

Laboratory Procedure Manual

Analyte: **HIV-1 RNA**

Matrix: **Serum/Plasma**

Method: **Aptima HIV-1 RNA Qualitative Assay**

as performed by: *HIV Laboratory Branch*
 Division of HIV/AIDS Prevention
 National Center for HIV/AIDS, Viral Hepatitis, STD, and TB
 Prevention

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Important Information for Users

CDC periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each protocol before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:

File Name	Variable Name	SAS Label
HIV_I	LBXHNAT	HIV-1 Confirmatory Test

Aptima HIV-1 RNA Qualitative Assay

1. Summary of Test Principle and Clinical Relevance – Nucleic acid amplification

The APTIMA[®] HIV-1 RNA Qualitative Assay is an in vitro nucleic acid assay system for the detection of human immunodeficiency virus (HIV-1) in human plasma. It is intended for use as an aid in the diagnosis of HIV-1 infection, including acute or primary infection. Presence of HIV-1 RNA in the plasma of patients without antibodies to HIV-1 is indicative of acute or primary HIV-1 infection. The APTIMA HIV-1 RNA Qualitative Assay may also be used as an additional test, when it is reactive, to confirm HIV-1 infection in an individual whose specimen is repeatedly reactive for HIV-1 antibodies. 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-1 is transmitted primarily by exposure to infected blood or blood products, certain body fluids or tissues, and from mother to fetus or child.

Current detection of HIV-1 infection is based on serologic testing for anti-viral antibodies by enzyme immunoassay (EIA) with confirmation by supplemental antibody tests such as Western blot or immunofluorescence assays. Although sensitivity of HIV-1 antibody detection has increased in the last few years and sensitive tests for p24 antigen (p24Ag) have been developed and implemented, a window period between infection and detectable serological markers still exists (8, 9, 17).

Following a recent exposure to HIV-1, it may take several months for the antibody response to reach detectable levels, during which time, testing for antibodies to HIV-1, including the use of rapid antibody tests, will not be indicative of true infection status. Several studies suggest that the addition of nucleic acid-based amplification tests would allow for earlier detection of HIV-1 infection (8).

Biological Principles of the Procedure

The APTIMA HIV-1 RNA Qualitative Assay involves three main steps which take place in a single tube: sample preparation; HIV-1 RNA target amplification by Transcription-Mediated Amplification (TMA) (10); and detection of the amplification products (amplicon) by the Hybridization Protection Assay (HPA) (11).

During sample preparation, RNA is isolated from specimens via the use of target capture. Specimens are treated with a detergent to solubilize the viral envelope, denature proteins and release viral genomic RNA. Oligonucleotides (“capture oligonucleotides”) that are homologous to highly conserved regions of HIV-1, are hybridized to the HIV-1 RNA target, if present, in the

test specimen. The hybridized target is then captured onto magnetic microparticles that are separated from plasma in a magnetic field. Wash steps are utilized to remove extraneous plasma components from the reaction tube. Magnetic separation and wash steps are performed with the GEN-PROBE[®] Target Capture System (TCS).

Target amplification occurs via TMA, which is a transcription-based nucleic acid amplification method that utilizes two enzymes, MMLV reverse transcriptase and T7 RNA polymerase. The reverse transcriptase is used to generate a DNA copy (containing a promoter sequence for T7 RNA polymerase) of the target RNA sequence. T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template.

Detection is achieved by HPA using single-stranded nucleic acid probes with chemiluminescent labels that are complementary to the amplicon. The labeled nucleic acid probes hybridize specifically to the amplicon. The Selection Reagent differentiates between hybridized and unhybridized probes by inactivating the label on unhybridized probes. During the detection step, the chemiluminescent signal produced by the hybridized probe is measured in a luminometer and is reported as Relative Light Units (RLU).

Internal Control is added to each test specimen, external quality control, or assay calibrator tube via the Target Capture Reagent that contains the Internal Control. The Internal Control in this reagent controls for specimen processing, amplification and detection steps. Internal Control signal in each tube or assay reaction is discriminated from the HIV-1 signal by the differential kinetics of light emission from probes with different labels (12). Internal Control specific amplicon is detected using a probe with rapid emission of light (termed flasher signal). Amplicon specific to HIV-1 is detected using probes with relatively slower kinetics of light emission (termed glower signal). The Dual Kinetic Assay (DKA) is a method used to differentiate between the signals from flasher and glower labels (12).

2. Safety Precautions

A. For in vitro diagnostic use.

B. Specimens may be infectious. Use Universal Precautions (13, 17) when performing the assay. Proper handling and disposal methods should be established according to local, state and federal regulations (14 - 16). Only personnel qualified as proficient in the use of the APTIMA HIV-1 RNA Qualitative Assay, and trained in handling infectious materials should perform this type of diagnostic procedure.

C. CAUTION: Some components of this kit contain human blood products. The Positive Calibrator in this kit contains human plasma that is HIV-1 positive and has been heat-treated to inactivate the virus. The Negative Calibrator has been assayed by FDA licensed tests and found non-reactive for the presence of hepatitis B surface antigen (HBsAg), HIV-1 p24Ag and antibodies to HIV-1/-2 and HCV. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. All human blood sourced materials should be considered potentially infectious and should be handled with Universal Precautions (13, 17). If spillage occurs, immediately disinfect, then wipe up with a 0.5% (final concentration) sodium hypochlorite solution (diluted bleach) or follow appropriate site procedures.

- D. Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink or smoke in designated work areas. Wear disposable gloves and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.
- E. This product contains sodium azide as a preservative. Do not use metal tubing for reagent transfer. If solutions containing 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.
- F. Avoid contact of Auto Detect Reagents 1 and 2 with skin, eyes and mucous membranes. Wash with water if contact with these reagents occurs. If spills of these reagents occur, dilute with water before wiping dry and follow appropriate site procedures.
- G. Dispose of all materials that have come in contact with specimens and reagents according to local, state and federal regulations (14, 15). Thoroughly clean and disinfect all work surfaces.
- H. Use only supplied or specified required disposables.
- I. Do not use this kit after its expiration date. DO NOT interchange, mix, or combine reagents from kits with different master lot numbers.
- J. Avoid microbial and ribonuclease contamination of reagents.
- K. Store all assay reagents at specified temperatures. The performance of the assay may be affected by use of improperly stored assay reagents. See Specimen Collection, Storage and Handling and Reagent Preparation sections below.
- L. Do not combine any assay reagents or fluids without specific instruction.
- M. Some reagents of this kit are labeled with risk and safety symbols. 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.

3. Computerization; Data Management System

The Aptima test results are entered manually into a Microsoft Excel spreadsheet. After a run is completed and verified in the laboratory, the Excel sheet is finalized and transmitted to Westat's ISIS computer system for review and analysis.

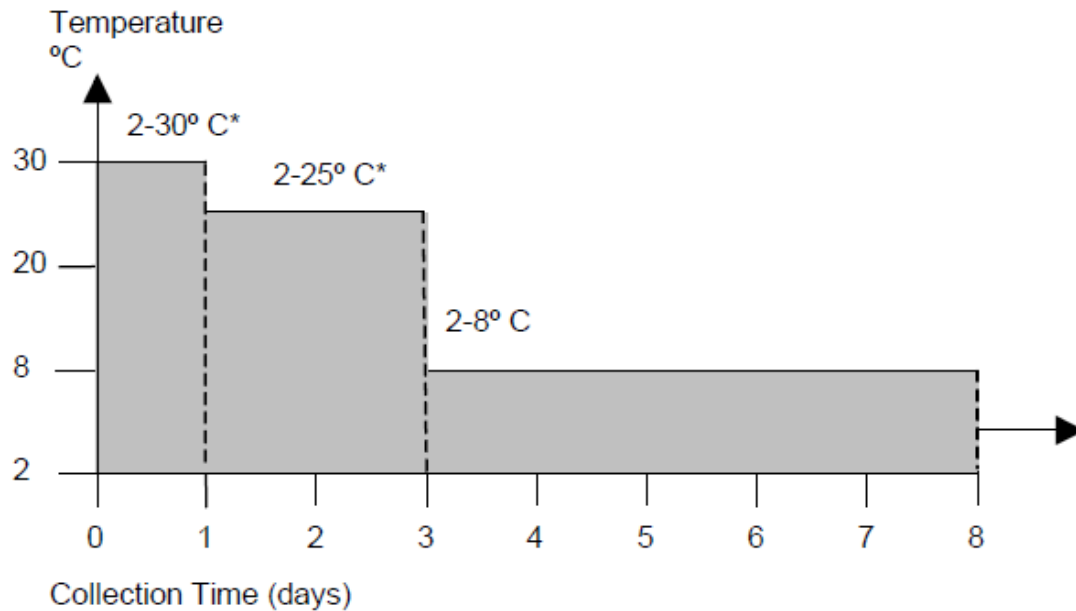
4. Specimen Collection, Storage, and Handling Procedures; Criteria for Specimen Rejection

Note: Handle all specimens as if they are potentially infectious agents.

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

A. Blood specimens collected in glass or plastic tubes may be used.

B. Specimens collected in K2EDTA, K3EDTA, ACD, sodium citrate, Becton-Dickinson EDTA Plasma Preparation Tubes (BD PPT), or serum tubes may be used. Follow sample tube manufacturer's instructions. Specimen stability is affected by elevated temperature. Whole blood, plasma or serum may be stored for up to 72 hours from time of draw at $< 25^{\circ}\text{C}$; temperatures not to exceed 30°C are acceptable for no more than 24 hours. Specimens may be stored an additional five days at 2°C to 8°C following centrifugation. Plasma separated from the cells may be stored for longer periods of time at $< -20^{\circ}\text{C}$ before testing. Long-term storage of serum has not been evaluated. Do not freeze whole blood.



*The 2-30° and 2-25°C periods indicated above may occur at any time.

C. No adverse effect on assay performance was observed when plasma or serum was subjected to three freeze-thaw cycles.

- D. Specimens with visible precipitates or fibrinous material should be clarified by centrifugation for 10 minutes at 1000 to 3000 x g prior to testing. Do not test specimens that do not have sufficient sample volume above the gel separator or red cell interface.
- E. Mix thawed plasma or serum thoroughly and centrifuge for 10 minutes at 1000 to 3000X g before testing. Centrifugation times and speeds for thawed PPT tubes must be validated by the user.
- F. Other collection and storage conditions should be validated by the user. If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiologic agents (16).
- G. False positive results may occur if cross contamination of specimens is not adequately controlled during specimen handling and processing.

5. Preparation for Microscopic Examination; Criteria for Rejection of Inadequately Prepared Slides

Not applicable for this procedure.

6. Equipment and Instrumentation, Materials, Reagent Preparation, Calibrators (Standards), and Controls

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 RNA Qualitative Assay, Cat No. 302178 (3 boxes)

Aptima HIV-1 RNA Qualitative Assay Box 1 (store at -15°C to -35°C upon receipt)

Symbol	Component	Quantity
IC	Internal Control Reagent <i>RNA transcript in HEPES buffer with detergent.</i>	1 x 1 mL
A	Amplification Reagent <i>Primers, dNTPs, NTPs, and co-factors in TRIS buffer with preservative</i>	1 x 8.5 mL
E	Enzyme Reagent <i>MMLV Reverse Transcriptase and T7 RNA Polymerase in HEPES/TRIS buffer with sodium azide (0.05%)</i>	1 x 2.8 mL
P	Probe Reagent <i>Chemiluminescent oligonucleotide probes in succinate buffer with detergent</i>	1 x 14 mL
NCAL	Negative Calibrator <i>Defibrinated normal human plasma with gentamicin and sodium azide (0.2%)</i>	4 x 2 mL
PCAL	Positive Calibrator <i>Inactivated HIV-1 positive plasma in defibrinated normal human plasma with gentamicin and sodium azide (0.2%)</i>	4 x 2 mL

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

Symbol	Component	Quantity
TCR	Target Capture Reagent <i>Capture oligonucleotides and magnetic microparticles in HEPES buffer with detergent</i>	1 x 50 mL

Aptima HIV-1 RNA Qualitative Assay Box 3 (store at 15°C to 30°C upon receipt)

Symbol	Component	Quantity
S	Selection Reagent <i>Borate buffer with surfactant.</i>	1 x 30 mL
W	Wash Solution <i>Primers, dNTPs, NTPs, and co-factors in TRIS buffer with preservative.</i>	1 x 400 mL
O	Oil <i>Silicone oil.</i>	1 x 24 mL
DF	Buffer for Deactivation Fluid <i>Sodium bicarbonate buffer, pH 9.2 to 9.4.</i>	1 x 400 mL
	Master Lot Sheet	1 sheet

Materials Required But Available Separately

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

	<u>Cat. No.</u>
Leader® HC+ Luminometer	104747
Hologic Target Capture System (TCS)	104555
Aptima HIV-1 and HCV Auto Detect Reagents	302162
Pipettor: 1000 uL	901715
1 mL serological pipets	—
Disposable 1000 uL filter tips in rack	—
Eppendorf COMBITIPS repeat pipettor tips <i>(0.5 mL, 2.5 mL, 5.0 mL, 25 mL) or equivalent</i>	—
Sterile, polypropylene conical tubes with sealing caps <i>Freestanding tubes are recommended in two different sizes (5 mL to 10 mL tube and □ 30 mL tube). The tubes must be able to accommodate the diameter of an eppendorf repeat pipettor tip.</i>	—
Ten Tip Cassettes (TTC)	104578
Ten Tube Units (TTU)	TU0022
Sealing cards	102085
Aptima HIV-1 RNA Qualitative Assay Calibrators Kit	302782
Aptima HIV-1 Proficiency Panel 302160 <i>For training purposes only</i>	
Bleach	—
<i>For use in final concentration of 5% sodium hypochlorite and 0.5% sodium hypochlorite.</i>	

3 Circulating water baths	104586
3 Water bath inserts	104627
2 Multi-tube vortex mixers	102160
3 eppendorf repeat pipettors or equivalent	105725
Worklist Editor Software	901019
Aptima HIV-1 & HCV Assay Software	901012

7. Examination (Test) Procedure

Prior to performing the assay, ALL work surfaces in the Pre and Post areas should be decontaminated with 10% bleach followed by Deionized water.

Turn the water baths on in both areas, remove the covers and allow them to reach the appropriate temperatures.

Target Capture System (TCS)

Startup:

- Check the Wash Solution levels in the Wash Solution bottle & refill if necessary. Switch the DI water bottle with Wash Solution bottle & prime the dispense pump with Wash Solution. Confirm that there are no bubbles in the line & all 10 nozzles are delivering a steady stream of liquid in the priming trough.

Check dispense volume

- Confirm that the pump dial is set to 10 ml
- Load an empty priming TTU into the separation bay.
- Dispense 1 ml of wash into an empty TTU & visually verify that 100 + 0.1 ml's are dispensed into each tube.

Check aspiration:

- Turn on the vacuum pump & observe the vacuum gauge.
- Confirm that the gauge reads between 4.5 in. to 14 in. Hg on the outside ring of the gauge.
- Load a TTC into the TTC rack
- Using the aspiration manifold, pick up the TTC tips and aspirate the wash previously dispensed into the TTU.
- Remove the TTU from the TCS & visually verify that all of the wash solution has been completely aspirated from each tube.

Creating a RUN

- Secure a specimen TTU rack
- Determine the appropriate amount of TTU's needed to perform the RUN.
- Open the Aptima assay protocol from the computer desk top.
- Scan the TTU's - then SAVE the file
- Starting in position 1 of the TTU work list, scan 3 negative calibrators followed by 3 positive calibrators
- Manually enter the sample ID's and any additional controls
- SAVE THE DATA
- Right click on the start button, select Explore, then Work list
- Insert a 3.5 floppy disk, and then drag the saved work list data to floppy.
- Transfer the floppy disk to the post amp computer
- Right click on the start button, select Explore, then 3.5 floppy
- Drag the saved floppy data to Work list.

Target Capture

- Pipette 400 ul of the working TCR into each tube.
- Pipette 500 ul of the Negative Calibrators into tubes 1 - 3
- Pipette 500 ul of the Positive Calibrators into tubes 4 - 6
- Pipette 500ul of the specimens and any additional controls into the remaining TTU's

CHANGE GLOVES HERE

- Cover the TTU's with sealing cards.
- Vortex for 20 seconds

The operator has the option to leave the tubes at room temp, for up to 75 min, before proceeding to the following 60°C incubation.

- Incubate the rack in a $60 \pm 1^\circ\text{C}$ water bath for 20 ± 1 min
- Incubate the rack at room temp (14 to 20 min)
- Incubate the rack in the TCS (9-20 min)
- Remove the sealing cards
- Aspirate; lift the rack out & visually check to make sure tubes are dry
- Add 1.0 ± 0.1 ml Wash Solution
- Cover TTU's with sealing cards and vortex 20 seconds
- Incubate in TCS (4 min to 10 min)
- Aspirate the liquid from the TTU
- Lift the rack of TTU's out & visually check to make sure that the tubes are dry
- Add 1.0 ± 0.1 ml of Wash Solution
- Cover with sealing cards and vortex 20 seconds

- Incubate in the TCS (4 min to 10 min)
- Aspirate the liquid from the TTU
- Lift the rack of TTU's out & visually check to make sure that the tubes are dry

Amplification

CHANGE GLOVES HERE

- Pipette 75 ul of Amplification Reagent into the TTU's
- Pipette 200 ul of Oil (at an angle) into the TTU's
- Cover the TTU's with sealing cards and vortex 20 seconds
- Incubate the TTU's in $60 \pm 1^\circ\text{C}$ water bath for 10 ± 1 min
- Incubate the TTU's in $41.5 \pm 1^\circ\text{C}$ water bath for 9 min to 20 min

The operator has the option to start the TCS shut down during this 9 to 20 min incubation.

CHANGE GLOVES HERE

- While the rack is in the $41.5 \pm 1^\circ\text{C}$ water bath~ pipette 25 ul of Enzyme Reagent into the TTU's .

Do not pipette at an angle – (The pipette should be held in an upright position).
Cover with sealing cards and shake the rack to mix - DO NOT VORTEX

- Incubate in $41.5 \pm 1^\circ\text{C}$ water bath for 60 ± 5 min

TCS Shutdown:

- Switch the Wash Solution bottle with a DI water bottle.
- Verify that the dispense pump is in DI water and prime the lines with DI water
- Fill the priming trough with about 200ml of 10% household bleach
- Using the priming TTC, aspirate all the bleach from the trough
- Fill the priming trough with DI water
- Aspirate the DI water from the through
- Refill the priming trough with DI water
- Aspirate again. Leave the vacuum pump ON

- Remove & discard the TTC from the TTC rack, then place the TTC rack in bleach tub.
- Place the priming trough in the bleach tub
- Verify that the aspiration manifold tubing is completely dry
- Turn OFF the vacuum pump.
- Decontaminate the TCS

- Rinse the racks with water and allow them to air dry

Proceed to HPA (Post Amplification Area)

After the 60 ± 5 min incubation in the pre amplification area, the operator has the option to leave the tubes at room temp, for up to 30 min, in the post amplification area, before the addition of probe reagent.

Start Up -- Leader HC+ computer:

- Turn on the computer, followed by the monitor, then the printer.
- Press Ctrl/Alt/Del when the computer boots
- Enter “username” as the password to enter the windows program
- Turn on the Luminometer
- Select OK
- Enter your name and password
- Check the volume of detection reagents
- Load 1 TTU, then press Wash on the Leader.
- Check the volume of reagent dispensed. (It should be even with the lower rim of the TTU)
- Place 13 cassettes on the right and 12 on the left side of the leader.
- On the leader control panel, press UTILITIES, enter (0), the select STOP.
- Verify the placement of the cassettes.

CHANGE GLOVES HERE

- Pipette 100 ul of the Probe Reagent (at an angle) into each tube
- Cover the TTU's with sealing cards and vortex for 20 seconds
- Incubate the TTU's in a $60 \pm 1^\circ\text{C}$ water bath for 15 ± 1 min – Prepare a 1:1 dilution of Deactivation Buffer if necessary.

Deactivation buffer is prepared by pouring 1 full bottle of deactivation buffer into a designated bin or receptacle. Then refilling that same bottle with bleach and pouring it into the same bin or receptacle.

CHANGE GLOVES HERE

- Pipette 250 ul of the Selection Reagent (at an angle)
- Cover the TTU's with sealing cards and vortex for 20 seconds
- Incubate the TTU's in a $60 \pm 1^\circ\text{C}$ water bath for 10 ± 1 min
- Incubate the TTU's in a $23 \pm 4^\circ\text{C}$ water bath for a minimum of 10 min

- Remove the sealing cards

DETECTION

NOTE: Readings must be completed within 75 min of completing the Selection Reagent incubation

- Wipe the TTUs with a wet kimwipe as you load them into the right side of the luminometer .
- Close and lock the door of the luminometer
- Select F3 New Run.
- Verify that “dHIV-1” is selected as the protocol; enter the number of tubes, master lot number and expiration date of the kit.
- Select F8 – START – Then NO
- At the end of the run, select OK

DECONTAMINATION

- Remove the TTUs from the Luminometer & place them in the Deactivation buffer for a minimum of 30 minutes, then discard accordingly.
- Decontaminate the work surfaces and equipment

Shut Down Leader

- Select MAIN MENU on the Leader panel, then OK
- Select LOG OFF
- Select EXIT PROGRAM

After the PC has powered down, turn off the Leader

Procedural Notes

- RUN SIZE
Each run of up to 100 tests must contain 3 replicates each of the Negative Calibrator and the Positive Calibrator.
- EQUIPMENT PREPARATION
 - Three dedicated circulating water baths must be used: one for target capture and pre-amplification ($60^{\circ}\text{C} \pm 1^{\circ}\text{C}$), one for amplification ($41.5^{\circ}\text{C} \pm 1^{\circ}\text{C}$) and one for hybridization and selection ($60^{\circ}\text{C} \pm 1^{\circ}\text{C}$). An additional water bath is required to be maintained at $23^{\circ}\text{C} \pm 4^{\circ}\text{C}$ for the step preceding detection.
 - Equilibrate circulating water baths to $60^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for target capture and $41.5^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for amplification incubations.

- Prepare the Hologic TCS for use according to instructions in the Operator's Manual.
 - Wipe work surfaces and pipettors daily with diluted bleach (0.5% sodium hypochlorite in water). Allow bleach to contact surfaces and pipettors for at least 15 minutes and then follow with a water rinse.
 - Equilibrate a circulating water bath to $60^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for hybridization and selection incubations. Prepare an additional container of water at $23^{\circ}\text{C} \pm 4^{\circ}\text{C}$ for cool down prior to detection.
 - Setup procedures for the Leader HC+ Luminometer are given in the Operator's Manual.
- REAGENTS
- Add all reagents using an eppendorf repeat pipettor (or equivalent) capable of delivering specified volume with $\pm 5\%$ accuracy and a precision of $\square 5\%$ CV. Check pipettor functionality monthly and calibrate regularly.
 - To minimize waste of Amplification, Oil, Enzyme, Probe, and Selection Reagents, aliquot each reagent for a given run size. Aliquoting must be performed after reagent preparation using sterile, polypropylene conical tubes with sealing caps in an area that is template and amplicon free. The aliquoting area must be wiped down with diluted bleach (0.5% sodium hypochlorite in water) before and after the aliquoting process. The aliquoted reagents must be used the same day the aliquoting was performed. DO NOT store reagents in the aliquot conical tubes.
 - If using Aptima HIV-1 Calibrators from a separate Aptima HIV-1 Calibrators Kit, ensure that the Master Lot number of the calibrators kit matches that of the main assay kit.
- WORK FLOW
- To minimize the possibility of laboratory areas from becoming contaminated with amplicon, the laboratory area should be arranged with a uni-directional workflow. Proceed from reagent preparation to sample preparation to amplification and then to detection areas. Samples, equipment and reagents should not be returned to the area where a previous step was performed. Also, personnel may not move from the dedicated HPA area back into previous work areas without proper anti-contamination safeguards.

Procedural Notes

- Perform reagent preparation in a template free area.
 - Perform Target Capture and Pre-Amplification steps in an amplicon-free area.
 - Perform Hybridization Protection Assay in an area separate from the reagent preparation and amplification areas.
- TEMPERATURE
- The Target Capture, Amplification, Hybridization and Selection steps are temperature dependent. Therefore, it is imperative that the water baths are maintained within the specified temperature range. Use a calibrated thermometer.

- Room temperature is defined as 15°C to 30°C.
- Detection is sensitive to temperature. The laboratory temperature in the detection area must be 21°C to 27°C.
- TIME
The Target Capture, Amplification, and Hybridization Protection Assay steps are all time dependent. Adhere to specific times outlined in *Quality Control Procedures* on page 14. Use a calibrated timer.
- VORTEXING
Proper vortexing is important to the successful performance of the Aptima HIV-1 RNA Qualitative Assay. Vortex equipment speed settings may vary. Start the vortexor at low speed and then adjust upward to allow reaction mixture to reach and maintain a height within the upper half of all tubes. The reaction mixture should never touch the sealing cards. **It is critical to have a homogeneous mixture after the additions of the Probe and Selection Reagent.**
- PIPETTING
 - All pipettors used in the Target Capture, Amplification and HPA steps must be dedicated.
 - Take care to deliver reagents, excluding working TCR, to each tube without inserting pipette tip into the tube or touching the rim of the tube to minimize the chance of carryover from one tube to another.
- SPECIMEN PIPETTING
 - Improper pipetting technique will affect the results of the assay. In order to avoid the loss of Positive ID Tracking, verification of correct sample ID by a second individual is recommended.
 - Ensure that the TTU is oriented in the rack with the pointed end on the left side and the rounded end on the right side of the rack. Pipette the first calibrator into the first tube next to the pointed end of the TTU. Samples are pipetted from left to right.
 - Use a new pipette tip for each sample and dispose of the tip in a biological waste container after use. Take care to avoid cross-contamination by pipetting the specimens and discarding the used pipette tips without passing over open tubes or touching laboratory surfaces or other pieces of equipment.
 - To avoid the risk of contamination, clean and decontaminate sample pipettors between assay runs.
 - Ensure proper sample placement into the correct TTU position as indicated on the manual work list record.
- DECONTAMINATION
 - The extremely sensitive nature of the test makes it imperative to take all possible precautions to avoid contamination. Laboratory bench surfaces, and pipettes must be decontaminated daily with diluted bleach (0.5% sodium hypochlorite in water). Allow bleach to contact surfaces for at least 15 minutes and then follow with a water rinse. Chlorine solutions may pit equipment

and metal. Thoroughly rinse bleached equipment to avoid pitting. Reactions must be decontaminated with Deactivation Fluid as described in the detection procedure.

- **SEALING CARDS**
 - When applying sealing cards, cover the TTUs with the sealing card and press gently to ensure complete contact with all of the tubes. Always use a new sealing card. **DO NOT** reuse sealing cards.
 - When removing sealing cards, carefully lift and peel in one continuous motion to avoid aerosols and cross contamination. Immediately dispose of card in appropriate waste container.

8. Method Performance Specifications

Reproducibility

For determination of the reproducibility of the Aptima HIV-1 RNA Qualitative Assay five panel members were tested. Four of these panel members were HIV-1 RNA positive, and one was HIV-1 RNA negative. (Table 1).

The reproducibility panels were tested by a total of six operators (two at each site) with three different Clinical Lots over at least 18 nonconsecutive days.

Inter- and intra-assay variability and inter-lot variability were determined.

Mean S/CO, standard deviation (SD) and coefficient of variation (%CV) results are shown for panel members and for the Negative and Positive Calibrators.

Since HIV-1 RNA positive samples containing 90 copies/mL or greater gave high (saturated) signals, results on multiple panel members are combined. (Table 1)

Table 1. Reproducibility of the Aptima HIV-1 RNA Qualitative Assay (excludes 10 false positive results)

Specimen	N	Concentration Copies/mL	Number of replicates	% Agreement	Mean S/CO	Intra-Assay		Inter-Assay		Inter-Lot	
						SD	% CV	SD	% CV	SD	% CV
Nonreactive	1	0	322	100	0.19	0.050	26.3	0.029	15.3	0.024	12.3
HIV-1	4	150, 500, 1500, 10000	1289	100	19.69	2.391	12.1	1.114	5.7	0.883	4.5
Specimen	Number of replicates	% Agreement	Mean RLU	Intra-Assay		Inter-Assay		Inter-Lot			
				SD	% CV	SD	% CV	SD	% CV		
Negative Calibrator	323	N/A	8900	2121	23.8	1824	20.5	1470	16.5		
Positive Calibrator	320	N/A	894464	57091	6.4	63756	7.1	30695	3.4		

N = Number of panel members combined for this analysis.

Specificity

Clinical Specificity

The specificity of the Aptima HIV-1 RNA Qualitative Assay was determined in two different studies from different donor populations. The first was from a population of individual donor specimens obtained at eight volunteer blood donor sites and the second was from paid source plasma donors collected at two different sites. Testing for both studies was conducted with three different clinical lots. In the first study with volunteer blood donors, the initial reactive rate was 0.24% (6/2508). In this study, initially reactive specimens were not retested. In the second study with paid source plasma donors, 1,012 specimens were tested. Of the 1,007 valid results, none were reactive, for a 0% initial false reactive rate. The overall specificity rate between the two studies was 99.83% (6/3515 initial reactive). In an additional study conducted in-house, in which EIA nonreactive specimens from volunteer blood donors were tested in the Aptima HIV-1 RNA Qualitative Assay with three reagent lots, the initial reactive rate was 0.12% (2/1701). Both initial reactive specimens were nonreactive upon retest.

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Performance Characteristics

To assess the specificity of the Aptima HIV-1 RNA Qualitative Assay in serum specimens, 211 serum specimens were tested in an in-house study. Of the 211 serum specimens tested, 1 (or 0.47%) was initially reactive but found to be non-reactive in duplicate retesting. The resolved specificity of 100% showed that assay specificity was not affected by the use of serum as a specimen type.

Sixteen of 16 HIV-1 seroreactive, Western blot positive specimens identified from the screening of volunteer blood donors were also Aptima HIV-1 RNA Qualitative Assay reactive. One HIV-1 seroreactive, Western blot indeterminate specimen was nonreactive in the Aptima HIV-1 RNA Qualitative Assay.

Non-Specificity Studies

When tested with the Aptima HIV-1 RNA Qualitative Assay, no cross-reactivity or interference was observed for naturally occurring icteric, hemolyzed or lipemic specimens or plasma containing the following substances: serum albumin (up to 225 g/L), hemoglobin (up to 5000 mg/L), bilirubin (up to 200 mg/L) and lipids (up to 2,752 mg/dL). No cross-reactivity or interference was observed in specimens from patients with autoimmune diseases or with liver diseases not caused by HCV infection. Autoimmune conditions included rheumatoid arthritis (n = 10), rheumatoid factor (n = 10), antinuclear antibody (n = 10), multiple sclerosis (n = 10), lupus (n = 10) and multiple myeloma (n = 9). Also tested were flu vaccinees (n = 10), hepatitis B vaccinees (n = 10), elevated IgM (n = 6), elevated IgG (n = 11), alcoholic liver cirrhosis (n = 10) and elevated ALT (n = 10).

No cross-reactivity or interference was observed in bacterially contaminated plasmas

or in plasmas infected with other blood borne pathogens, including herpes simplex virus-1 (n = 10), herpes simplex virus-2 (n = 1), CMV (n = 10), EBV (n = 10), hepatitis A virus (n = 10), HTLV-I (n = 10), HTLV-II (n = 10), hepatitis B virus (n = 10), HIV-2 (n = 10), rubella (n = 10) and parvovirus B-19 (n = 10).

Clinical Sensitivity

Testing of Specimens from HIV-1 Infected Individuals

A total of 1041 specimens positive by commercial HIV-1 RNA assay (sensitivity ≥ 100 copies/mL) were obtained from four commercial vendors. Three Clinical Lots were used for all testing. These specimens were classified as HIV-1 RNA positives (N = 868) and both HIV-1 and HCV RNA (coinfected) positives (N = 174) based on alternate nucleic acid testing. These positive samples were tested undiluted (neat) in the Aptima HIV-1 RNA Qualitative Assay. During the study, specimens known to contain < 100 copies/mL of viral RNA were excluded from this analysis and therefore the sensitivity presented herein is for samples with viral RNA concentrations equal to or greater than 100 copies/mL, or of unknown viral concentration.

All 1041 known positive specimens were reactive in the Aptima HIV-1 RNA Qualitative Assay. The overall clinical sensitivity of the Aptima HIV-1 RNA Qualitative Assay in this study was 100% (95% CI: 99.6-100%).

Performance Characteristics

The data from the above study were further analyzed according to the disease stages of the patients from whom the specimens were obtained as shown in Table 3. A total of 296 samples were from AIDS patients (as defined by AIDS-indicative conditions and/or a CD4 count of $<200/\text{mm}^3$), 338 from

asymptomatic patients (asymptomatic, persistent generalized lymphadenopathy, or acute HIV-1 infection), 168 from symptomatic but non-AIDS patients (not AIDS and not asymptomatic) and 239 from individuals with unknown HIV disease state (18). Treatment history was known for 613 individuals, 500 of which were on anti-viral medication. Of these 500, 251 were AIDS patients, 145 were symptomatic but non-AIDS patients, and 104 were asymptomatic patients. The sensitivity of the Aptima HIV-1 RNA Qualitative Assay in confirming HIV-1 infection in this study was 100%. All HIV-1 p24 Ag reactive specimens were also reactive with the Aptima HIV-1 RNA Qualitative Assay. The disease stages for HIV-1 infected individuals did not significantly affect the sensitivity of the Aptima HIV-1 RNA Qualitative Assay.

More information on assay performance is available in the Aptima Product insert,

9. Reportable Range of Results

Reportable results are expressed as positive or negative.

10. Quality Control (QC) Procedures

Acceptance Criteria for the Aptima HIV-1 RNA Qualitative Assay

Run Validity Criteria

A run is invalid and must be repeated if any of the following conditions occur:

- More than one positive and one negative calibrator values are invalid. Cutoff values will not be calculated for Internal Control and Analyte signals.
- Operator observes and documents technical, operator, or instrument difficulties while performing the assay.

Acceptance Criteria for the Calibration and Calculation

of Cutoff Negative Calibrator Acceptance Criteria

- Each individual Negative Calibrator must also have an Analyte value less than or equal to 40,000 RLU and greater than or equal to 0 RLU.
- Each individual Negative Calibrator (NC) must have an Internal Control (IC) value greater than or equal to 75,000 RLU and less than or equal to 300,000 RLU.
- If one of the Negative Calibrator values is invalid due to an IC value or Analyte value that is outside of these limits, the Negative Calibrator mean (NC_x) will be recalculated based upon the two acceptable values.
- The run is invalid and must be repeated if two or more of the three Negative Calibrator values have IC values or Analyte values that are outside of these limits.

Determination of the mean of the Negative Calibrator (NC_x) values for Internal Control [NC_x (Internal Control)].

Determination of the mean of the Negative Calibrator values (NC_x) for Analyte [NC_x (Analyte)].

Example:

Negative Calibrator	Internal Control Relative Light Units
1	124,000
2	126,000
3	125,000
Total Internal Control RLU =	375,000
NC_x (Internal Control) = $\frac{\text{Total Internal Control RLU}}{3} = 125,000$	

Example:



Acceptance Criteria for the Aptima HIV-1 RNA Qualitative Assay

Negative Calibrator	Analyte Relative Light Units
1	12,000
2	11,000
3	13,000
Total Analyte RLU =	36,000
$NC_x (\text{Analyte}) = \frac{\text{Total Analyte RLU}}{3} = 12,000$	

Positive Calibrator Acceptance Criteria

- Individual Positive Calibrator (PC) Analyte values must be less than or equal to 1,800,000 RLU and greater than or equal to 300,000 RLU.
- IC values may not exceed 475,000 RLU.
- If one of the Positive Calibrator values is outside these limits, the Positive Calibrator mean (HIV-1 PC_x) will be recalculated based upon the two acceptable Positive Calibrator values.
- The run is invalid and must be repeated if two or more of the three Positive Calibrator Analyte values are outside these limits.

Determination of the mean of the Positive Calibrator (HIV-1 PC_x) values for Analyte [HIV-1 PC_x (Analyte)].

Example:

Positive Calibrator	Analyte Relative Light Units
1	690,000
2	700,000
3	710,000
Total Analyte RLU =	2,100,000

$$\begin{aligned} \text{HIV-1 PC}_x (\text{Analyte}) &= \frac{\text{Total Analyte RLU}}{3} \\ &= \frac{2,100,000}{3} \\ &= 700,000 \end{aligned}$$

Calculation of the Internal Control Cutoff Value

Internal Control Cutoff Value = $0.5 \times [NC_x \text{ (Internal Control)}]$. Using values given in the Negative Calibrator example above: Internal Control Cutoff Value = $0.5 \times (125,000)$
Internal Control Cutoff Value = 62,500 RLU

Calculation of the Analyte Cutoff Value

Analyte Cutoff Value = $NC_x \text{ (Analyte)} + [0.04 \times \text{HIV-1 PCx (Analyte)}]$
Using values given in the Negative Calibrator and Positive Calibrator examples above: Analyte Cutoff Value = $12,000 + (0.04 \times 700,000)$
Analyte Cutoff Value = 40,000 RLU

Interpretation of Results

All calculations described above are performed by the Aptima HIV-1 & HCV Assay Software. Two cutoffs are determined for each assay: one for the Analyte signal (glower signal) termed the Analyte Cutoff and one for the Internal Control signal (flasher signal) termed the Internal Control Cutoff. The calculation of these cutoffs is shown above. For each sample, an Analyte signal RLU value and Internal Control signal RLU value is determined. Analyte signal RLU divided by the Analyte Cutoff is abbreviated as the Analyte Signal/Cutoff (S/CO) on the report.

For a sample with Analyte signal less than the Analyte Cutoff (i.e., Analyte S/CO < 1), the Internal Control (IC) signal must be greater than or equal to the Internal Control Cutoff (IC Cutoff) and less than 475,000 RLU for the result to be valid. In this case the Internal Control result will be reported as Valid and the sample is reported as Nonreactive. For a sample with the Analyte signal less than the Analyte Cutoff (i.e., Analyte S/CO < 1) and the Internal Control signal less than the Internal Control Cutoff, the Internal Control Result will be reported as Invalid and the sample result is reported as Invalid. For a sample with the Analyte signal greater than the Analyte Cutoff (i.e., Analyte S/CO \geq 1) and the IC signal less than 475,000 RLU, the sample result is reported as Reactive.

Summary of Sample Validity

Sample Interpretation	Criteria
Nonreactive	Analyte S/CO < 1 and IC \geq IC Cutoff and IC \leq 475,000 RLU
Reactive	Analyte S/CO \geq 1 and IC \leq 475,000 RLU
Invalid	IC > 475,000 or Analyte S/CO < 1 and IC < IC Cutoff

Interpretation of Specimen Results

1. A specimen with an overall interpretation of **Invalid** should be retested. If the same result is generated in repeat testing, the interpretation remains invalid.
 - A **Reactive** result indicates that HIV-1 RNA was detected. For a specimen that is repeatedly reactive on an HIV-1 antibody test and reactive in the Aptima HIV-1 RNA Qualitative Assay, the individual is considered confirmed infected with HIV-1 (see Row A in the table below). The individual should be referred for medical follow-up.
 - If a specimen is nonreactive on an HIV-1 antibody test and reactive in the Aptima HIV-1 RNA Qualitative Assay, then the specimen should be retested in duplicate in the Aptima HIV-1 RNA Qualitative Assay.
 - If the antibody nonreactive specimen is reactive in at least one of the two retest replicates in the Aptima HIV-1 RNA Qualitative Assay, the specimen is repeatedly reactive. This would indicate possible acute or primary HIV-1 infection (see Row B in the table below). The individual should be referred for medical follow-up and additional testing.
 - If the antibody nonreactive specimen is nonreactive in both retest replicates in the Aptima HIV-1 RNA Qualitative Assay, this would indicate an initial false positive and the result is interpreted as nonreactive (see Row D in the table below). HIV-1 RNA was not detected.
 - A **Nonreactive** result indicates that HIV-1 RNA was not detected. A specimen that is nonreactive in the Aptima HIV-1 RNA Qualitative Assay and repeatedly reactive in a test for HIV-1 antibodies should be tested by Western blot or immunofluorescent assay (IFA) to confirm the presence of HIV-1 antibodies (see Row C in the table below). The individual should be referred for medical follow-up and additional testing.
 - A specimen that is nonreactive in the Aptima HIV-1 RNA Qualitative Assay and nonreactive for HIV-1 antibodies (or a test for HIV-1 antibodies has not been done) should be interpreted that HIV-1 RNA was not detected (see row D in the table below). However, a nonreactive test result does not preclude the possibility of exposure to or infection with HIV-1.
 - A specimen that is reactive in the Aptima HIV-1 RNA Qualitative Assay but that has not been tested in an HIV-1 antibody test should be further tested using a licensed test for HIV-1 antibodies.

Summary of Interpretation of Specimen Results

* The individual should be referred for medical follow-up and additional testing.

** Antibody results should be confirmed with Western blot or IFA.

*** A nonreactive test result does not preclude the possibility of exposure to or infection with HIV-1.

12. Limitations of the Procedure

The concentrations for HIV-1 subtype N and group O virus used for assessing analytical sensitivity were determined by an in-house quantitative test, which used the same technology as the Aptima assays. This may result in inaccurate assessment of analytical sensitivity for these viral subtypes. The effect of anti-retroviral drugs has not been evaluated analytically; no effect from these drugs was observed on detection of HIV-1 RNA in clinical specimens.

13. Reference Ranges (Normal Values)

A normal sample is negative for HIV-1 RNA.

14. Critical Call Results (Panic Values)

Not applicable to this assay method.

15. Specimen Storage and Handling during Testing

Specimens are stored at $\leq -70^{\circ}\text{C}$ until testing. After an aliquot of the thawed sample has been removed for testing, the residual is refrozen and stored at $\leq -70^{\circ}\text{C}$.

16. Alternate Methods for Performing Test or Storing Specimens if Test System Fails

If the analytical system fails, store specimens at $\leq -70^{\circ}\text{C}$ until the system is investigated and the problem is resolved. .

17. Test Result Reporting System; Protocol for Reporting Critical Calls (If Applicable)

Not applicable to this assay method.

18. Transfer or Referral of Specimens; Procedures for Specimen Accountability and Tracking

Standard record keeping involves using the computerized database and the hard copy results themselves to track specimens. Records are maintained indefinitely. Only numerical identifiers (e.g., case ID numbers) should be used. All personal identifiers should be available only to the medical supervisor or project coordinator to safeguard confidentiality.

For the NHANES study, residual serum is retained at $\leq -70^{\circ}\text{C}$ for 1 year and then returned to NCHS serum bank.

19. Summary Statistics and QC Graphs

Assay controls are monitored for proper performance on each run.

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