

APTIMA® HIV-1 RNA Qualitative Assay

For *in vitro* diagnostic use.

100 test kit.

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Intended Use

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 and serum. 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 or serum 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).

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).

Materials Provided

APTIMA® HIV-1 RNA Qualitative Assay - P/N 302178

Box 1

| Symbol | Component | Quantity | Description | Storage |
|--------|--------------------------|------------|---|-------------------------|
| IC | Internal Control Reagent | 1 x 1 mL | RNA transcript in HEPES buffer with detergent | -15°C to -35°C unopened |
| A | Amplification Reagent | 1 x 8.5 mL | Primers, dNTPs, NTPs, and co-factors in TRIS buffer with preservative | -15°C to -35°C unopened |
| E | Enzyme Reagent | 1 x 2.8 mL | MMLV Reverse Transcriptase and T7 RNA Polymerase in HEPES/TRIS buffer with sodium azide (0.05%) | -15°C to -35°C unopened |
| P | Probe Reagent | 1 x 14 mL | Chemiluminescent oligonucleotide probes in succinate buffer with detergent | -15°C to -35°C unopened |
| NCAL | Negative Calibrator | 4 x 2 mL | Defibrinated normal human plasma with gentamicin and sodium azide (0.2%) | -15°C to -35°C |
| PCAL | Positive Calibrator | 4 x 2 mL | Inactivated HIV-1 positive plasma in defibrinated normal human plasma with gentamicin and sodium azide (0.2%) | -15°C to -35°C |

Box 2

| Symbol | Component | Quantity | Description | Storage |
|--------|------------------------|-----------|---|------------|
| TCR | Target Capture Reagent | 1 x 50 mL | Capture oligonucleotides and magnetic microparticles in HEPES buffer with detergent | 2°C to 8°C |

Box 3

| Symbol | Component | Quantity | Description | Storage |
|--------|-------------------------------|------------|---|--------------|
| S | Selection Reagent | 1 x 30 mL | Borate buffer with surfactant | 15°C to 30°C |
| W | Wash Solution | 1 x 400 mL | HEPES buffer with detergent and preservatives | 15°C to 30°C |
| O | Oil | 1 x 24 mL | Silicone oil | 15°C to 30°C |
| DF | Buffer for Deactivation Fluid | 1 x 400 mL | Sodium bicarbonate buffer, pH 9.2 to 9.4 | 15°C to 30°C |

Materials Required, Sold Separately

APTIMA® HIV-1 Proficiency Panel (Cat. No. 302160)

APTIMA® HIV-1 and HCV Auto Detect Reagents (Cat. No. 302162)

Bleach

For use in final concentration of 5% sodium hypochlorite and 0.5% sodium hypochlorite.

Disposables

(Disposables are single use only, do not reuse. Use of other disposables is not recommended.)

1 mL serological pipets

Disposable 1000 µL filter tips in rack

Eppendorf COMBITIPS repeat pipettor tips (2.5 mL, 5.0 mL, 25 mL) or equivalent

Sterile, polypropylene conical tubes with sealing caps

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.

Ten Tip Cassettes (TTC) (Cat. No. 104578)

Ten Tube Units (TTU) (Cat. No. TU0022)

Sealing cards (Cat. No. 102085)

Equipment and Software

Pipettor: 1000 µL (Cat. No. 901715)

3 Circulating water baths (Cat. No. 104586)

3 Water bath inserts (Cat. No. 104627)

2 Multi-tube vortex mixers (Cat. No. 102160)

3 Eppendorf repeat pipettors (Cat. No. 105725) or equivalent

GEN-PROBE® LEADER® HC+ Luminometer (Cat. No. 104747)


GEN-PROBE® Target Capture System (TCS) (Cat. No. 104555)

Worklist Editor Software (Cat. No. 901019)

APTIMA® HIV-1 & HCV Assay Software (Cat. No. 901012)

Storage Instructions

A. Room temperature is defined as 15°C to 30°C.

B.  The APTIMA HIV-1 RNA Qualitative Assay Probe Reagent is light sensitive. Protect this reagent from light during storage and preparation for use.

C. Target Capture Reagent (TCR) is stable when stored unopened at 2°C to 8°C until the expiration date. Do not use after expiration date. If a precipitate forms in the Target Capture Reagent during storage, see instructions under *Reagent Preparation* on page 4. DO NOT VORTEX. DO NOT FREEZE Target Capture Reagent.

Note: If after removing the TCR from storage at 2°C to 8°C, the precipitate is allowed to settle to the bottom of the container, the likelihood of the formation of a gelatinous precipitate is increased substantially.

D. Selection Reagent is stable when stored unopened at room temperature until the expiration date. Do not use after expiration date. Mix thoroughly prior to use.

E. The following reagents are stable when stored unopened at room temperature until the expiration date.

Wash Solution

Oil

Auto Detect 1

Auto Detect 2

Buffer for Deactivation Fluid

Do not use after expiration date.

- F. Once opened, Wash Solution, Oil, Selection Reagent, Buffer for Deactivation Fluid, Auto Detect 1 and Auto Detect 2 are stable for 30 days when stored at room temperature.
- G. The following reagents are stable when stored unopened at -15°C to -35°C until the expiration date:
- Internal Control Reagent
Amplification Reagent
Enzyme Reagent
Probe Reagent
Negative Calibrator
Positive Calibrator
Do not use after expiration date.
- H. After thawing, the Amplification Reagent, Enzyme Reagent, and Probe Reagent are stable when stored at 2°C to 8°C for 30 days. Once completely thawed, these reagents may be kept at room temperature up to 8 hours per 24-hour period while in use, not to exceed 80 hours at room temperature. Do not refreeze Amplification, Enzyme, and Probe Reagents after the initial thaw.
- I. After thawing, Negative and Positive Calibrators may be kept at room temperature up to 8 hours. These are single use vials and must be discarded after use.
- J. After addition of Internal Control Reagent, the working Target Capture Reagent is stable when stored at 2°C to 8°C for 30 days and may be kept at room temperature up to 8 hours per 24-hour period while in use, not to exceed 80 hours at room temperature.
- K. If precipitate forms in the Wash Solution, Amplification Reagent, or Probe Reagent, warm to 15°C to 30°C and mix thoroughly prior to use. See instructions under *Reagent Preparation* on page 4.
- L. If precipitate forms in the Selection Reagent during storage, see instructions under *Reagent Preparation* on page 4.
- M. Changes in the physical appearance of the reagents supplied may indicate instability or deterioration of these materials. If changes in the physical appearance of the reagents are observed (e.g., obvious changes in reagent color or cloudiness apparent with microbial contamination), they should not be used.

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* on page 4 and *Reagent Preparation* on page 4.
- 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 according to the European Directive 1999/45/EC and should be handled accordingly.

Material Safety Data Sheets are available upon request.

The following reagents contain 0.2% sodium azide as a preservative:

Negative Calibrator

Positive Calibrator



Xn. Harmful

R22/R32/S2
S13/S36/S46

| | |
|-----|--|
| R22 | Harmful if swallowed |
| R32 | Contact with acid liberates very toxic gas |
| S2 | Keep out of reach of children |
| S13 | Keep away from food, drink, and animal feeding stuffs |
| S36 | Wear suitable protective clothing |
| S46 | If swallowed, seek medical advice immediately and show this container or label |

Refer to *Warnings* and *Precautions* in other applicable APTIMA Assay package inserts and in APTIMA and Gen-Probe System Operator's Manuals.

Reagent Preparation

Note: This step should be performed prior to beginning Target Capture in an area that is free of template and amplicon.

- Warm all reagents to room temperature and mix thoroughly prior to use. A dedicated water bath at room temperature may be used to aid this process. Ensure that precipitates are dissolved. Do not use a reagent if precipitate or cloudiness is present. See step 7 for Target Capture Reagent preparation.
- DO NOT heat the Probe Reagent above 30°C.
- Thaw reagents upright.
- If necessary, thaw Amplification, Probe, and Enzyme Reagents at room temperature or at 2°C to 8°C. Amplification and Probe Reagents may be mixed by vortexing. Enzyme Reagent should be mixed thoroughly by gentle inversion taking care to avoid excessive foaming. Once completely thawed, these reagents may be kept at room temperature up to 8 hours per 24-hour period while in use. These reagents are stable for 30 days when stored at 2°C to 8°C. Record date of thaw (THAW DATE) for Amplification, Probe, and Enzyme Reagents in the space provided on the label.
- Precipitate will form in the Probe Reagent when stored at 2°C to 8°C. Probe Reagent may be warmed in a water bath to facilitate dissolution of precipitate, but temperature in the bath should not exceed 30°C. The Probe Reagent may take up to 4 hours with periodic mixing to allow complete dissolution of precipitate if thawing is conducted on the lab bench. Ensure that precipitates in the Probe Reagent are dissolved. Do not use if precipitate or cloudiness is present.
- Selection Reagent is stored at room temperature. If Selection Reagent has been inadvertently stored at 2°C to 8°C or the temperature of the laboratory falls below 15°C, precipitate may form. If precipitate forms in the Selection Reagent during storage, heat at 60°C ± 1° C for no more than 45 minutes, shaking the bottle frequently (every 5 to 10 minutes). Once all precipitate has gone back into solution, place the bottle in a room temperature water bath and allow the bottle to equilibrate for at least 1 hour. Do not use the Selection Reagent until it has equilibrated. The Selection Reagent must be at room temperature before use. Do not use if precipitate or cloudiness is present.
- Prepare working Target Capture Reagent: thaw one vial of Internal Control Reagent at room temperature or 2°C to 8°C. Mix the Internal Control Reagent thoroughly by inversion. Remove Target Capture Reagent (TCR) from 2°C to 8°C storage. IMMEDIATELY upon removing from storage, mix vigorously (at least 10 inversions). DO NOT VORTEX. After mixing, place the TCR bottle at 22°C to 30°C. Approximately every 10 minutes shake the bottle until all precipitate has disappeared. TCR precipitate should normally dissolve in about 30 minutes. If a gel is observed after performing this procedure, a new bottle must be used according to the handling recommendations above. Return the bottle with gel back to 2°C to 8°C storage for subsequent use. When the Internal Control Reagent and TCR have reached room temperature, mix TCR thoroughly by inversion. Using a serological pipettor, add 1 mL of Internal Control Reagent into the TCR bottle. The total time for each of these reagents at room temperature must not exceed 8 hours, in the first 24-hour period. This is now the working Target Capture Reagent. Mix thoroughly. Use the space indicated on the TCR bottle to record the date Internal Control Reagent was added and lot number used (IC LOT). Record the expiration date of the working TCR in the space provided on the label.
- Thaw calibrators at room temperature. These are single use vials and must be thawed prior to each run. Once thawed, use calibrators within 8 hours. Mix thoroughly by inversion.
- Wash Solution is shipped at ambient temperature and stored at room temperature. Precipitates may form in the Wash Solution during shipment or during storage when temperatures fall below 15°C. Wash Solution may be incubated in a warm water bath to facilitate dissolution of precipitate. Temperature in the bath should not exceed

30°C. Ensure that precipitates in the Wash Solution are dissolved prior to use. Do not use if precipitate or cloudiness is present.

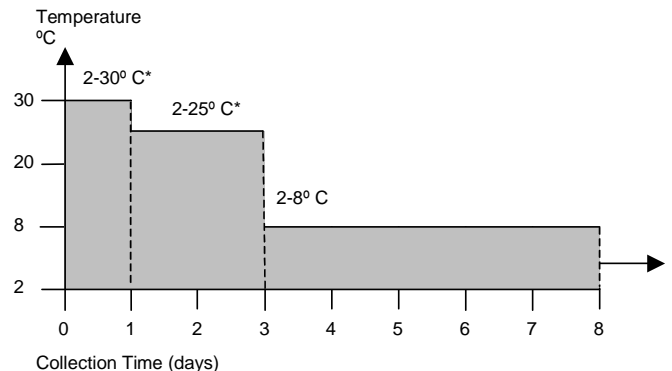
- Once opened, Wash Solution, Oil, Selection Reagent, Buffer for Deactivation Fluid, Auto Detect 1 and Auto Detect 2 are stable for 30 days when stored at room temperature. Record the date the reagent was first opened (OPEN DATE) in the space provided on the label.
- To prepare Deactivation Fluid, mix one part Buffer for Deactivation Fluid with one part 5% sodium hypochlorite. Deactivation Fluid is stable for 30 days when stored at room temperature.

Specimen Collection, Storage and Handling

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.

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

- No adverse effect on assay performance was observed when plasma or serum was subjected to three freeze-thaw cycles.
- 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.
- Mix thawed plasma or serum thoroughly and centrifuge for 10 minutes at 1000 to 3000 x g before testing. Centrifugation times and speeds for thawed PPT tubes must be validated by the user.
- 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).
- False positive results may occur if cross contamination of specimens is not adequately controlled during specimen handling and processing.

Procedural Notes

A. RUN SIZE

Each run of up to 100 tests must contain 3 replicates each of the Negative Calibrator and the Positive Calibrator.

B. EQUIPMENT PREPARATION

1. 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.
2. 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.
3. Prepare the GEN-PROBE TCS for use according to instructions in the Operator's Manual.
4. 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.
5. 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.
6. Setup procedures for the LEADER HC+ Luminometer are given in the Operator's Manual.

C. REAGENTS

1. Add all reagents using an Eppendorf repeat pipettor (or equivalent) capable of delivering specified volume with $\pm 5\%$ accuracy and a precision of $\leq 5\%$ CV. Check pipettor functionality monthly and calibrate regularly.
2. 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.

D. WORK FLOW

1. 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.
2. Perform reagent preparation in a template free area.
3. Perform Target Capture and Pre-Amplification steps in an amplicon-free area.
4. Perform Hybridization Protection Assay in an area separate from the reagent preparation and amplification areas.

E. TEMPERATURE

1. 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.
2. Room temperature is defined as 15°C to 30°C .
3. Detection is sensitive to temperature. The laboratory temperature in the detection area must be 21°C to 27°C .

F. TIME

The Target Capture, Amplification, and Hybridization Protection Assay steps are all time dependent. Adhere to specific times outlined in *Instructions for Use* on page 6. Use a calibrated timer.

G. 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.**

H. PIPETTING

1. All pipettors used in the Target Capture, Amplification and HPA steps must be dedicated.
2. 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.

I. SPECIMEN PIPETTING

1. 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.
2. 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.
3. 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.
4. To avoid the risk of contamination, clean and decontaminate sample pipettors between assay runs.
5. Ensure proper sample placement into the correct TTU position as indicated on the manual work list record.

J. DECONTAMINATION

1. 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.
2. Reactions must be decontaminated with Deactivation Fluid as described in the detection procedure.

K. SEALING CARDS

1. 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.
2. 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.

Instructions for Use

Do not use this kit after its expiration date. DO NOT interchange, mix, or combine reagents from kits with different master lot numbers.

Note: All process steps described below are intended to be completed in a continuous flow with a minimal, if any, delay between steps.

Note: Refer to the applicable Procedural Note for each step.

A. TARGET CAPTURE

The assay results within the run report will be marked "M" indicating that the specimens were manually pipetted.

1. For sample tracking, an electronic worklist must be created using the Worklist Editor software. Refer to the APTIMA Assay Software Operator's Manual for instructions. Verification of correct sample ID on the worklist with the specimen tubes and with the detailed assay run report by a second individual is recommended.
2. Load sufficient Ten Tube Units (TTUs) for the run into a TTU rack.
3. Thoroughly mix working TCR immediately before use to resuspend microparticles.
4. Refer to the worklist and carefully pipette 400 μL of working TCR to each reaction tube that will contain a specimen. **To dispense, insert the tip approximately one quarter of the way into the tube at an angle and pipette working TCR down the side of the tube. Always pipette the working TCR first, followed by the specimen.**
5. Pipette specimens.
 - a. Refer to the worklist to identify the TTU number with the corresponding calibrator and test specimen identification numbers.
 - b. Ensure that the Negative Calibrator is pipetted into the first three tubes. Ensure that the Positive Calibrator is pipetted into the second three tubes.
 - c. Aspirate 500 μL of each calibrator or test sample from its collection tube using a single channel pipettor with corresponding filtered disposable tip. Insert only the end of the pipette tip into the specimen. Do not disturb the sediment, if any.
 - d. To dispense, insert the pipette tip halfway into the tube taking care not to touch the sides of the upper half of the tube with the pipette tip. At an angle, pipette the specimen down the side of the bottom half of the tube. Hold down the plunger of the pipettor while removing it from the tube. Take care to avoid touching the rim or the side of the tube with the pipette tip when removing it from the tube.
6. Replace pipette tip with a new tip and repeat Step 5 until all specimens have been pipetted.
7. Visually inspect tubes to ensure proper specimen volume and working TCR volume have been dispensed.
8. Cover the TTUs with sealing cards.
9. Vortex the rack of TTUs a minimum of 20 seconds and until magnetic microparticles are resuspended.
10. Rack may remain at room temperature up to 75 minutes prior to proceeding to the 60°C \pm 1°C incubation.
11. Incubate the tubes in a water bath at 60°C \pm 1°C for 20 minutes \pm 1 minute.
12. Remove the rack of TTUs and transfer to target capture area.
13. Incubate the rack of TTUs on the lab bench at room temperature for 14 minutes to 20 minutes.
14. Transfer the rack of TTUs to the Gen-Probe TCS separation bay for 9 to 20 minutes.

15. Carefully remove and dispose of the sealing cards.
16. Aspirate the solution from each tube according to the Gen-Probe TCS Operator's Manual.
17. Add 1 mL of Wash Solution to each tube. Cover the TTUs with sealing cards. Remove the rack of TTUs from the Gen-Probe TCS separation bay and vortex to resuspend the microparticle pellets.
18. Place the rack of TTUs on the Gen-Probe TCS separation bay for 4 to 10 minutes.
19. Carefully remove and dispose of the sealing cards.
20. Aspirate the solution from each tube according to the Gen-Probe TCS Operator's Manual.
21. Add 1 mL of Wash Solution to each tube. Cover the TTUs with sealing cards. Remove the rack of TTUs from the Target Capture System separation bay and vortex to resuspend the microparticle pellets.
22. Place the rack of TTUs on the Gen-Probe TCS separation bay for 4 to 10 minutes.
23. Carefully remove and dispose of the sealing cards.
24. Completely aspirate the solution from each tube according to the Gen-Probe TCS Operator's Manual. Cover the TTUs with a sealing card.
25. Remove the rack of TTUs from the Gen-Probe TCS separation bay and proceed directly to Amplification.

B. AMPLIFICATION

The repeat pipettors used in this step must be dedicated for use only in AMPLIFICATION steps.

1. Deliver 75 μL of Amplification Reagent to the bottom of each tube using the dedicated repeat pipettor. Take care to deliver the reagent to the bottom of each tube without inserting the pipette tip into the tube or touching the rim of the tube.
2. Add 200 μL of Oil to each reaction tube using the dedicated repeat pipettor. Angle the pipette tip toward the sides of the tubes, not straight to the bottom, to avoid splashback.
3. Cover the TTUs with sealing cards.
4. Vortex the rack of TTUs a minimum of 20 seconds and until all microparticles are resuspended. Ensure that magnetic particles are no longer adhering to the walls of the tube, and are evenly dispersed in the aqueous phase.
5. Incubate the TTUs in a water bath at 60°C \pm 1°C for 10 minutes \pm 1 minute, then at 41.5°C \pm 1°C for 9 to 20 minutes.
6. Leaving the rack of TTUs at 41.5°C \pm 1°C, carefully remove and dispose of the sealing cards. Proceed immediately to enzyme addition. Add 25 μL of the Enzyme Reagent into each tube using the dedicated repeat pipettor. Take care to deliver the reagent to the bottom of each tube without inserting the pipette tip into the tube or touching the rim of the tube. Place new sealing cards over the TTUs. Remove the rack of TTUs from the water bath and shake to mix. DO NOT VORTEX. Minimize the time the tubes are out of the water bath.
7. Incubate the rack of TTUs in the water bath at 41.5°C \pm 1°C for 60 minutes \pm 5 minutes.
8. Remove the rack of TTUs from the water bath and transfer it to the Hybridization Protection Assay area. Rack may remain at room temperature for up to 30 minutes prior to the addition of Probe Reagent.

C. HYBRIDIZATION PROTECTION ASSAY (HPA)

The repeat pipettor used in this step must be dedicated for use only in HYBRIDIZATION PROTECTION ASSAY.

A separate, dedicated location for the Hybridization Protection Assay (HPA) step is recommended to minimize amplicon contamination in

the assay. This dedicated area should be on a separate bench in a separate area from the reagent and sample preparation and amplification areas.

1. Hybridization

- a. Carefully remove and dispose of the sealing cards.
- b. Add 100 μL of Probe Reagent into each tube using the dedicated repeat pipettor. Take care to deliver the reagent to the bottom of each tube without inserting the pipette tip into the tube or touching the rim of the tube. Angle the pipette tip toward the sides of the tubes, not straight to the bottom, to avoid splashback.
- c. Cover the TTUs with sealing cards.
- d. Vortex the rack of TTUs a minimum of 20 seconds and until a homogeneous solution is achieved. To avoid possible contamination, do not allow reaction mixture to come in contact with the sealing card.
- e. Incubate the rack of TTUs in a dedicated water bath at $60^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 15 minutes \pm 1 minute.

2. Selection

- a. Remove the rack of TTUs from the $60^{\circ}\text{C} \pm 1^{\circ}\text{C}$ water bath. Carefully remove and dispose of the sealing cards.
- b. Add 250 μL of Selection Reagent to each tube using a repeat pipettor. Take care to deliver the reagent to the bottom of each tube without inserting the pipette tip into the tube or touching the rim of the tube. Angle the pipette tip toward the sides of the tubes, not straight to the bottom, to avoid splash back.
- c. Cover the TTUs with sealing cards. Vortex the rack of TTUs a minimum of 20 seconds and until a homogeneous solution is achieved. To avoid possible contamination, do not allow reaction mixture to come in contact with the sealing card.
- d. Return the rack of TTUs to the $60^{\circ}\text{C} \pm 1^{\circ}\text{C}$ water bath for 10 minutes \pm 1 minute.
- e. Remove the rack of TTUs from the $60^{\circ}\text{C} \pm 1^{\circ}\text{C}$ water bath.
- f. Cool the rack of TTUs in a $23^{\circ}\text{C} \pm 4^{\circ}\text{C}$ container of water for a minimum of 10 minutes while preparing for Detection (step 3a).
- g. Remove the rack of TTUs from the $23^{\circ}\text{C} \pm 4^{\circ}\text{C}$ container of water onto absorbent material.

3. Detection

- a. Prepare the LEADER HC+ Luminometer for operation as indicated in the Operator's Manual. Ensure that there are sufficient volumes of Auto Detect 1 and Auto Detect 2 to complete the tests.
- b. Open the APTIMA HIV-1 assay protocol from the APTIMA HIV-1 & HCV Assay Software.
- c. Carefully remove and dispose of the sealing cards.
- d. Before transferring TTUs to the luminometer, wipe the outside of the tubes using an absorbent tissue dampened with deionized water. This will ensure that no residue is present on the outside of the tubes and will help reduce static electricity that may affect luminometer readings.
- e. Transfer TTUs to the luminometer according to the software instructions. Note: Tube reading should be completed within 75 minutes after completing the selection reaction (step 2e in Selection procedure).
- f. When the analysis is complete, remove the TTUs from the luminometer.

- g. After removing the TTUs from the luminometer, add at least 1 mL Deactivation Fluid to each tube. Allow to sit at room temperature for at least 30 minutes before disposing the contents of the tubes. This will help to prevent contamination of the laboratory environment with amplicon.
- h. TTU racks should be decontaminated by complete immersion in diluted bleach (0.5% sodium hypochlorite in water) for a minimum of 15 minutes. The bleach should then be rinsed off with water and the rack may be allowed to air dry or may be wiped dry.

Quality Control 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:

- A. More than one positive and one negative calibrator values are invalid. Cutoff values will not be calculated for Internal Control and Analyte signals.
- B. 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

- 1. 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.
- 2. 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.
- 3. 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.
- 4. 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)].

Example:

| Negative Calibrator | Internal Control Relative Light Units |
|------------------------------|--|
| 1 | 124,000 |
| 2 | 126,000 |
| 3 | 125,000 |
| Total Internal Control RLU = | 375,000 |

$$NC_x \text{ (Internal Control)} = \frac{\text{Total Internal Control RLU}}{3} = 125,000$$

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

Example:

| 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

- 1. 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.
- 2. IC values may not exceed 475,000 RLU.
- 3. 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.
- 4. 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 |

$$HIV-1 \text{ PC}_x \text{ (Analyte)} = \frac{\text{Total Analyte RLU}}{3} = 700,000$$

Calculation of the Internal Control Cutoff Value

$$\text{Internal Control Cutoff Value} = 0.5 \times [NC_x \text{ (Internal Control)}]$$

Using values given in the Negative Calibrator example above:

$$\text{Internal Control Cutoff Value} = 0.5 \times (125,000)$$

$$\text{Internal Control Cutoff Value} = 62,500 \text{ RLU}$$

Calculation of the Analyte Cutoff Value

$$\text{Analyte Cutoff Value} = NC_x \text{ (Analyte)} + [0.04 \times HIV-1 \text{ PC}_x \text{ (Analyte)}]$$

Using values given in the Negative Calibrator and Positive Calibrator examples above:

$$\text{Analyte Cutoff Value} = 12,000 + (0.04 \times 700,000)$$

$$\text{Analyte Cutoff Value} = 40,000 \text{ RLU}$$

Summary of Acceptance Criteria for the APTIMA HIV-1 RNA Qualitative Assay

| | |
|----------------------------|-------------------------------|
| Negative Calibrator | |
| Analyte | ≥ 0 and ≤ 40,000 RLU |
| Internal Control | ≥ 75,000 and ≤ 300,000 RLU |
| Positive Calibrator | |
| Analyte | ≥ 300,000 and ≤ 1,800,000 RLU |
| Internal Control | ≤ 475,000 RLU |

Summary of Cutoff Calculations for the APTIMA HIV-1 RNA Qualitative Assay

| | |
|---------------------------|--|
| Analyte Cutoff = | NC Analyte Mean RLU + 0.04 x (HIV-1 PC Analyte Mean RLU) |
| Internal Control Cutoff = | 0.5 x (Negative Calibrator IC Mean 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 ≥ 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 ≥ IC Cutoff and IC ≤ 475,000 RLU |
| Reactive | Analyte S/CO ≥ 1 and IC ≤ 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.
2. 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.
3. 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.
 - a. 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.
 - b. 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.
4. 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.

5. 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.
6. 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

| | APTIMA HIV-1 RNA Qualitative Assay Result | HIV-1 Antibody Result | Specimen Interpretation |
|---|---|-------------------------|---|
| A | Reactive | Repeatedly Reactive | Confirmed HIV-1 infection* |
| B | Repeatedly Reactive | Nonreactive | Possible acute/primary HIV-1 infection* |
| C | Nonreactive | Repeatedly Reactive | Unconfirmed HIV-1 antibody positive** |
| D | Nonreactive | Nonreactive or Not Done | HIV-1 RNA not detected*** |

* 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.

Performance Characteristics

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.

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 (Table 2). 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%).

Table 2. The Sensitivity of the APTIMA HIV-1 RNA Qualitative Assay for HIV-1 Positive Specimens with RNA Concentrations ≥ 100 Copies/mL or Unknown

| Sensitivity | | | | |
|-------------|------|---------------------------|-----|----------------|
| Sample | N | Number Reactive on APTIMA | % | (95% C.I.) |
| All | 1041 | 1041 | 100 | (99.6 - 100.0) |
| HIV-1 Only | 867 | 867 | 100 | (99.6 - 100.0) |
| HIV-1 & HCV | 174 | 174 | 100 | (97.9 - 100.0) |

N = Number of samples; C.I. = Confidence Interval

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/mm³), 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.

Table 3. Sensitivity of the APTIMA HIV-1 RNA Quantitative Assay for HIV-1 Positive Specimens From Individuals at Various Disease States*

| Disease | APTIMA HIV-1 RNA Qualitative Assay | | | HIV-1 Antibody (EIA) | | | HIV-1 Western blot | | | | | HIV-1 p24 Ag | | | |
|-----------------------------------|------------------------------------|------------|--------|----------------------|------------|------------|--------------------|------------|-----------|------------|------------|--------------|------------|------------|------------|
| | Tested | R (%) | NR (%) | Tested | RR (%) | NR(%) | Tested | POS (%) | NEG (%) | IND (%) | Not Tested | Tested | POS (%) | NEG (%) | Not Tested |
| AIDS N = 296 | 296 | 296 (100) | 0 (0) | 296 | 296 (100) | 0 (0) | 0 | - | - | - | 296 | 226 | 44 (19.5) | 182 (80.5) | 70 |
| Asymptomatic** N = 338 | 338 | 338 (100) | 0 (0) | 338 | 297 (87.9) | 41^ (12.1) | 0 | - | - | - | 338 | 234 | 73 (31.2) | 161 (68.8) | 104 |
| Symptomatic*** N = 168 | 168 | 168 (100) | 0 (0) | 168 | 168 (100) | 0 (0) | 0 | - | - | - | 168 | 138 | 14 (10.1) | 124 (89.6) | 30 |
| Unknown N = 239 | 239 | 239 (100) | 0 (0) | 239 | 232 (97.1) | 7^^ (2.9) | 151 | 143 (94.7) | 5^^ (3.3) | 3^^^ (2.0) | 88 | 201 | 29 (14.4) | 172 (85.6) | 38 |
| Total N = 1041 | 1041 | 1041 (100) | 0 (0) | 1041 | 993 (95.4) | 48 (4.6) | 151 | 143 (94.7) | 5 (3.3) | 3 (2.0) | 890 | 799 | 160 (20.0) | 639 (80.0) | 242 |

N = Number; R = Reactive; NR = Nonreactive; RR = Repeat Reactive NEG = Negative; POS = Positive; IND = Indeterminate; % is percentage of specimens tested for given test

* Samples with confirmed viral loads < 100 copies/mL excluded.

** Asymptomatic (asymptomatic, persistent generalized lymphadenopathy, or acute HIV-1 infection).

*** Symptomatic (not asymptomatic and not AIDS).

^ 8 specimens were p24 Ag negative and 33 were p24 Ag positive.

^^ 4 specimens Western blot neg/p24 Ag pos; 1 specimen Western blot Indeterminate/p24 Ag pos; 1 specimen not tested Western blot/p24 Ag pos; 1 specimen Western blot neg/p24 Ag neg.

^^^ 4 specimens were EIA nonreactive and p24 Ag positive; 1 specimen was EIA nonreactive and p24 Ag negative.

^^^^ 2 specimens were EIA repeat reactive and p24 Ag negative; 1 specimen was EIA nonreactive and p24 Ag positive.

Prospective Study of Individuals at High Risk for HIV-1 Infection

Specimens from 300 individuals at high risk for infection with HIV-1 were tested in the APTIMA HIV-1 RNA Qualitative Assay and the results are shown in Table 4. Of the 300 individuals tested, 274 (91.3%) had IV drug use (IVDU) as one of their risk factors. Risk factors other than IVDU included having unprotected sex, men having sex with men, occupational exposure, having sex with an HIV-1 positive partner, and having transfusion of blood or blood products.

The APTIMA HIV-1 RNA Qualitative Assay detected all 22 samples that were repeat reactive by EIA and positive by Western blot, as well as 1 confirmed acute infected sample that was EIA non-reactive but p24 Ag positive and polymerase chain reaction (PCR) positive. The resulting sensitivity of the APTIMA HIV-1 RNA Qualitative Assay in confirming HIV-1 infection in this study was 100%.

Table 4. Clinical Sensitivity of the APTIMA HIV-1 RNA Qualitative Assay in a High Risk Population

| APTIMA HIV-1 RNA Qualitative Assay | | | | | |
|------------------------------------|----------|-------------|----|-----|-------------------------|
| | Reactive | Nonreactive | TP | TN | Sensitivity (95% C. I.) |
| EIA RR | 22* | 0 | 23 | 277 | 100 (85.2 - 100.0) |
| EIA NR | 1** | 277 | | | |

RR = Repeat Reactive; NR = Nonreactive

TP = True Positive (EIA RR/WB positive or p24 Ag positive); TN = True Negative (EIA nonreactive); C.I. = Confidence Interval

* Western blot positive, 11 PCR positive, 11 not tested with PCR assay

** p24 Ag positive, PCR positive

Clinical Sensitivity in Serum Specimens

To assess the clinical sensitivity of the APTIMA HIV-1 RNA Qualitative Assay in serum specimens, 105 serum specimens from individuals known to be infected with HIV-1 were tested in an in-house study. The serum specimens had viral loads ranging from unquantifiable (< 48 copies/mL) to 938,040 copies/mL; 15 of the 105 samples had either unquantifiable viral titers or reported titers that were less than 100 copies/mL. Of the 105 samples tested in the APTIMA HIV-1 RNA Qualitative Assay, 4 (or 3.8%) were initially non-reactive. All 4 initially non-reactive samples had HIV-1 viral loads that were unquantifiable, with reported titers of < 48 copies/mL. Repeat testing showed either non-reactivity or unreliable detection in the initially non-reactive samples, most likely due to the low viral titers. All samples with quantifiable viral loads ≥ 100 copies/mL (N = 90) gave reactive results, for 100% sensitivity in this subset of the samples, indicating that the clinical sensitivity of the APTIMA HIV-1 RNA Qualitative Assay was not affected by serum as a specimen type.

Analytical Sensitivity

HIV-1 Dilution Panel

To determine the analytical sensitivity of the APTIMA HIV-1 RNA Qualitative Assay for detection of HIV-1 RNA, HIV-1 panel members were prepared by serial dilution of negative human plasma spiked with HIV-1 (type B isolate) tissue culture supernatant. The RNA levels in viral stocks used to make the HIV-1 panel were value assigned using an in-house quantitative HIV-1 assay calibrated to the VQA standard (Rush-Presbyterian Hospital, Chicago, IL).

The panel members were tested with ten clinical lots of reagents and the test results are presented in Table 5. The APTIMA HIV-1 RNA Qualitative Assay achieved 100% detection for panel members containing 300 and 100 copies/mL and >98.5% detection for panel members containing 30 copies/mL of HIV-1 RNA. The lower bound of 95% CI for HIV-1 at 30, 100 and 300 copies/mL exceeded 95%.

Table 5. Detection of HIV-1 B RNA in Analytical Sensitivity Panels

| HIV-1 Copies/mL | Number of reactive/ tested* | % Positive | 95% Confidence Limits | |
|--------------------|-----------------------------|---------------|--------------------------|-------|
| | | | Lower | Upper |
| 300 | 715/715 | 100 | 99.5 | 100 |
| 100 | 718/718 | 100 | 99.5 | 100 |
| 30 | 702/713 | 98.5 | 97.3 | 99.2 |
| 10 | 592/717 | 82.6 | 79.6 | 85.3 |
| 3 | 305/717 | 42.5 | 38.9 | 46.3 |
| 1 | 139/718 | 19.4 | 16.5 | 22.4 |

* Invalid reactions were not retested.

CBER HIV-1 RNA Panel

Panel A (5 members) and Panel B (8 members) were tested using the APTIMA HIV-1 RNA Qualitative Assay. Results for both Panel A and B are shown in Table 6. For Panel A, testing showed reproducible detection of HIV-1 RNA at copy levels ranging from 250,000 to 100 copies/mL; the panel member at 0 copies/mL was non-reactive. Results for Panel B demonstrated reproducible detection of HIV-1 RNA at copy levels ranging from 250,000 to 10 copies/mL and non-reactive results with both negative panel members (B4 and B8).

Table 6. Detection of HIV-1 RNA in CBER panel members

| | Panel members tested and positivity rates | | | | | | | | | | | | |
|-------------------------------------|---|--------------|-------------|-----------|---------|-------------|----------|---------------|---------|-----------|----------|--------------|---------|
| CBER HIV-1 RNA Panel (copies/mL) | A1 250,000 | A2 25,000 | A3 1,000 | A4 100 | A5 0 | B1 2,500 | B2 10 | B3 250,000 | B4 0 | B5 100 | B6 50 | B7 25,000 | B8 0 |
| APTIMA HIV-1 RNA Qualitative Assay* | 100% | 100% | 100% | 100% | 0% | 100% | 100% | 100% | 0% | 100% | 100% | 100% | 0% |

* N=6

WHO International Standard for HIV-1

The WHO International Standard for HIV-1 RNA (NIBSC code 97/656) with a concentration of 100,000 IU/mL was serially diluted and tested with the APTIMA HIV-1 RNA Qualitative Assay. The results shown in Table 7 demonstrated reproducible detection of HIV-1 RNA ranging from 300 to 33.3 IU/mL.

Table 7. Testing of International Standards for HIV-1 RNA (NIBSC code 97/656)

| | Concentrations tested and positivity rates | | | | | | |
|-------------------------------------|--|--------------|---------------|---------------|--------------|---------------|------------|
| WHO HIV-1 (97/656) | 300 IU/mL | 100 IU/mL | 33.3 IU/mL | 11.1 IU/mL | 3.7 IU/mL | 1.23 IU/mL | 0 IU/mL |
| APTIMA HIV-1 RNA Qualitative Assay* | 100% | 100% | 100% | 80% | 27.6% | 26.6% | 0% |

* N=30

Subtype Detectability

Since there are no recognized international standards for HIV-1 other than HIV-1 subtype B, multiple specimens and isolates (59 different HIV-1 specimens) were tested to determine detectability of these viral subtypes (19). HIV-1 specimens of subtypes A, B, C, D, E, F, and G were quantified for HIV-1 RNA concentrations using commercial quantitative HIV-1 RNA assays or an in-house developed quantitative test, the latter using the same technology as the APTIMA assays. HIV-1 subtypes N and O were quantified with an in-house quantitative HIV-1 RNA test. Specimens were diluted into negative human plasma to target viral concentrations of 300 or 100 copies/mL and diluted specimens were tested in the APTIMA HIV-1 RNA Qualitative Assay. All HIV-1 subtypes were reactive with the APTIMA HIV-1 RNA Qualitative Assay at 300 and 100 copies/mL. (Table 8).

Table 8. HIV-1 Subtype Detectability

| Subtype | Copies/mL | APTIMA HIV-1 RNA Qualitative Assay Reactive/Total |
|---------|-----------|--|
| A* | 300 | 11/11 |
| | 100 | 9/9 |
| B | 300 | 11/11 |
| | 100 | 11/11 |
| C | 300 | 9/9 |
| | 100 | 9/9 |
| D | 300 | 6/6 |
| | 100 | 6/6 |
| E | 300 | 8/8 |
| | 100 | 8/8 |
| F | 300 | 5/5 |
| | 100 | 5/5 |
| G | 300 | 3/3 |
| | 100 | 3/3 |
| N | 300 | 1/1 |
| | 100 | 1/1 |
| O | 300 | 6/6 |
| | 100 | 6/6 |

* Two samples were quantified at < 1000 copies/mL and were reactive when tested undiluted and at 1:3 dilution.

Reactivity in Seroconverting Donors

Commercially available seroconversion panels collected from plasmapheresis donors were tested with the APTIMA HIV-1 RNA Qualitative Assay. Ten seroconversion panels for HIV-1 were tested with one Clinical Lot. The test results were compared with those of Abbott HIV-1/2 antibody and Abbott or Coulter HIV-1 p24 antigen test for HIV-1 seroconversion panels. (Table 9).

The APTIMA HIV-1 RNA Qualitative Assay was able to detect infection with median values of 12 and 6 days earlier than the Abbott HIV-1/2 antibody and HIV-1 p24 Ag tests, respectively. Reduction of the window period was observed in 9 of 10 panels when the APTIMA HIV-1 RNA Qualitative Assay was used as compared to the use of the Abbott HIV-1/2 Ab test alone. When compared to the HIV-1 p24 Ag assay, the APTIMA HIV-1 RNA Qualitative Assay detected the infection earlier in 8 of 10 panels and at the same time as HIV-1 p24 Ag in the other two panels. In all cases, HIV-1 p24 Ag reactive specimens were reactive with the APTIMA HIV-1 RNA Qualitative Assay.

Table 9. Testing for HIV-1 RNA with the APTIMA HIV-1 RNA Qualitative Assay on HIV-1 Seroconversion Panels

| Panel ID | Days Earlier Detection Than HIV-1 Antibody | Days Earlier Detection Than HIV-1 p24 Ag |
|---------------|--|--|
| BCP 6240 | 12 | 7 |
| BCP 6248 | 11 | 4 |
| PRB923* | 17 | 7 |
| PRB926** | 27 | 7 |
| PRB929*** | 11 | 0 |
| PRB932 | 0 | 0 |
| PRB943 | 9 | 2 |
| PRB945** | 13 | 13 |
| PRB946^ | 7 | 3 |
| PRB950** | 28 | 18 |
| Median | 12 | 6 |

* Intermittent HIV-1 reactivity at 11 and 35 days prior to ramp up is not used for this calculation.

** HIV-1 positive in first bleed of seroconversion panel.

*** Single reactive in the APTIMA HIV-1 RNA Qualitative Assay 14 days prior to ramp up is not used for this calculation.

^ No seroconversion to HIV-1 antibody in this panel which spans 11 days.

The data from the seroconversion panels described above was further analyzed to compare EIA, Western blot and APTIMA HIV-1 RNA Qualitative Assay results. Results are summarized in Table 10. Of the 27 EIA repeat reactive specimens, all were reactive in the APTIMA HIV-1 RNA Qualitative Assay, 12 of which were Western blot positive, 7 were Western blot indeterminate, and 8 were Western blot negative. Of the 49 EIA nonreactive specimens, 29 were reactive in the APTIMA HIV-1 RNA Qualitative Assay (1 Western blot positive, 1 Western blot indeterminate, and 27 Western blot negative). The other 20 EIA nonreactive specimens were nonreactive in the APTIMA HIV-1 RNA Qualitative Assay and negative by Western blot.

Table 10. Comparison of APTIMA APTIMA HIV-1 RNA Qualitative Assay, EIA and Western blot Data from Seroconversion Panels

| APTIMA HIV-1 RNA Qualitative Assay Results | | | |
|---|------------|-----------------|--------------------|
| EIA | WB | Reactive | Nonreactive |
| Repeat Reactive N=27 | POS | 12 | 0 |
| | IND | 7 | 0 |
| | NEG | 8 | 0 |
| Nonreactive N=49 | POS | 1 | 0 |
| | IND | 1 | 0 |
| | NEG | 27 | 20 |

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.

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