Panther Waste Bin Cover	902714
Or Panther Run Kit for Real Time Assays <i>contains MTUs, waste bags, waste bin covers, and assay fluids</i>	PRD-03455 (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	—

Replacement non-penetrable caps	103036A
Reagent replacement caps	
<i>Amplification, Enzyme, and Promoter reagent</i>	
<i>reconstitution solutions</i>	CL0041 (100 caps)
TCR	CL0040 (100 caps)
Plastic-backed laboratory bench covers	—
Lint-free wipes	—
Pipettor	—
Tips	—
Primary collection tubes 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.
Specimen Aliquot Tubes (SAT) (100 pack)	503762
Transport Tube Cap (100 pack)	504415
<i>cap for SAT</i>	
Aptima Specimen Diluent	PRD-03003
Additional Aptima HIV-1 Quant Dx Assay Controls Kit	PRD-03002
Additional Aptima HIV-1 Quant Dx Assay Calibrator Kit	PRD-03001
Transfer pipets	—
Commercially available panels, for example:	—
<i>HIV-1 from Quality Control for Molecular Diagnostics or</i>	
<i>College of American Pathologists HIV viral load survey panel or</i>	
<i>SeraCare ACCURUN HIV Panels</i>	
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.

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) and discard (Figure 5, Step 8).
 - e. Record operator initials and reconstitution date on the label. Load bottles onto the Reagent Rack for immediate use; otherwise, cap the bottles and store (2°C to 8°C) (Figure 5, Step 7).

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

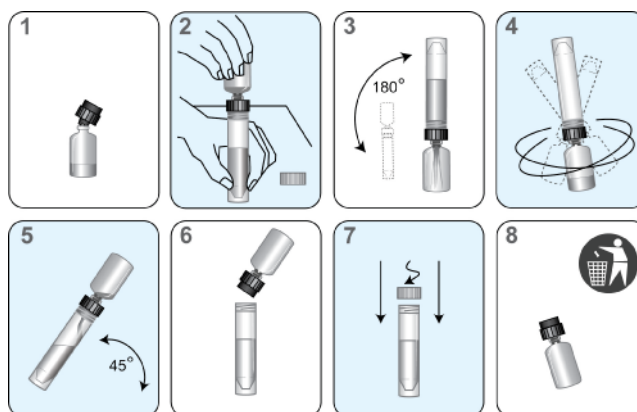


Figure 5. Reagent Reconstitution Process

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, 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 specimen. Ensure that each 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 3000g 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 a SAT

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

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

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

a. Specimen 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 a 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. Specimen 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 μ L of specimen in a SAT.
- ii. Add 2970 μ L of specimen diluent.
- iii. Cap the tube.
- iv. Gently invert 5 times to mix.
- v. Multiply the result by 100.

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 pop any bubbles or foam.
 - f. When the last cap has been removed, secure the Sample Retainer, if running other assays and sample types at the same time, and load the Sample Rack into a Sample Bay.
 - g. Repeat steps 2.a to 2.f for the next Sample Rack.

Procedural Notes

A. Calibrator

1. The qHIV-1 positive calibrator 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 is currently being processed by the system.
 - b. A valid result for the calibrator is registered on the system.
2. Once the calibrator tube has been pipetted and is processing for the Aptima HIV-1 Quant Dx assay reagent kit, specimens can be tested with the associated, reconstituted kit for up to 24 hours **unless**:
 - a. The calibrator result is 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 tube can be tested once. Attempts to pipette more than three replicates from the tube can lead to processing errors.

B. Controls

1. The qHIV-1 low positive control, high positive control, and 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 controls are currently being processed by the system.
 - b. Valid results for the controls are registered on the system.
2. Once the control tubes have been pipetted and are processing for the Aptima HIV-1 Quant Dx reagent kit, specimens can be tested with the associated, reconstituted kit for up to 24 hours **unless**:
 - a. Controls 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. Each control tube can be tested once. Attempts to pipette more than once from the tube can lead to processing errors.

C. 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.

Assay Calibration

To generate valid results, an assay calibration must be completed. A single positive calibrator is run in triplicate (i) each time a reagent kit is loaded on the Panther system, (ii) with each newly reconstituted reagent kit, and (iii) every 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 (i) each time a reagent kit is loaded on the Panther system, (ii) with each newly reconstituted reagent kit, and (iii) every 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 "HIV-1 RNA 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 the IC result is invalid, the sample result is invalidated. Every sample with an invalid IC result must be retested to obtain a valid result.

Test Interpretation

Note: Quantitative results of the Aptima HIV-1 Quant Dx assay has been evaluated with plasma. Serum may not be used to obtain quantitative results. Qualitative results have been evaluated with both plasma and serum.

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 log₁₀ copies/mL. The interpretation of results is provided in Table 1. If the 1:3 Option is used for 1:3 diluted specimens, the Panther system automatically calculates the HIV-1 concentration for the neat specimen by multiplying the concentration results by the dilution factor.

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 or LLOQ, respectively. 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 HIV-1 RNA concentration into a qualitative result (Table 1). Specimens with results listed as “Not Detected” are nonreactive for HIV-1 RNA. Specimens with results listed as “<30 detected” or specimens with results listed within the linear range indicate HIV-1 RNA was detected and these specimens are reactive for HIV-1 RNA.

Table 1: Result Interpretation

Reported Aptima HIV-1 Quant Dx Result ^a		HIV-1 RNA Concentration Interpretation ^a	User’s Diagnostic Qualitative Interpretation ^c
Copies /mL ^b	Log ₁₀ Value		
Not Detected	Not Detected	HIV-1 RNA not detected.	Non-reactive for HIV-1 RNA.
<30 detected	<1.47	HIV-1 RNA is detected but at a level below the Lower Limit of Quantitation (LLOQ).	Reactive for HIV-1 RNA.
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.	Reactive for HIV-1 RNA.
>10,000,000	>7.00	HIV-1 RNA concentration is above the Upper Limit of Quantitation (ULOQ).	Reactive for HIV-1 RNA.
Invalid	Invalid	There was an error in the generation of the result. Specimen should be retested.	Invalid. ^d

^a Assay performance for quantitation of HIV-1 RNA has not been established with serum specimens. Do not report quantitative results for HIV-1 RNA using serum specimens.

^b 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.

^c A diagnostic interpretation may be made from either serum or plasma specimens that have not been diluted.

Note: For diluted specimens, “Not Detected” or “<30 detected” may be generated by diluting a specimen with a concentration above, but close to the LOD/LLOQ. It is recommended to collect and test another neat specimen if a quantitative result is not obtained.

^d 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. This assay has been validated for use as a quantitative assay with only human plasma.
- D. This assay has been validated for use as a qualitative assay with human plasma and serum.
- E. Contamination can only be avoided by adhering to good laboratory practices and to the procedures specified in this package insert.
- F. 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 Dx assay may result in underquantification of or failure to detect the virus.

Performance

Limit of Detection

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 results from the reagent lot with the highest concentration for the predicted detection limit are defined as LOD and are shown in Table 2. Through Probit analysis, the LOD for the Aptima HIV-1 Quant Dx assay is 13 copies/mL (37 IU/mL; 0.35 copies = 1 IU).

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

Predicted Detection Limit	Concentration (copies/mL)
10%	1.3
20%	1.8
30%	2.3
40%	2.7
50%	3.2
60%	3.9
70%	4.9
80%	6.5
90%	9.5
95%	13.1

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 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%	7.6
CRF02_AG	50%	3.4
	95%	15.4
C	50%	2.2
	95%	11.8
D	50%	4.2
	95%	14.8
F	50%	2.0
	95%	8.3
G	50%	3.5
	95%	18.2
N	50%	1.3
	95%	7.9
O	50%	2.0
	95%	8.0

Linear Range

The linear range of the Aptima HIV-1 Quant Dx 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 copies/mL. Testing was performed on seven Panther systems with two reagent lots of Aptima HIV-1 Quant Dx assay. As shown in Figure 6, the Aptima Quant Dx assay demonstrated linearity across the range tested.

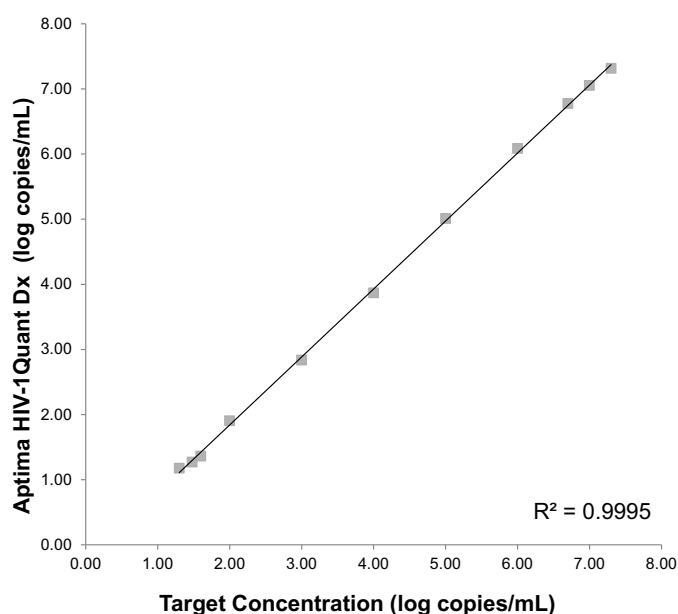


Figure 6. Linearity of the Aptima HIV-1 Quant Dx Assay

Linearity across HIV-1 Subtypes and Groups

The linear response of the Aptima HIV-1 Quant Dx 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 6.50 log copies/mL. Testing was performed on four Panther systems and six runs. Linearity was demonstrated across the range tested (Figure 7).

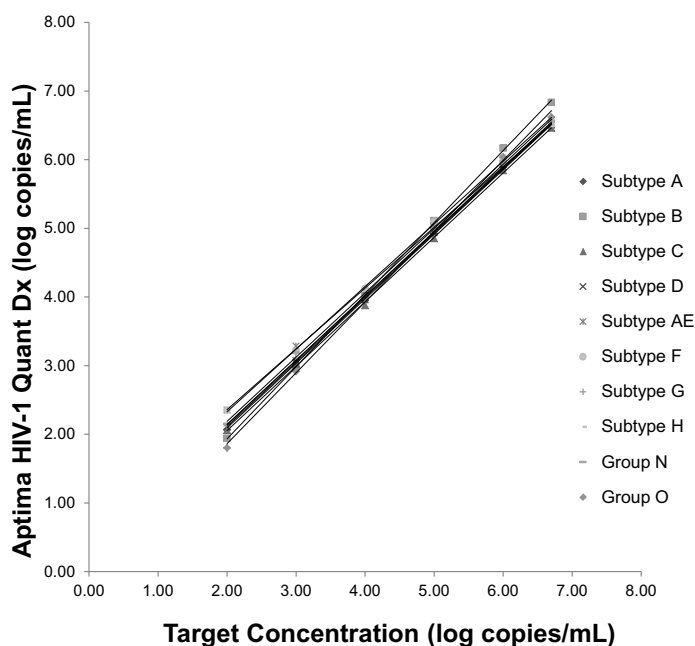


Figure 7. Linearity across Group M (Subtypes A, B, C, D, F, G, H, CRF01_AE) and Groups N and O

Determination of the Lower Limit of Quantitation Using the 3rd 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 ($TE = \text{bias} + 2SD$). To ensure accuracy and precision of measurements, the TE of the Aptima HIV-1 Quant Dx 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 plasma. Per CLSI EP17-A2, panels were tested with three reagent lots in replicates of 30 for each lot from 26 runs. The results are shown in Table 4. The highest LLOQ for the three lots tested on the Aptima HIV-1 Quant Dx assay using the 3rd WHO International Standard is 19 copies/mL (1.27 log copies/mL) (Table 5).

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

Reagent Lot	Target Concentration (log copies/mL)	Aptima HIV-1 Quant Dx (log copies/mL)	SD (log copies/mL)	Bias (log copies/mL)	Calculated TE (log copies/mL)
1	1.15	0.97	0.36	0.18	0.90
	1.24	0.86	0.34	0.39	1.06
	1.42	1.27	0.35	0.15	0.85
	1.54	1.36	0.23	0.19	0.64
	1.94	1.90	0.15	0.05	0.34
	2.42	2.40	0.08	0.01	0.18
2	1.15	0.49	0.30	0.65	1.26
	1.24	0.73	0.41	0.51	1.33
	1.42	0.88	0.38	0.54	1.30
	1.54	1.20	0.25	0.34	0.84
	1.94	1.74	0.22	0.21	0.64
	2.42	2.30	0.19	0.12	0.50
3	1.15	0.86	0.40	0.29	1.09
	1.24	0.95	0.37	0.29	1.03
	1.42	1.10	0.33	0.32	0.98
	1.54	1.27	0.36	0.27	0.99
	1.94	1.79	0.18	0.15	0.51
	2.42	2.35	0.12	0.07	0.31

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

Reagent Lot	LLOQ (log copies/mL)	LLOQ (copies/mL)
1	1.27	19
2	1.20	16
3	1.10	13

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 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 Dx 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	15
Subtype C	30
Subtype D	20
Subtype F	15
Subtype G	30
Group N	10
Group O	15

Precision

To assess precision of the Aptima HIV-1 Quant Dx 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 Dx 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 (%)
132	1.74	0.00	0.00	0.03	1.76	0.00	0.00	0.00	0.00	0.16	9.08	0.16	9.25
157	2.32	0.00	0.00	0.05	2.17	0.02	0.91	0.09	3.75	0.15	6.57	0.18	7.92
158	2.42*	0.00	0.00	0.02	0.61	0.05	1.93	0.07	2.81	0.13	5.31	0.15	6.34
162	2.92	0.02	0.53	0.08	2.58	0.04	1.28	0.09	3.23	0.09	3.02	0.16	5.30
162	3.80	0.02	0.57	0.04	0.93	0.05	1.29	0.05	1.38	0.08	2.10	0.12	3.03
159	4.99	0.01	0.19	0.03	0.51	0.05	0.94	0.05	1.09	0.04	0.79	0.09	1.73
162	5.73	0.00	0.00	0.02	0.38	0.04	0.78	0.04	0.65	0.07	1.28	0.10	1.67
161	6.75	0.00	0.00	0.02	0.26	0.04	0.62	0.04	0.54	0.05	0.80	0.08	1.17

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.

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

Potentially Interfering Substances

The susceptibility of the Aptima HIV-1 Quant Dx assay to interference by elevated levels of endogenous substances and by drugs commonly prescribed to HIV-1 infected individuals was evaluated. HIV-1 negative plasma samples and samples spiked to a concentration of 3 log copies/mL of HIV-1 RNA were tested.

No interference in performance of the Aptima HIV-1 Quant Dx 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 Dx 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	PegInterferon 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 Dx assay with and without the presence of 3 log 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 Eryth (SLE)
5	Rheumatoid Arthritis (RA)
6	Multiple Sclerosis (MS)
7	Hyperglobulinemia (IgG or IgM is acceptable)
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 Dx assay was determined using 120 fresh and 510 frozen HIV-1 negative plasma specimens, and using 120 fresh and 510 frozen HIV-1 negative serum specimens. All results were non-reactive (specificity of 100%; 95% CI: 99.4-100%).

Table 10: Serum and Plasma Specificity

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

Analytical Specificity

Potential cross-reactivity to pathogens (Table 11) was evaluated in the Aptima HIV-1 Quant Dx assay in the presence or absence of 3 log copies/mL HIV-1 RNA in HIV-1 negative plasma. No interference in the performance of the assay was observed in the presence of the pathogens.

Table 11: Pathogens Tested for Analytical Specificity

Pathogen	Concentration
Hepatitis A	100,000 PFU/mL ¹
Hepatitis B	100,000 IU/mL ²
Hepatitis C	100,000 IU/mL
Hepatitis G	100,000 copies/mL
Herpes Simplex Virus 1	100,000 PFU/mL
Herpes Simplex Virus 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
HTLV	100,000 vp/mL ³
West Nile Virus	100,000 copies/mL
Parvo 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	100,000 copies/mL
Influenza A	100,000 PFU/mL
<i>Staphylococcus aureus</i>	1,000,000 CFU/mL ⁴
<i>Propionibacterium acnes</i>	1,000,000 CFU/mL
<i>Staphylococcus epidermis</i>	1,000,000 CFU/mL
<i>Neisseria gonorrhoeae</i>	1,000,000 CFU/mL
<i>Chlamydia trachomatis</i>	300,000 IFU/mL ⁵
<i>Candida albicans</i>	1,000,000 CFU/mL

¹PFU/mL = Plaque Forming Units per mL.

²IU/mL = International Units per mL.

³vp/mL = Viral Particles per mL.

⁴CFU/mL = Colony Forming Units per mL.

⁵IFU/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 Dx assay. The average concentration and standard deviation is shown in Table 12.

Table 12: Repeatability of Clinical Specimens

Specimen	Average Concentration (log copies/mL)	SD
1	2.51	0.08
2	3.14	0.02
3	3.17	0.02
4	3.95	0.03
5	4.20	0.06
6	4.94	0.02
7	5.19	0.08
8	5.55	0.03
9	5.86	0.02
10	6.67	0.01

Sample Dilution Using Specimen Diluent

To assess sample dilution, a panel that consisted of 11 samples with concentrations that spanned the linear range of the Aptima HIV-1 Quant Dx assay and that consisted of two samples above the Upper Limit of Quantitation 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 Factor	Average Neat Concentration (log copies/mL)	Average Reported Concentration ^a (log copies/mL)	Difference
1:3	2.51	2.65	0.14
	3.14	3.31	0.17
	3.17	3.51	0.34
	3.95	4.02	0.07
	4.20	4.21	0.01
	4.94	4.81	0.13
	5.19	5.11	0.08
	5.55	5.38	0.17
	5.86	5.99	0.13
	6.67	6.66	0.01
	2.39 ^b	2.17 ^c	0.22
1:100	>7.00 (7.13)	7.51	0.38
1:100	>7.00 (7.46) ^b	7.45	0.01

^aReported concentration is the value calculated after the dilution factor has been applied.

^bSpiked specimen.

^c Result from two out of three replicates tested. One replicate reported result was non-numerical (< 30 detected).

Note. All results > 7.00 log copies/mL were estimated using additional analysis.

Method Correlation

The performance of the Aptima HIV-1 Quant Dx assay was assessed against a CE-marked comparator assay by testing undiluted clinical plasma specimens from HIV-1 infected patients on four Panther systems with two reagent lots. A total of 340 frozen and 103 fresh plasma specimens with quantifiable results in both the Aptima HIV-1 Quant Dx assay and the comparator assay were used for the linear regression (Figure 8). The specimens included HIV-1 group M (subtypes A, B, C, D, F, G, H, CRF01_AE, CRF02_AG).

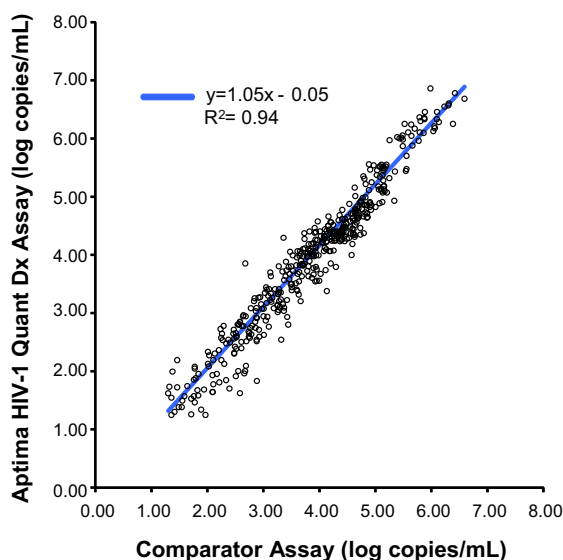


Figure 8. Correlation between the Aptima HIV-1 Quant Dx Assay and Comparator Assay

Diagnostic Agreement

To assess diagnostic agreement, specimens from HIV-1 positive individuals were tested using the Aptima HIV-1 Quant Dx assay and a comparator CE-marked HIV-1 qualitative assay: 413 specimens had valid results (Table 14). The results for both assays were categorized as follows. Any result giving a quantifiable or detectable result was categorized as “Detected.” Any result of target not detected was categorized as “Target Not Detected.”

Table 14: Diagnostic Agreement between Aptima HIV-1 Quant Dx Assay and Comparator Assay

		Aptima HIV-1 Quant Dx Assay	
		Detected	Target Not Detected
Comparator Assay	Detected	213	0
	Target Not Detected	0	200

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 copies/mL) dispersed between HIV-1 negative samples in a checkerboard pattern. Testing was carried out over five runs. The overall carryover rate was 0% (n=470).

Seroconversion Panel

Nineteen HIV-1 seroconversion panel sets, consisting of 204 samples, were tested using the Aptima HIV-1 Quant Dx assay. Detection of HIV-1 RNA was compared to detection with p24 antigen tests and with HIV-1/2 antibody tests. The number of days to the first reactive result using p24 antigen tests, anti-HIV 1/2 antibody tests, and the Aptima HIV-1 Quant Dx assay is listed in Table 15. The Aptima HIV-1 Quant Dx assay detected HIV-1 RNA an average of 5.58 and 10.89 days before p24 antigen and anti-HIV 1/2 antibody tests, respectively.

Table 15: 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 HIV-1 Quant Dx	HIV p24 Antigen	Anti-HIV 1/2 Antibody	Aptima HIV-1 Quant Dx	HIV p24 Antigen	Anti-HIV 1/2 Antibody	Days Earlier Detection Than HIV p24 Antigen	Days Earlier Detection Than Anti-HIV 1/2 Antibody
6248	7	3	2	1	14	18	25	4	11
6243	10	6	3	2	18	25	32	7	14
6247	9	4	4	1	21	21	30	0	9
9016	10	3	2	0	27	30	34**	3	7
9018	11	5	3	2	21	28	32	7	11
9020	22	5	4	1	83	87	97	4	14
9021	17	5	4	1	43	47	57	4	14
9022	9	3	2	1	23	25	32	2	9
9023	22	5	3	0	71	78	85**	7	9
9030	16	5	3	1	40	47	54	7	14
9034	13	4	3	1	41	46	53	5	12
9089	6	5	3	2	7	16	20	9	13
12008	13	7	4	4	21	28	33	7	12
PRB962	6	4	2	0	7	14	17**	7	10
PRB963	7	4	2	0	9	17	21**	8	12
PRB966	10	5	3	2	35	44	48	9	13
PRB974*	4	3	2	1	7	9	16	2	9
PRB975*	5	3	1	0	7	14	14**	7	7
PRB978*	7	3	1	0	26	33	33**	7	7
Total	204	82	51	20	Mean			5.58	10.89
					Median			7	11

Anti-HIV-1/2 Antibody testing was completed with Abbott Anti-HIV 1/2, with the following exceptions:

*Panels PRB974, PRB975, and PRB978 were tested with Siemens Anti-HIV 1/2 test.

HIV-1 p24 Antigen testing was completed with Coulter HIV-1 p24 Ag, with the following exceptions:

*Panels PRB974, PRB975, and PRB978 were tested with BioMerieux p24 Ag test.

**All bleeds in this panel were non-reactive for Anti-HIV 1/2 Antibody. The last bleed day was used as the "Days to First Reactive Result."

Serum, Plasma Equivalency Study

To assess equivalency, matched sets of serum and plasma (25 HIV-1 positive and 25 HIV-1 negative), and 40 samples that were spiked with cultured HIV-1 (50-1,000,000 copies/mL in HIV-1 negative plasma and serum) were tested with the Aptima HIV-1 Quant Dx assay. The negative agreement was 100.0% (95% CI: 96.6%-100.0%). The positive agreement was 98.4% (95% CI: 95.4%-99.5%).

Bibliography

1. **Barre-Sinoussi, F., J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Vezinet-Brun, C. Rouziuz, W. Rozenbaum, and L. Montagnier.** 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for Acquired Immune Deficiency Syndrome (AIDS). *Science* **220**:868–871.
2. **Popovic, M., M. G. Sarngadharan, E. Read, and R. C. Gallo.** 1984. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science* **224**:497–500.
3. **Gallo R. C., S. Z. Salahuddin, M. Popovic, G. M. Streater, M. Kaplan, D. F. Haynas, T. J. Palker, R. Redfield, J. Oleske, B. Safai, G. White, P. Foster, and P. D. Markham.** 1984. Frequent detection and isolation of cytopathic retroviruses (HTLV III) from patients with AIDS and at risk for AIDS. *Science* **224**:500–503.
4. **Piot, P., F. A. Plummer, F. S. Mhalu, J-L. Lamboray, J. Chin, and J. M. Mann.** 1988. AIDS: An international perspective. *Science* **239**:573–579.
5. **Sarngadharan, J. G., M. Popovic, L. Broch, J. Scupbach, and R. C. Gallo.** 1984. Antibodies reactive with human T-lymphotropic retroviruses (HTLV-III) in the serum of patients with AIDS. *Science* **224**:506–508.
6. **Gallo, D., J. S. Kimpton, and P. J. Dailey.** 1987. Comparative studies on use of fresh and frozen peripheral blood lymphocyte specimens for isolation of human immunodeficiency virus and effects of cell lysis on isolation efficiency. *J. Clin. Microbiol.* **25**:1291–1294.
7. **Clavel, F., D. Guetard, F. Brun-Vezinet, S. Chamaret, M. Rey, M. O. Santos-Ferraira, A. G. Laurent, C. Dauguet, C. Katlama, C. Rouzioux, D. Klatzmann, J. L. Champalimaud, and L. Montagnier.** 1986. Isolation of a new human retrovirus from West African patients with AIDS. *Science* **233**:343–346.
8. **Curran, J. W., H. W. Jaffe, A. M. Hardy, W. M. Morgan, R. M. Selik, and T. J. Dondero.** 1988. Epidemiology of HIV Infection and AIDS in the United States. *Science* **239**:610–616.
9. **Gaines, H., M. A. von Sydow, and L.V. von Stedingk.** 1990. Immunological changes in primary HIV-1 infection. *AIDS* **4**:995–999.
10. **Tindall, B., and D. A. Cooper.** 1991. Primary HIV-1 infection: host responses and intervention strategies. *AIDS* **5**:1–14.
11. **Daar, E. S., T. Moudgil, R. D. Meyer, and D. D. Ho.** 1991. Transient high levels of viremia in patients with primary human immunodeficiency virus type 1 infection. *N. Engl. J. Med.* **324**:961–964.
12. **Clark, S. J., M. S. Saag, and W. D. Decker.** 1991. High titers of cytopathic virus in plasma of patients with symptomatic primary HIV-1 infection. *N. Engl. J. Medicine* **324**:954–960.
13. **Albert J., B. Abrahamsson, K. Nagy, E. Aurelius, H. Gaines, G. Nystrom, and E. M. Fenyo.** 1990. Rapid development of isolate-specific neutralizing antibodies after primary HIV-1 infection and consequent emergence of virus variants which resist neutralization by autologous sera. *AIDS* **4**:107–112.
14. **Horsburgh, C. R. Jr., C. Y. Ou, J. Jason, S. D. Holmberg, I. M. Longini Jr., C. Schable, K. H. Mayer, A. R. Lifson, G. Schochetman, J. W. Ward, et al.** 1989. Duration of human immunodeficiency virus infection before detection of antibody. *Lancet* **16**:637–640.
15. **Schnittman, S. M., M. C. Psallidopoulos, H. C. Lane, L. Thompson, M. Baseler, F. Massari, C. H. Fox, N. P. Salzman, and A. S. Fauci.** 1989. The reservoir for HIV-1 in human peripheral blood is a T cell that maintains expression of CD4. *Science* **245**:305–308. Erratum in: *Science* 1989 **245**, preceding 694.
16. **Schnittman, S. M., J. J. Greenhouse, M. C. Psallidopoulos, M. Baseler, N. P. Salzman, A. S. Fauci, and H.C. Lane.** 1990. Increasing viral burden in CD4+ T cells from patients with human immunodeficiency virus (HIV) infection reflects rapidly progressive immunosuppression and clinical disease. *Ann. Intern. Med.* **113**:438–443.
17. **Pantaleo, G., C. Graziosi, and A. S. Fauci.** 1993. New concepts in the immunopathogenesis of human immunodeficiency virus (HIV) infection. *N. Engl. J. Med.* **328**:327–335.
18. **Piatak, M. Jr., M. S. Saag, L. C. Yang, S. J. Clark, J. C. Kappes, K. C. Luk, B. H. Hahn, G. M. Shaw, and J. D. Lifson.** 1993. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. *Science* **259**:1749–1754.
19. **Fauci, A. S., S. M. Schnittman, G. Poli, S. Koenig, and G. Pantaleo.** 1991. NIH conference: immunopathogenic mechanisms in human immunodeficiency virus (HIV) infection. *Ann. Intern. Med.* **114**:678–693.
20. **Coffin, J. M.** 1995. HIV-1 population dynamics in vivo: implications for genetic variation, pathogenesis, and therapy. *Science* **267**:483–489.
21. **Ho, D. D., A. U. Neumann, A. S. Perelson, W. Chen, J. M. Leonard, and M. Markowitz.** 1995. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* **373**:123–126.
22. **Wei, X., S. K. Ghosh, M. E. Taylor, V. A. Johnson, E. A. Emini, P. Deutsch, J. D. Lifson, S. Bonhoeffer, M. A. Nowak, B. H. Hahn et al.** 1995. Viral dynamics in human immunodeficiency virus type 1 infection. *Nature* **373**:117–122.
23. **O'Brien, W. A., P. M. Hartigan, D. Martin, J. Esinhart, A. Hill, S. Benoit, M. Rubin, M. S. Simberkoff, and J. D. Hamilton.** 1996. Changes in plasma HIV-1 RNA and CD4 lymphocyte counts and the risk of progression to AIDS. Veterans Affairs Cooperative Study Group on AIDS. *N. Engl. J. Med.* **334**:426–431.
24. **Welles, S. L., J. B. Jackson, B. Yen-Lieberman, L. Demeter, A. J. Japour, L. M. Smeaton, V. A. Johnson, D. R. Kuritzkes, R. T. D'Aquila, P. A. Reichelderfer, D. D. Richman, R. Reichman, M. Fischl, R. Dolin, R. W. Coombs, J. O. Kahn, C. McLaren, J. Todd, S. Kwok, and C. S. Crumpacker.** 1996. Prognostic value of plasma Human Immunodeficiency Virus Type I (HIV-1) RNA

- levels in patients with advanced HIV-1 disease and with little or no zidovudine therapy. AIDS Clinical Trials Group Protocol 116A/116B/117 Team. *J. Infect. Dis.* **174**:696–703.
25. **Coombs, R. W., S. L. Welles, C. Hooper, P. S. Reichelderfer, R. T. D'Aquila, A. J. Japour, V. A. Johnson, D. R. Kuritzkes, D. D. Richman, S. Kwok, J. Todd, J. B. Jackson, V. DeGruttola, C. S. Crumpacker, and J. Kahn.** 1996. Association of plasma Human Immunodeficiency Virus Type 1 RNA level with risk of clinical progression in patients with advanced infection. AIDS Clinical Trials Group (ACTG) 116B/117 Study Team. ACTG Virology Committee Resistance and HIV-1 RNA Working Groups. *J. Infect. Dis.* **174**:704–712.
 26. **Hammer, S., C. Crumpacker, R. D'Aquila, B. Jackson, J. Lathey, D. Livnat, and P. Reichelderfer.** 1993. Use of virologic assays for detection of human immunodeficiency virus in clinical trials: Recommendations of the AIDS Clinical Trials Group Virology Committee. *J. Clin. Microbiol.* **31**:2557–2564.
 27. **Schochetman, G., and J. R. George,** ed. 1994. *AIDS Testing: A Comprehensive Guide To Technical, Medical, Social, Legal and Management Issues*, 2nd ed. Springer-Verlag, New York.
 28. **Mulder, J., N. McKinney, C. Christopherson, J. Sninsky, L. Greenfield, and S. Kwok.** 1994. Rapid and simple PCR assay for quantitation of human immunodeficiency virus type 1 RNA in plasma: application to acute retroviral infection. *J. Clin. Microbiol.* **32**:292–300.
 29. **Dewar, R. L., H. C. Highbarger, M. D. Sarmiento, J. A. Todd, M. B. Vasudevachari, R. T. Davey, Jr., J. A. Kovacs, N. P. Salzman, H. C. Lane, and M. S. Urdea.** 1994. Application of branched DNA signal amplification to monitor human immunodeficiency virus type 1 burden in human plasma. *J. Infect. Dis.* **170**:1172–1179.
 30. **van Gemen, B., T. Kievits, R. Schukkink, D. van Strijp, L. T. Malek, R. Sooknanan, H. G. Huisman, and P. Lens.** 1993. Quantification of HIV-1 RNA in plasma using NASBA during HIV-1 primary infection. *J. Virol. Methods* **43**:177–187.
 31. **Centers for Disease Control and Association of Public Health Laboratories.** 2014. Laboratory testing for the diagnosis of HIV infection: Updated recommendations.
 32. **Pandori, M. W., J. Hackett Jr., B. Louie, A. Vallari, T. Dowling, S. Liska, and J. D. Klausner.** 2009. Assessment of the ability of a fourth-generation immunoassay for human immunodeficiency virus (HIV) antibody and p24 antigen to detect both acute and recent HIV infections in a high-risk setting. *J. Clin. Microbiol.* **47**:2639-2642.
 33. **Clinical and Laboratory Standards Institute (CLSI).** 2005. Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline. CLSI Document MM13-A. Wayne, PA.
 34. **29 CFR Part 1910.1030.** Occupational Exposure to Bloodborne Pathogens; current version.
 35. **Centers for Disease Control and Prevention/National Institutes of Health.** Biosafety in Microbiological and Biomedical Laboratories (BMBL); current version.
 36. **Clinical and Laboratory Standards Institute (CLSI).** 2002. Clinical Laboratory Waste Management. CLSI Document GP5-A2. Villanova, PA.
 37. **Clinical and Laboratory Standards Institute (CLSI).** 2012. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition. CLSI Document EP17-A2. Clinical and Laboratory Standards Institute, Wayne, PA.
 38. **Clinical and Laboratory Standards Institute (CLSI).** 2003. Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. CLSI document EP06-A. Clinical and Laboratory Standards Institute, Wayne, PA.



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



EMERGO EUROPE
Molenstraat 15
2513 BH, The Hague
The Netherlands

Customer Support: +1 844 Hologic (+1 844 465 6442)
customersupport@hologic.com
Technical Support: +1 888 484 4747
molecularsupport@hologic.com

For more contact information, visit www.hologic.com.

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

Armored RNA is a trademark of Asuragen, Inc.

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

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

© 2014 Hologic, Inc. All rights reserved.

AW-11853-001 Rev. 001

2014-11