

Abbott
RealTime

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RealTime HIV-1

REF 6L18

51-602146/R2

Abbott RealTime HIV-1

Customer Service: 1-800-553-7042

This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Key to symbols used

REF

List Number

CAL A

Calibrator A

LOT

Lot Number

CAL B

Calibrator B

IVDFor *In Vitro* Diagnostic Use**CONTROL -**

Negative Control



Store at -10°C or colder

CONTROL L

Low Positive Control



Manufacturer

CONTROL H

High Positive Control



Consult instructions for use

INTERNAL CONTROL

Internal Control



Expiration Date

AMPLIFICATION REAGENT PACK

Amplification Reagent Pack



CAUTION: Handle human sourced materials as potentially infectious. Consult instructions for use. (Infection Risk)

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See **REAGENTS** section for a full explanation of symbols used in reagent component naming.

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NAME

Abbott RealTime HIV-1

INTENDED USE

The Abbott RealTime HIV-1 assay is an *in vitro* reverse transcription-polymerase chain reaction (RT-PCR) assay for the quantitation of Human Immunodeficiency Virus type 1 (HIV-1) on the automated *m2000* System in human plasma from HIV-1 infected individuals over the range of 40 to 10,000,000 copies/mL. The Abbott RealTime HIV-1 assay is intended for use in conjunction with clinical presentation and other laboratory markers for disease prognosis and for use as an aid in assessing viral response to antiretroviral treatment as measured by changes in plasma HIV-1 RNA levels. This assay is not intended to be used as a donor screening test for HIV-1 or as a diagnostic test to confirm the presence of HIV-1 infection.

SUMMARY AND EXPLANATION OF THE TEST

Human Immunodeficiency Virus (HIV) is the etiologic agent of Acquired Immunodeficiency Syndrome (AIDS).^{1,2,3} It can be transmitted through sexual contact, exposure to infected blood or blood products, or from an infected mother to the fetus.⁴ Acute HIV syndrome, characterized by flu-like symptoms, develops three to five weeks after initial infection and is associated with high levels of viremia.^{5,6} Within four to six weeks of the onset of symptoms, HIV specific immune response is detectable.^{7,8} After seroconversion, viral load in peripheral blood declines and most patients enter an asymptomatic phase that can last for years.⁹

Quantitative measurement of HIV levels in peripheral blood has greatly contributed to the understanding of the pathogenesis of HIV infection^{10,11} and has been shown to be an essential parameter in prognosis and management of HIV infected individuals.¹²⁻¹⁷ Decisions regarding initiation or changes in antiretroviral therapy are guided by monitoring plasma HIV RNA levels (viral load), CD4+ T cell count, and the patient's clinical condition.^{17,18} The goal of antiretroviral therapy is to reduce the HIV virus in plasma to below detectable levels of available viral load tests.^{17,19}

HIV RNA levels in plasma can be quantitated by nucleic acid amplification or signal amplification technologies.^{20,21,22} The Abbott RealTime HIV-1 assay uses Polymerase Chain Reaction (PCR) technology with homogenous real-time fluorescent detection. Partially double-stranded fluorescent probe design allows detection of diverse group M subtypes and group O isolates. The assay is standardized against a viral standard from the Virology Quality Assurance (VQA) Laboratory of the AIDS Clinical Trial Group,²³ and against World Health Organization (WHO) 1st International Standard for HIV-1 RNA (97/656).^{24,25} The assay results can be reported in copies/mL or International Units/mL (IU/mL).

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The Abbott RealTime HIV-1 assay uses RT-PCR²⁶ to generate amplified product from the RNA genome of HIV-1 in clinical specimens. An RNA sequence that is unrelated to the HIV-1 target sequence is introduced into each specimen at the beginning of sample preparation. This unrelated RNA sequence is simultaneously amplified by RT-PCR, and serves as an internal control (IC) to demonstrate that the process has proceeded correctly for each sample. The amount of HIV-1 target

sequence that is present at each amplification cycle is measured through the use of fluorescent-labeled oligonucleotide probes on the Abbott *m2000rt*TM instrument. The probes do not generate signal unless they are specifically bound to the amplified product. The amplification cycle at which fluorescent signal is detected by the Abbott *m2000rt* is proportional to the log of the HIV-1 RNA concentration present in the original sample.

Sample Preparation

The purpose of sample preparation is to extract and concentrate the target RNA molecules to make the target accessible for amplification, and to remove potential inhibitors of amplification from the extract.

The Abbott *m2000sp* instrument prepares samples for the Abbott RealTime HIV-1 assay using the Abbott *m*TM Sample Preparation System (4 x 24 Preps) reagents. The *m2000sp* uses magnetic particle technology to capture nucleic acids and washes the particles to remove unbound sample components. The bound nucleic acids are eluted and transferred to a 96 deep-well plate. The nucleic acids are then ready for amplification. The IC is taken through the entire sample preparation procedure along with the calibrators, controls, and specimens.

Reagent Preparation and Reaction Plate Assembly

The Abbott *m2000sp* combines the Abbott RealTime HIV-1 amplification reagent components (HIV-1 Oligonucleotide Reagent, Thermostable rTth Polymerase Enzyme, and Activation Reagent). The Abbott *m2000sp* dispenses the resulting master mix to the Abbott 96-Well Optical Reaction Plate along with aliquots of the nucleic acid samples prepared by the Abbott *m2000sp*. The plate is ready, after manual application of the optical seal, for transfer to the Abbott *m2000rt*.

Amplification

During the amplification reaction on the Abbott *m2000rt*, the target RNA is converted to cDNA by the reverse transcriptase activity of the thermostable rTth DNA polymerase. First, the HIV-1 and IC reverse primers anneal to their respective targets and are extended during a prolonged incubation period. After a denaturation step, in which the temperature of the reaction is raised above the melting temperature of the double-stranded cDNA:RNA product, a second primer anneals to the cDNA strand and is extended by the DNA polymerase activity of the rTth enzyme to create a double-stranded DNA product.

During each round of thermal cycling, amplification products dissociate to single strands at high temperature allowing primer annealing and extension as the temperature is lowered. Exponential amplification of the product is achieved through repeated cycling between high and low temperatures, resulting in a billion-fold or greater amplification of target sequences. Amplification of both targets (HIV-1 and IC) takes place simultaneously in the same reaction.

The target sequence for the Abbott RealTime HIV-1 assay is in the *pol* integrase region of the HIV-1 genome. This region is highly conserved.²⁷

The IC target sequence is derived from the hydroxypyruvate reductase gene from the pumpkin plant, *Cucurbita pepo*, and is delivered in an Armored RNA[®] particle that has been diluted in negative human plasma.

Detection

During the read cycles of amplification on the Abbott *m2000rt*, the temperature is lowered further to allow fluorescent detection of amplification products as the HIV-1 and IC probes anneal to their

targets (real-time fluorescence detection). The HIV-1 probe has a fluorescent moiety that is covalently linked to the 5' end. A short oligonucleotide (quencher oligonucleotide) is complementary to the 5' end of the HIV-1 probe and has a quencher molecule at its 3' end. In the absence of HIV-1 target, the HIV-1 probe fluorescence is quenched through hybridization to the quencher oligonucleotide. In the presence of the HIV-1 target sequence, the HIV-1 probe preferentially hybridizes to the target sequence, dissociating from the quencher oligonucleotide, allowing fluorescent detection.

The IC probe is a single-stranded DNA oligonucleotide with a fluorophore at the 5' end and a quencher at the 3' end. In the absence of IC target sequences, probe fluorescence is quenched. In the presence of IC target sequences, probe hybridization to complementary sequences separates the fluorophore and the quencher and allows fluorescent emission and detection.

The HIV-1 and IC specific probes are each labeled with a different fluorophore, thus allowing for simultaneous detection of both amplified products at each cycle. The amplification cycle at which fluorescent signal is detected by the Abbott *m2000rt* is proportional to the log of the HIV-1 RNA concentration present in the original sample.

Quantitation

A calibration curve is required to quantitate the HIV-1 RNA concentration of specimens and controls. Two assay calibrators are run in replicates of three to generate a calibration curve. The calibration curve slope and intercept are calculated from the assigned HIV-1 RNA concentration and the median observed threshold cycle for each calibrator and are stored on the instrument. The concentration of HIV-1 RNA in specimens and controls is calculated from the stored calibration curve, and the results are automatically reported on the *m2000rt* workstation. The Abbott RealTime HIV-1 Negative Control, Low Positive Control, and High Positive Control must be included in each

run to verify run validity. The *m2000* System verifies that the controls are within the assigned ranges.

PREVENTION OF NUCLEIC ACID CONTAMINATION

The possibility of nucleic acid contamination is minimized because:

- Reverse transcription, PCR amplification, and oligonucleotide hybridization occur in a sealed 96-Well Optical Reaction Plate.
- Detection is carried out automatically without the need to open the 96-Well Optical Reaction Plate.
- Aerosol barrier pipette tips are used for all pipetting. The pipette tips are discarded after use.
- Separate dedicated areas are used to perform the Abbott RealTime HIV-1 assay. Refer to the **SPECIAL PRECAUTIONS** section of this package insert.

REAGENTS

The Abbott RealTime Reagents are intended for single-use only and unused reagents should be discarded.

Abbott RealTime HIV-1 Amplification Reagent Kit (List No. 6L18-90)

1. **INTERNAL CONTROL** Abbott RealTime HIV-1 Internal Control (List No. 2G31Y)
(4 vials, 1.2 mL per vial).

Noninfectious Armored RNA with internal control sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HIV RNA, HCV RNA, HBV DNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin® 300 and 0.15% ProClin 950.

2. **AMPLIFICATION REAGENT PACK** Abbott RealTime HIV-1 Amplification Reagent Pack (List No. 6L18).

Four packs of single-use reagents, 24 tests/pack. **Unused reagents should be discarded.**

Each pack contains:

- 1 bottle (0.141 mL) Thermostable rTth Polymerase Enzyme (2.9 to 3.5 Units/μL) in buffered solution.
- 1 bottle (1.10 mL) HIV-1 Oligonucleotide Reagent. Synthetic oligonucleotides (4 primers, 2 probes, and 1 quencher oligonucleotide), and dNTPs in a buffered solution with a reference dye. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.
- 1 bottle (0.40 mL) Activation Reagent. 30 mM manganese chloride solution. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

Abbott RealTime HIV-1 Control Kit (List No. 6L18-80) and Lot-specific Kit Card.

1. **CONTROL -** Abbott RealTime HIV-1 Negative Control (List No. 2G31Z) (8 vials, 1.8 mL per vial).

Negative human plasma tested and found to be nonreactive for HBsAg, HIV RNA, HCV RNA, HBV DNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

2. **CONTROL L** Abbott RealTime HIV-1 Low Positive Control (List No. 2G31W)
(8 vials, 1.8 mL per vial).
Noninfectious Armored RNA with HIV-1 sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HIV RNA, HCV RNA, HBV DNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.
3. **CONTROL H** Abbott RealTime HIV-1 High Positive Control (List No. 2G31X)
(8 vials, 1.8 mL per vial).
Noninfectious Armored RNA with HIV-1 sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HIV RNA, HCV RNA, HBV DNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

Abbott RealTime HIV-1 Calibrator Kit (List No. 6L18-70) and Lot-specific Kit Card.

1. **CAL A** Abbott RealTime HIV-1 Calibrator A (List No. 2G31A)
(12 vials, 1.8 mL per vial).
Noninfectious Armored RNA with HIV-1 sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HIV RNA, HCV RNA, HBV DNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.
2. **CAL B** Abbott RealTime HIV-1 Calibrator B (List No. 2G31B)
(12 vials, 1.8 mL per vial).
Noninfectious Armored RNA with HIV-1 sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HIV RNA, HCV RNA, HBV DNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

NOTE: Control kit lots, calibrator kit lots, and amplification reagent kit lots can be used interchangeably. If a new amplification reagent kit lot is used, then the assay needs to be recalibrated. Do not interchange kit components from different kit lots. For example, do not use the negative control from control kit lot X with the positive controls from control kit lot Y.

WARNINGS AND PRECAUTIONS

IVD For *In Vitro* Diagnostic Use Only.

- This assay is not intended to be used as a screening test for HIV-1 or as a diagnostic test to confirm the presence of HIV-1 infection.
- The Abbott RealTime HIV-1 reagents are intended to be used only on the Abbott *m2000* System consisting of the *m2000sp* for sample processing and the *m2000rt* for amplification and detection.
- Do not use expired reagents.
- The Abbott *m2000sp* Master Mix Addition protocol must be initiated within one hour after completion of Sample Preparation. If the Abbott *m2000sp* master mix addition protocol is aborted, seal the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the *m2000sp* Operations Manual, Hazards section, along with the gloves used to handle the plate. Do not import the test order onto the *m2000rt*.
- The appropriate PCR plate must be selected when samples are loaded into the *m2000rt* instrument.

- The *m2000rt* protocol must be started within 40 minutes of the initiation of the Master Mix Addition protocol. If the Abbott *m2000rt* instrument run is not initiated within 40 minutes, or is interrupted or aborted, seal the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott *m2000rt* Operations Manual along with the gloves used to handle the plate.
- The Abbott RealTime HIV-1 assay was evaluated using frozen plasma samples. Fresh plasma samples were not evaluated.

Safety Precautions

- Refer to the Abbott *m2000sp* and Abbott *m2000rt* Operations Manuals, Hazard Section, for instructions on safety precautions.



- **CAUTION:** This product contains human sourced and/or potentially infectious components. For a specific listing, refer to the **REAGENTS** section of this package insert. Human sourced material has been tested and found to be nonreactive to HBsAg, HCV RNA, HIV RNA, HBV DNA, anti-HIV-1/HIV-2, and anti-HCV. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens.²⁸ Biosafety Level 2²⁹ or other appropriate biosafety practices^{30,31} should be used for materials that contain or are suspected of containing infectious agents. These precautions include, but are not limited to, the following:

- Wear gloves when handling specimens or reagents.
- Do not pipette by mouth.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where these materials are handled.
- Clean and disinfect spills of specimens by including the use of a tuberculocidal disinfectant such as 1.0% sodium hypochlorite or other suitable disinfectant.^{32,33}
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and federal regulations.^{34,35}
- The Abbott RealTime HIV-1 Calibrator Kit, Control Kit, Internal Control, HIV-1 Oligonucleotide Reagent, and Activation Reagent contain a mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one which are components of ProClin. The components are classified per applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases:



R43 May cause sensitization by skin contact.

S24 Avoid contact with skin.

S35 This material and its container must be disposed of in a safe way.

S37 Wear suitable gloves.

S46 If swallowed, seek medical advice immediately and show this container or label.

SPECIAL PRECAUTIONS

Handling Precautions

- The Abbott RealTime HIV-1 assay is only for use with plasma specimens that have been handled

and stored in capped tubes as described in the **SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE** section.

- During preparation of samples, compliance with good laboratory practices is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of ribonucleases (RNases) into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with RNA.
- Amplification technologies such as PCR are sensitive to accidental introduction of product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the RealTime reagents used in the amplification step become contaminated by accidental introduction of even a few molecules of amplification product. Measures to reduce the risk of contamination in the laboratory include physically separating the activities involved in performing PCR in compliance with good laboratory practices.

Work Areas

- Two dedicated areas, Sample Preparation Area and Amplification Area, are recommended.
 - The **Sample Preparation Area** is dedicated to processing samples (specimens, Abbott RealTime HIV-1 Controls, and Calibrators), and to adding processed specimens, controls, and calibrators to the Abbott 96-Well Optical Reaction Plate. **All reagents used in the Sample Preparation Area should remain in this dedicated area at all times. Laboratory coats, pipettes, pipette tips, and vortexers used in the Sample Preparation Area must remain in this area and not be moved to the Amplification Area. Do not bring amplification product into the Sample Preparation Area.**

- The **Amplification Area** is dedicated to the amplification and detection of amplified product. Laboratory coats and equipment used in the **Amplification Area** must remain in this area and not be moved to the **Sample Preparation Area**.
- Control kit lots, calibrator kit lots, and amplification reagent kit lots can be used interchangeably. If a new amplification reagent kit lot is used, then the assay needs to be recalibrated. Do not interchange kit components from different kit lots. For example, do not use the negative control from control kit lot X with the positive controls from control kit lot Y.
- The Amplification Reagent Kit, Control Kit, and Calibrator Kit can be thawed and re-frozen up to three times before use.
- Work area and instrument platforms must be considered potential sources of contamination. Change gloves after contact with potential contaminants (specimens, eluates, and/or amplified product) before handling unopened reagents, negative control, positive controls, calibrators, or specimens. Refer to the Abbott *m2000sp* and *m2000rt* Operations Manuals for instrument cleaning procedures.
- If the Abbott *m2000sp* instrument run is aborted, dispose of all commodities and reagents according to the Abbott *m2000sp* Operations Manual. If the Abbott *m2000sp* master mix addition protocol is aborted, seal the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the *m2000sp* Operations Manual, Hazards section, along with the gloves used to handle the plate.
- If the Abbott *m2000rt* instrument run is interrupted or aborted, seal the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott *m2000rt* Operations

Manual along with the gloves used to handle the plate.

- Decontaminate and dispose of all potentially biohazardous materials in accordance with local, state, and federal regulations.^{34,35} All materials should be handled in a manner that minimizes the chance of potential contamination of the work area. **Note: Autoclaving the sealed Reaction Plate will not degrade the amplified product and may contribute to the release of the amplified product by opening the sealed plate. The laboratory area can become contaminated with amplified product if the waste materials are not carefully handled and contained.**

Aerosol Containment

To reduce the risk of nucleic acid contamination due to aerosols formed during manual pipetting, aerosol barrier pipette tips must be used for all manual pipetting. The pipette tips must be used only one time. Clean and disinfect spills of specimens and reagents as stated in the Abbott *m2000sp* and Abbott *m2000rt* Operations Manuals.

Contamination and Inhibition

The following precautions should be observed to minimize the risks of RNase contamination, cross-contamination between samples, and inhibition:

- Wear appropriate personal protective equipment at all times.
- Use powder-free gloves.
- Change gloves after having contact with potential contaminants (such as specimens, eluates, and/or amplified product).
- To reduce the risk of nucleic acid contamination due to aerosols formed during pipetting,

pipettes with aerosol barrier tips must be used for all pipetting. The length of the tip should be sufficient to prevent contamination of the pipette barrel. While pipetting, care should be taken to avoid touching the pipette barrel to the inside of the sample tube or container. The use of extended aerosol barrier pipette tips is recommended.

- Change aerosol barrier pipette tips between ALL manual liquid transfers.
- The Abbott *m*Sample Preparation System (4 x 24 Preps) reagents are single use only. Use new reagent vessels, reaction vessels, and newly opened reagents for every new Abbott RealTime HIV-1 assay run. At the end of each run, discard all remaining reagents from the worktable as stated in the Abbott *m*2000*sp* Operations Manual and the Abbott *m* Sample Preparation System (4 x 24 Preps) product information sheet.

STORAGE INSTRUCTIONS

Abbott RealTime HIV-1 Amplification Reagent Kit (List No. 6L18-90).

-  -10°C The Abbott RealTime HIV-1 Amplification Reagent Pack and Internal Control vials must be stored at -10°C or colder when not in use. Care must be taken to separate the Abbott RealTime HIV-1 Amplification Reagent Pack that is in use from direct contact with samples, calibrators and controls.

Abbott RealTime HIV-1 Control Kit (List No. 6L18-80).

-  -10°C The Abbott RealTime HIV-1 Negative and Positive Controls must be stored at -10°C or colder.

Abbott RealTime HIV-1 Calibrator Kit (List No. 6L18-70).

-  -10°C The Abbott RealTime HIV-1 Calibrator A and Calibrator B must be stored at -10°C or colder.

SHIPPING CONDITIONS

- Abbott RealTime HIV-1 Amplification Reagent Kit: Ship on dry ice.
- Abbott RealTime HIV-1 Control Kit: Ship on dry ice.
- Abbott RealTime HIV-1 Calibrator Kit: Ship on dry ice.

INDICATION OF INSTABILITY OR DETERIORATION OF REAGENTS

When a positive or negative control value is out of the expected range, it may indicate deterioration of the reagents. Associated test results are invalid and samples must be retested. Assay recalibration may be necessary.

INSTRUMENT PROCEDURE

The Abbott RealTime HIV-1 application files must be installed on the Abbott *m2000sp* and Abbott *m2000rt* systems from the Abbott RealTime HIV-1 *m2000™* System Combined Application CD-ROM prior to performing the assay. For detailed information on application file installation, refer to the Abbott *m2000sp* and Abbott *m2000rt* Operations Manuals, Operating Instructions section.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE

Specimen Collection and Storage

Human plasma (ACD-A and EDTA) specimens may be used with the Abbott RealTime HIV-1 assay. Follow the manufacturer's instructions for processing plasma collection tubes.

Freshly drawn specimens (whole blood) may be held at 15-30°C for up to 6 hours or at 2-8°C for up to 24 hours, prior to centrifugation.

After centrifugation, remove plasma from cells and store at -70°C or lower.^{36,37} Multiple freeze-thaw cycles should be avoided and should not exceed three freeze/thaw cycles. Thaw plasma specimens at 15-30°C or at 2-8°C. Once thawed, if plasma specimens are not being processed immediately, they can be stored at 2-8°C for up to 6 hours.

Fresh plasma has not been validated for use in the assay.

Specimen Transport

Ship specimens frozen on dry ice. For domestic shipments, specimens should be packaged and labeled in compliance with applicable local, state, and federal regulations covering the transport of clinical, diagnostic, or biological specimens.

ABBOTT REALTIME HIV-1 ASSAY PROCEDURE

The Abbott RealTime HIV-1 assay provides three sample volume options (0.2 mL, 0.5 mL, and 1.0 mL). (See assay protocol step 9 and **Interpretation of Results** section).

Materials Provided

- Abbott RealTime HIV-1 Amplification Reagent Kit (List No. 6L18-90)

Materials Required But Sold Separately

- Abbott RealTime HIV-1 Calibrator Kit (List No. 6L18-70)
- Abbott RealTime HIV-1 Control Kit (List No. 6L18-80)

Materials Required But Not Provided

(Each available separately)

Sample Preparation Area

- Abbott *m2000sp* instrument (List No. 9K14)
- Abbott *m* Sample Preparation System (4 x 24 Preps) (List No. 02K02-24)
- 5 mL Reaction Vessels (List No. 4J71-20)
- 13 mm to 16 mm sample tubes
- 200 mL Reagent Vessels (List No. 4J71-60)
- 200 μ L (List No. 4J71-15) Disposable Tips
- 1000 μ L (List No. 4J71-10) Disposable Tips
- Abbott Optical Adhesive Covers (List No. 4J71-75)
- Abbott Adhesive Cover Applicators (List No. 9K32-01)

- Abbott Splash-Free Support Base (List No. 9K31-01)
- Abbott 96-Deep-Well Plate (List No. 4J71-30)
- Abbott RealTime HIV-1 *m2000* System Combined Application CD-ROM (List No. 6L83)
- Abbott 96-Well Optical Reaction Plate (List No. 4J71-70)
- Aerosol Barrier Pipette Tips for 20-1000 μL pipettes
- Calibrated Pipettes capable of delivering 20-1000 μL
- Centrifuge capable of 2000g
- Master Mix Tube (List No. 4J71-80)
- Vortex mixer

Amplification Area

- Abbott *m2000rt* instrument (List No. 9K15)
- Abbott RealTime HIV-1 *m2000* System Combined Application CD-ROM (List No. 6L83)
- Abbott *m2000rt* Optical Calibration Kit (List No. 4J71-93)

Other Materials

- Biological safety cabinet approved for working with infectious materials
- Sealable plastic bags
- RNase-free water (Eppendorf or equivalent)[†]
- 1.7 mL RNase-free Microcentrifuge Tubes (Dot Scientific, Inc. or equivalent)[†]
- Cotton Tip Applicators (Puritan or equivalent)[†]

†Note: These three items are used in the procedure for **Monitoring the Laboratory for the Presence of Contamination**. Refer to the **QUALITY CONTROL PROCEDURES** section of this package insert.

Procedural Precautions

- Read the instructions in this package insert carefully before processing samples.
- The Abbott RealTime HIV-1 Calibrators, Internal Control, Negative Control, Low Positive Control, and High Positive Control vials are intended for single-use only and should be discarded after use.
- The Abbott *m2000sp* Master Mix Addition protocol must be initiated within one hour after completion of Sample Preparation. If the Abbott *m2000sp* master mix addition protocol is not initiated, re-cap the Amplification Reagent vials and return the Amplification Reagent Pack to -10°C storage. Once thawed, the Abbott RealTime HIV-1 Amplification Reagent Pack can be frozen and thawed a maximum of three times. If the Abbott *m2000sp* master mix addition protocol is aborted, then discard the amplification reagents.
- The *m2000rt* protocol must be started within 40 minutes of the initiation of the Master Mix Addition protocol. If the Abbott *m2000rt* instrument run is not initiated within 40 minutes, or is interrupted or aborted, seal the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott *m2000rt* Operations Manual along with the gloves used to handle the plate.
- Use aerosol barrier pipette tips or disposable pipettes only one time when pipetting specimens or IC. To prevent contamination to the pipette barrel while pipetting, care should be taken to

avoid touching the pipette barrel to the inside of the sample tube or container. The use of extended aerosol barrier pipette tips is recommended.

- Monitoring procedures for the presence of amplification product can be found in the **QUALITY CONTROL PROCEDURES** section in this package insert.
- To reduce the risk of nucleic acid contamination, clean and disinfect spills of specimens by including the use of a tuberculocidal disinfectant such as 1.0% sodium hypochlorite or other suitable disinfectant.
- The Abbott RealTime HIV-1 Calibrators and Controls must be prepared in conjunction with the specimens to be tested. The use of the Abbott RealTime HIV-1 Controls and Calibrators is integral to the performance of the Abbott RealTime HIV-1 assay. Refer to the **QUALITY CONTROL PROCEDURES** section of this package insert for details.

ASSAY PROTOCOL

For a detailed description on how to operate the Abbott *m2000sp* instrument or the Abbott *m2000rt* instrument, refer to the Abbott *m2000sp* and *m2000rt* Operations Manuals, Operating Instructions sections.

Laboratory personnel must be trained to operate the Abbott *m2000sp* and *m2000rt* instruments. The operator must have a thorough knowledge of the applications run on the instruments and must follow good laboratory practices.

1. Thaw assay controls and IC at 15-30°C or at 2-8°C. Thaw calibrators at 15-30°C or at 2-8°C only if performing a calibration run; see **QUALITY CONTROL PROCEDURES** section of this

package insert for description of assay calibration. Once thawed, assay controls, IC, and calibrators can be stored at 2-8°C for up to 24 hours before use.

2. Vortex each assay calibrator and each control three times for 2-3 seconds. Ensure that the contents of each vial are at the bottom after vortexing by tapping the vials on the bench to bring liquid to the bottom of the vial. Ensure bubbles or foam are not generated; if present, remove with a sterile pipette tip, using a new tip for each vial.
3. Thaw amplification reagents at 15-30°C or at 2-8°C and store at 2-8°C until required for the amplification master mix procedure. Once thawed, the amplification reagents can be stored at 2-8°C for up to 24 hours if not used immediately.

NOTE: Use one bottle of *m*Lysis Buffer, one vial of IC, and one RealTime HIV-1 Amplification Reagent Pack to support up to 24 reactions. Use a second set of reagents to support 25 to 48 reactions. A maximum of 48 reactions can be performed per run.

4. Invert gently the Abbott *m* Sample Preparation bottles to ensure a homogeneous solution without generating any bubbles. If crystals are observed in any of the reagent bottles upon opening, allow the reagent to equilibrate at room temperature until the crystals disappear. Do not use the reagents until the crystals have dissolved. Ensure bubbles or foam are not generated; if present, remove with a sterile pipette tip, using a new tip for each bottle.
5. Vortex each IC vial three times for 2-3 seconds before use. Ensure bubbles or foam are not generated; if present, remove with a sterile pipette tip, using a new tip for each vial.
6. Use a calibrated precision **PIPETTE DEDICATED FOR INTERNAL CONTROL USE ONLY** to add 500 µL of IC to each bottle of *m*Lysis Buffer. Mix by gently inverting the container 5 to 10

times to minimize foaming.

- 7. Allow for a maximum of 48 samples for each run.** A negative control, a low positive control, and a high positive control are included in each run, therefore allowing a maximum of 45 specimens to be processed per run.
8. Thaw specimens at 15-30°C or at 2-8°C. Once thawed, specimens can be stored at 2-8°C for up to 6 hours if not processed immediately.
9. Check sample volume. The Abbott RealTime HIV-1 assay minimum sample volume and associated rack requirements on the Abbott *m2000sp* are described below.

CAUTION: Do not put a 13 mm tube in a 16 mm rack.

Abbott RealTime HIV-1 Minimum Sample Volume
Assay Application

| Rack | Tube Diameter ^a | 0.2 mL | 0.5 mL | 1.0 mL |
|-------|----------------------------|--------|--------|--------|
| 13 mm | 11.6 mm - 14.0 mm | 0.7 mL | 1.0 mL | 1.5 mL |
| 16 mm | 15.0 mm - 16.0 mm | 1.0 mL | 1.3 mL | 1.8 mL |

^aRefers to sample tube outer diameter.

CAUTION: Steps 10 and 11 must be done in the order described. Vortex the specimens first, and follow with centrifugation. If these two steps are not performed in this order, then invalid results may occur.

10. Vortex each specimen three times for 2-3 seconds.

11. Centrifuge specimens at 2,000g for 5 minutes before loading on the Abbott *m2000sp* worktable.

NOTE: The “g” refers to g force, not revolutions per minute (rpm).

12. Aliquot each specimen into clean tubes or vials if necessary. Refer to the Abbott *m2000sp* Operations Manual for tube sizes. Avoid touching the inside of the cap when opening tubes. Take care not to disturb contents of the tube while removing the tube from the centrifuge and that the bottom of the tube is not touched by the pipette tip. Ensure that the newly aliquotted sample retains the minimum volume indicated in the preceding table.

13. Place the low and high positive controls, the negative control, the calibrators, if applicable, and the patient specimens into the Abbott *m2000sp* sample rack.

14. Place the 5 mL Reaction Vessels into the *m2000sp* 1 mL subsystem carrier.

15. Load the Abbott *m* Sample Preparation System reagents and the Abbott 96 Deep-Well Plate on the Abbott *m2000sp* worktable as described in the Abbott *m2000sp* Operations Manual, Operating Instructions section.

16. Select the appropriate application file from the Run Sample Extraction screen that corresponds to the sample volume being tested. Initiate the sample extraction protocol as

described in the Abbott *m2000sp* Operations Manual, Operating Instruction section.

17. Enter calibrator (needed if a calibration curve has not been stored on the *m2000rt*) and control lot specific values in the **Sample Extraction: Worktable Setup, Calibrator and Control** fields. Lot specific values are specified in each Abbott RealTime HIV-1 Calibrator and Control Kit Card.

NOTE: Verify the values entered match the values provided in the lot specific kit cards.

NOTE: The Abbott *m2000sp* Master Mix Addition protocol (step 21) must be initiated within one hour after completion of Sample Preparation.

NOTE: Change gloves before handling the amplification reagents.

18. Load the amplification reagents and the master mix vial on the *m2000sp* worktable after sample preparation is completed. Each Amplification Reagent Pack supports up to 24 reactions.

NOTE: A second Amplification Reagent Pack is required if performing 25 to 48 reactions.

19. Ensure that the contents are at the bottom of the vials prior to opening the amplification reagents by tapping the vials in an upright position on the bench.
20. Remove and discard the amplification vial caps.
21. Select the appropriate deep well plate from the Run Master Mix Addition screen that matches

the corresponding sample preparation extraction. Initiate the Abbott *m2000sp* Master Mix Addition protocol. Follow the instructions as described in the Abbott *m2000sp* Operations Manual, Operating Instructions section. **The *m2000rt* protocol must be started within 40 minutes of the initiation of the Master Mix Addition protocol.**

22. Switch on and initialize the Abbott *m2000rt* instrument in the Amplification Area.

NOTE: The Abbott *m2000rt* requires 15 minutes to warm-up.

NOTE: Remove gloves before returning to the sample preparation area.

23. Seal the Abbott 96-Well Optical Reaction Plate after the Abbott *m2000sp* instrument has completed addition of samples and master mix according to the Abbott *m2000sp* Operations Manual, Operating Instructions section. Export completed PCR plate results to a CD.

24. Place the sealed optical reaction plate into the Splash Free Support Base for transfer to the Abbott *m2000rt* instrument.

25. Place the Abbott 96-Well Optical Reaction Plate in the Abbott *m2000rt* instrument. Import *m2000sp* test order via CD per the Import Order instructions in the Abbott *m2000rt* Operations Manual, Operating Instructions section.

POST PROCESSING PROCEDURES

1. Remove the Abbott 96 Deep-Well Plate from the worktable and dispose of according to the Abbott *m2000sp* Operations Manual.
2. Place the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose

according to the Abbott *m2000rt* Operations Manual along with the gloves used to handle the plate.

3. Clean the Splash Free Support Base before next use, according to the Abbott *m2000rt* Operations Manual.

QUALITY CONTROL PROCEDURES

Abbott *m2000rt* Optical Calibration

Refer to the Calibration Procedures section in the Abbott *m2000rt* Operations Manual for a detailed description of how to perform an Abbott *m2000rt* Optical Calibration.

Optical calibration of the Abbott *m2000rt* instrument is required for the accurate measurement and discrimination of dye fluorescence during the Abbott RealTime HIV-1 assay.

The following Abbott *m2000rt* Optical Calibration Plates are used to calibrate the Abbott *m2000rt* instrument for the Abbott RealTime HIV-1 assay:

- FAM™ Plate (Carboxyfluorescein)
- ROX™ Plate (Carboxy-X-rhodamine)
- VIC® Plate (Proprietary dye)

Assay Calibration

For a detailed description of how to perform an Assay Calibration refer to the Abbott *m2000sp* and *m2000rt* Operations Manuals, Operating Instructions sections.

A calibration curve is required to quantitate the HIV-1 RNA concentration of specimens and

controls. Two assay calibrators are run in replicates of three to generate a calibration curve (HIV-1 concentration versus the threshold cycle [C_t] at which a reactive level of fluorescent signal is detected). The calibration curve slope and intercept are calculated and stored on the instrument. The concentration of HIV-1 RNA in a sample is calculated from the stored calibration curve. Results are automatically reported on the *m2000rt* workstation.

Follow the procedure for sample extraction, master mix addition, amplification and detection protocols as stated in the Abbott *m2000sp* Operations Manual and the Abbott *m2000rt* Operations Manual.

Once an Abbott RealTime HIV-1 calibration is accepted and stored, it may be used for six months. During this time, all subsequent samples may be tested without further calibration unless:

- An Abbott RealTime HIV-1 Amplification Reagent Kit with a new lot number is used.
- An Abbott *m* Sample Preparation System (4 x 24 Preps) with a new lot number is used.
- An Abbott RealTime HIV-1 application file for a different sample volume is used.
- A new Abbott RealTime HIV-1 application specification file is installed.

Detection of Inhibition

An IC threshold cycle [C_t] assay validity parameter is established during a calibration run.

A defined, consistent quantity of IC is introduced into each specimen, calibrator, and control at the beginning of sample preparation and detected on the Abbott *m2000rt* instrument to demonstrate proper specimen processing and assay validity. The IC is comprised of an RNA sequence unrelated to the HIV-1 target sequence.

The median amplification cycle at which the IC target sequence fluorescent signal is detected in calibration samples establishes an IC C_t validity range to be met by all subsequent processed specimens and controls.

An error is displayed when a specimen or control fails to meet this specification. Refer to the *m2000rt* Operations Manual for an explanation of the corrective actions for the error. Specimens whose IC C_t value exceeds the established range must be retested starting with sample preparation.

Negative and Positive Controls

A negative control, a low positive control, and a high positive control are included in each test order to evaluate run validity.

The lot specific values for the low positive control and high positive control are specified on each Abbott RealTime HIV-1 Control Kit Card and must be entered into the assay test order when a run is performed.

An error is displayed when a control result is out of range. Refer to the Abbott *m2000rt* Operations Manual for an explanation of the corrective actions for the error. If negative or positive controls are out of range, all of the specimens and controls from that run must be reprocessed, beginning with sample preparation.

The presence of HIV-1 must not be detected in the negative control. HIV-1 detected in the negative control is indicative of contamination by other samples or by amplified product introduced during sample preparation or during preparation of the Abbott 96-Well Optical Reaction Plate. To avoid contamination, clean the Abbott *m2000sp* instrument and the Abbott *m2000rt* instrument and repeat sample processing for controls and specimens following the **Procedural Precautions**. If negative

controls are persistently reactive, contact your Abbott representative.

Monitoring the Laboratory for the Presence of Contamination

It is recommended that this test be done at least once a month to monitor laboratory surfaces and equipment for contamination by amplification product. It is very important to test all areas that may have been exposed to processed specimens, controls, and calibrators, and/or amplification product. This includes routinely handled objects such as pipettes, the Abbott *m2000sp* and Abbott *m2000rt* function keys, laboratory bench surfaces, microcentrifuges, and centrifuge adaptors.

1. Add 0.8 mL RNase-free water to a 1.7 mL RNase-free microcentrifuge tube.
2. Saturate the cotton tip of an applicator (Puritan or equivalent) in the RNase-free water from the microcentrifuge tube.
3. Using the saturated cotton tip of the applicator, wipe the area to be monitored using a sweeping motion. Place the applicator into the microcentrifuge tube.
4. Swirl the cotton tip in RNase-free water 10 times, and then press the applicator along the inside of the tube so that the liquid drains back into the solution at the bottom of the microcentrifuge tube. Discard the applicator.
5. Pipette 0.5 mL of *mWash* 1 buffer to a clean tube using the pipette dedicated for Internal Control use.
6. Add 20 μ L of the *mWash* 1 buffer to each microcentrifuge tube.
7. Cap the microcentrifuge tube.

8. Test the samples according to the assay procedure section of this package insert.
 - Transfer liquid from the microcentrifuge tube to a 5 mL Reaction Vessel.
 - Bring the volume to a minimum of 1.5 mL with RNase-free water.
9. The presence of contamination is indicated by the detection of HIV-1 nucleic acid in the swab samples.
10. If HIV-1 nucleic acid is detected on equipment, follow the cleaning and decontaminating guidelines given in that equipment's operations manual. If HIV-1 nucleic acid is detected on surfaces, clean the contaminated areas with 1.0% (v/v) sodium hypochlorite solution, followed by 70% ethanol or water.

NOTE: Chlorine solutions may pit equipment and metal. Use sufficient amounts or repeated applications of 70% ethanol or water until chlorine residue is no longer visible.
11. Repeat testing of the contaminated area by following Steps 1 through 10.

RESULTS

Calculation

The concentration of viral HIV-1 RNA in a sample or control is calculated from the stored calibration curve. The Abbott *m2000rt* instrument automatically reports the results on the Abbott *m2000rt* workstation. Assay results can be reported in Copies/mL, Log [Copies/mL], International Units (IU)/mL, or Log [IU/mL]; (1 IU = 0.58 copies, 1 copy = 1.74 IU), with WHO 1st International Standard for HIV-1 RNA (97/656).

INTERPRETATION OF RESULTS

| Sample Volume | Result | Interpretation |
|---------------|-------------------------------------|---------------------|
| 1.0 mL | Not Detected | Target not detected |
| | < 1.60 Log [Copies/mL] ^a | Detected |
| | 1.60 to 7.00 Log [Copies/mL] | |
| | > 7.00 Log [Copies/mL] | > ULQ ^d |
| 0.5 mL | Not Detected | Target not detected |
| | < 1.88 Log [Copies/mL] ^b | Detected |
| | 1.88 to 7.00 Log [Copies/mL] | |
| | > 7.00 Log [Copies/mL] | > ULQ |
| 0.2 mL | Not Detected | Target not detected |
| | < 2.18 Log [Copies/mL] ^c | Detected |
| | 2.18 to 7.00 Log [Copies/mL] | |
| | > 7.00 Log [Copies/mL] | > ULQ |

^a40 Copies/mL

^b75 Copies/mL

^c150 Copies/mL

^dULQ = upper limit of quantitation

- A result of “Not Detected” signifies that no target was detected.
- A result of “<1.60 or, < 1.88 or, < 2.18 Log [copies/mL]” indicates that target was detected but is less than lower limit of quantitation (LLQ) for the respective input volumes of 1.0, 0.5, and 0.2 mL.
- For 1.0 mL input volume, a result of “1.60 to 7.00 Log [copies/mL]” indicates that the target was detected and the concentration falls between 1.6 log copies per mL (LLQ) and 7.0 log copies per mL (ULQ). For a 0.5 mL input volume, a result of “1.88 to 7.00 Log [copies/mL]” indicates that the target was detected and the concentration falls between 1.88 log copies per mL (LLQ) and 7.0 log copies per mL (ULQ). For a 0.2 mL input volume, a result of “2.18 to 7.00 Log [copies/mL]” indicates that the target was detected and the concentration falls between 2.18 log copies per mL (LLQ) and 7.0 log copies per mL (ULQ). Note that no interpretation is reported on the *m2000rt* printout when results fall between LLQ and ULQ.
- A result of “>7.00 Log [copies/mL]” indicates that the target was detected and is greater than ULQ.

LIMITATIONS OF THE PROCEDURE

- **FOR *IN VITRO* DIAGNOSTIC USE ONLY.**
- Optimal performance of this test requires appropriate specimen collection, handling, preparation, and storage (refer to the **SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE** section of this package insert).
- Human plasma specimens (collected in ACD-A or EDTA tubes) may be used with the Abbott

RealTime HIV-1 assay.

- Use of the Abbott RealTime HIV-1 assay is limited to personnel who have been trained in the procedures of a molecular diagnostic assay and the Abbott *m2000sp* and the Abbott *m2000rt* instruments.
- The instruments and assay procedures reduce the risk of contamination by amplification product. However, nucleic acid contamination from the calibrators, positive controls, or specimens must be controlled by good laboratory practice and careful adherence to the procedures specified in this package insert.
- A specimen with a result of “Target not detected” cannot be presumed to be negative for HIV-1 RNA.
- As with any diagnostic test, results from the Abbott RealTime HIV-1 assay should be interpreted in conjunction with other clinical and laboratory findings.

SPECIFIC PERFORMANCE CHARACTERISTICS

The performance characteristics were determined using the RealTime HIV-1 assay with the Abbott *m2000* system and 1.0 mL sample volume, unless otherwise specified.

Limit of Detection (LOD)

The limit of detection is defined as the HIV-1 RNA concentration detected with a probability of 95% or greater.

Limit of Detection, 1.0 mL Sample Volume

The LOD claim for the Abbott RealTime HIV-1 assay is 40 copies/mL with the 1.0 mL sample volume procedure.

The LOD was determined by testing dilutions of a viral standard from the Virology Quality Assurance (VQA) Laboratory of the AIDS Clinical Trial Group. Dilutions were made in HIV-1 negative human plasma. Testing was performed with three lots of amplification reagents on three *m2000* Systems. The results, representative of the analytical sensitivity performance of the RealTime HIV-1 assay, are summarized in Table 1.

Table 1

| Conc. (copies/mL) | Number Tested | Number Detected | Percent Detected |
|-------------------|-----------------|-----------------|------------------|
| 100 | 57 | 57 | 100 |
| 75 | 57 | 57 | 100 |
| 60 | 57 | 57 | 100 |
| 50 | 57 | 57 | 100 |
| 40 | 57 | 57 | 100 |
| 30 | 57 | 55 | 96 |
| 20 | 57 | 50 | 88 |
| 10 | 56 ^a | 38 | 68 |
| 5 | 57 | 30 | 53 |

^aOne replicate generated an invalid replicate error message and was deleted from the data analysis.

Probit analysis of the data determined that the concentration of HIV-1 RNA detected with 95%

probability was 25 copies/mL (95% CI 20-33).

Limit of Detection, 0.5 mL Sample Volume

The LOD claim for the Abbott RealTime HIV-1 assay is 75 copies/mL with the 0.5 mL sample volume procedure.

The LOD for the 0.5 mL sample volume procedure was determined as described for the 1.0 mL sample volume procedure. The results, representative of the analytical sensitivity performance of the RealTime HIV-1 assay, are summarized in Table 2.

Table 2

| Conc. (copies/mL) | Number Tested | Number Detected | Percent Detected |
|--------------------------|----------------------|------------------------|-------------------------|
| 100 | 57 | 57 | 100 |
| 75 | 57 | 57 | 100 |
| 60 | 57 | 54 | 95 |
| 50 | 56 ^a | 52 | 93 |
| 40 | 57 | 47 | 82 |
| 30 | 57 | 46 | 81 |
| 20 | 57 | 42 | 74 |
| 10 | 57 | 26 | 46 |
| 5 | 57 | 21 | 37 |

^aOne replicate generated an invalid replicate error message and was deleted from the data analysis.

Probit analysis of the data determined that the concentration of HIV-1 RNA detected with 95% probability was 65 copies/mL (95% CI 51-88).

Limit of Detection, 0.2 mL Sample Volume

The LOD claim for the Abbott RealTime HIV-1 assay is 150 copies/mL with the 0.2 mL sample volume procedure.

The LOD for the 0.2 mL sample volume procedure was determined as described for the 1.0 mL sample volume procedure. The results, representative of the analytical sensitivity performance of the RealTime HIV-1 assay, are summarized in Table 3.

Table 3

| Conc. (copies/mL) | Number Tested | Number Detected | Percent Detected |
|--------------------------|----------------------|------------------------|-------------------------|
| 250 | 57 | 57 | 100 |
| 200 | 57 | 56 | 98 |
| 150 | 57 | 56 | 98 |
| 100 | 57 | 54 | 95 |
| 75 | 57 | 47 | 82 |
| 60 | 57 | 38 | 67 |
| 50 | 57 | 39 | 68 |
| 40 | 54 ^a | 30 | 56 |
| 30 | 52 ^a | 19 | 37 |

^aEight replicates were invalid due to an instrument error and were deleted from the data analysis.

Probit analysis of the data determined that the concentration of HIV-1 RNA detected with 95%

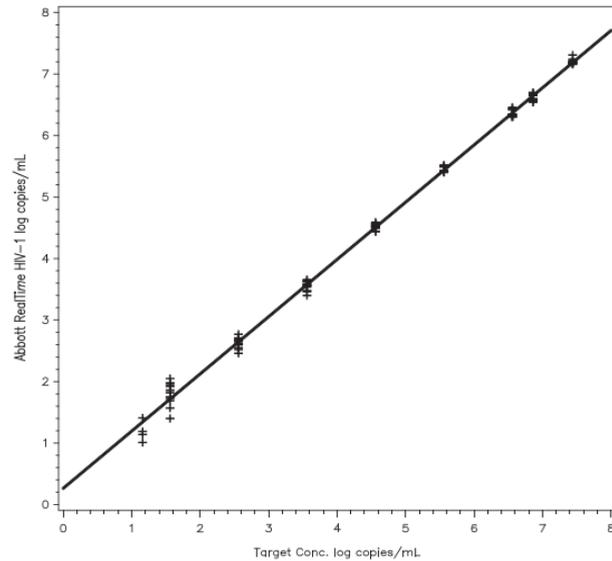
probability was 119 copies/mL (95% CI 102-150).

Linear Range

The upper limit of quantitation (ULQ) for the Abbott RealTime HIV-1 assay is 10 million copies/mL, and the lower limit of quantitation is equivalent to the LOD (40 copies/mL for the 1.0 mL sample volume procedure, 75 copies/mL for the 0.5 mL sample volume procedure, and 150 copies/mL for the 0.2 mL sample volume procedure).

A nine-member panel prepared by diluting armored HIV-1 RNA from 7.44 log copies/mL to 1.16 log copies/mL in HIV-1 negative human plasma was tested. Linearity analysis was performed following the NCCLS EP6-A³⁸ guideline. The results, representative of the RealTime HIV-1 assay linearity, are shown in Figure 1.

Figure 1



The RealTime HIV-1 assay was shown to be linear across the range tested ($n=99$, $r=0.999$, slope=0.93, and intercept=0.26).

Precision

The RealTime HIV-1 assay was designed to achieve an inter-assay standard deviation (SD) of less than or equal to 0.25 log copies/mL in samples that contain HIV-1 RNA concentrations between 5,000,000 to 500 copies/mL. Assay precision was demonstrated by testing a coded 45-member precision panel that consisted of nine unique members repeated five times within the panel. The panel was prepared by diluting an HIV-1 viral stock in HIV-1 negative human plasma. The mean RNA concentrations of the panel members ranged from 6.51 to 1.46 log copies/mL. Testing was conducted using the CLSI EP10-A2 guideline.³⁹ A total of three reagent lots were used. Each of the three external sites tested two of the lots for three days for a total of 18 runs. A total of 90 replicates was tested for each panel member. The results of a variance component analysis are in Table 4.

Table 4
Overall Precision Analysis

| Panel | n ^c | Mean Conc. (log copies/mL) | Within-Run | Between-Run | Inter-Assay | Between-Lot | Between-Site | Total |
|-------|-----------------|-------------------------------|------------|-------------|-----------------|-------------|--------------|-----------------|
| | | | Component | Component | | Component | Component | |
| | | | SD | SD | SD ^a | SD | SD | SD ^b |
| 1 | 88 | 6.51 | 0.06 | 0.04 | 0.07 | 0.00 | 0.14 | 0.16 |
| 2 | 86 | 5.83 | 0.06 | 0.03 | 0.06 | 0.02 | 0.11 | 0.13 |
| 3 | 87 | 5.21 | 0.05 | 0.03 | 0.06 | 0.04 | 0.11 | 0.13 |
| 4 | 87 | 4.58 | 0.06 | 0.03 | 0.06 | 0.06 | 0.07 | 0.11 |
| 5 | 88 | 3.96 | 0.06 | 0.00 | 0.06 | 0.07 | 0.03 | 0.09 |
| 6 | 87 | 3.38 | 0.06 | 0.01 | 0.06 | 0.04 | 0.05 | 0.09 |
| 7 | 88 | 2.77 | 0.07 | 0.02 | 0.08 | 0.06 | 0.06 | 0.11 |
| 8 | 89 | 2.13 | 0.13 | 0.04 | 0.13 | 0.10 | 0.07 | 0.18 |
| 9 | 86 ^d | 1.46 | 0.24 | 0.00 | 0.24 | 0.18 | 0.00 | 0.30 |

^aIncludes within-run and between-run components

^bIncludes within-run, between-run, between-lot, and between-site components

^cValid test results

^dOne replicate reported as "Target not detected"

Potentially Interfering Substances

The susceptibility of the Abbott RealTime HIV-1 assay to interference by elevated levels of endogenous substances and by drugs commonly prescribed to HIV-1 infected individuals was evaluated. HIV-1 negative samples and samples containing 10,000 copies/mL of HIV-1 RNA were tested.

No interference in the performance of the Abbott RealTime HIV-1 assay was observed in the presence of the following substances for all positive and negative samples tested:

- Hemoglobin 500 mg/dL
- Triglycerides 3000 mg/dL
- Bilirubin 20 mg/dL
- Protein 9 g/dL

Drugs at concentrations in excess of the peak plasma or serum levels were tested in five pools. No interference in the performance of the Abbott RealTime HIV-1 assay was observed in the presence of the following drug pools for all positive and negative samples tested:

| <u>Drug Pool</u> | <u>Drugs Tested</u> |
|------------------|--|
| 1 | Zidovudine, Saquinavir, Ritonavir, Clarithromycin, Interferon 2a, Interferon 2b |
| 2 | Abacavir sulfate, Amprenavir, Peginterferon 2a, Peginterferon 2b, Ribavirin |
| 3 | Tenofovir disoproxil fumarate, Lamivudine, Indinavir sulfate, Ganciclovir, Valganciclovir hydrochloride, Acyclovir |
| 4 | Stavudine, Efavirenz, Lopinavir, Enfuvirtide, Ciprofloxacin |
| 5 | Zalcitabine, Nevirapine, Nelfinavir, Azithromycin, Valacyclovir |

Specificity

The specificity of the RealTime HIV-1 assay was evaluated at three external sites by testing 514 HIV-1 seronegative plasma specimens from volunteer blood donors. The specimens were tested on three *m2000* instrument systems with four lots of amplification reagents.

In this representative study HIV-1 RNA was not detected for all 514 specimens and the RealTime HIV-1 assay specificity was estimated to be 100% (514/514), (95% CI 99.28 to 100%).

The specificity of the assay was further evaluated by testing 70 specimens that had been either obtained from individuals diagnosed or screened for an autoimmune disorder or serologically characterized as positive for the following markers: systemic lupus erythematosus (SLE), anti-nuclear antibodies (ANA), rheumatoid factor (RF), HBsAg, anti-HTLV-I/II, anti-HCV, and anti-HIV-2. HIV-1

RNA was not detected in any of the specimens tested. The results demonstrated that the presence of an autoimmune disorder or serologic markers for autoimmune disease or viral pathogens other than HIV-1 did not affect the Abbott RealTime HIV-1 assay.

Cross-Reactivity

The following viruses and microorganisms were evaluated for potential cross-reactivity in the RealTime HIV-1 assay. Purified nucleic acid or viral lysate from each organism was added at a targeted concentration of 5.0 log copies/mL into HIV-1 RNA negative samples and samples that contained 10,000 copies/mL HIV-1 RNA.

| | |
|--------------------------------|----------------------------|
| Human Immunodeficiency virus 2 | Vaccinia virus |
| Human T-lymphotropic virus 1 | BK human polyomavirus |
| Hepatitis C virus | Human papilloma virus 16 |
| Hepatitis B virus | Human papilloma virus 18 |
| Epstein-Barr virus | Neisseria gonorrhoeae |
| Herpes simplex virus 1 | Chlamydia trachomatis |
| Herpes simplex virus 2 | Candida albicans |
| Cytomegalovirus | Staphylococcus aureus |
| Human herpesvirus 6B | Staphylococcus epidermidis |
| Human herpesvirus 8 | Mycobacterium gordonae |
| Varicella-zoster virus | Mycobacterium smegmatis |

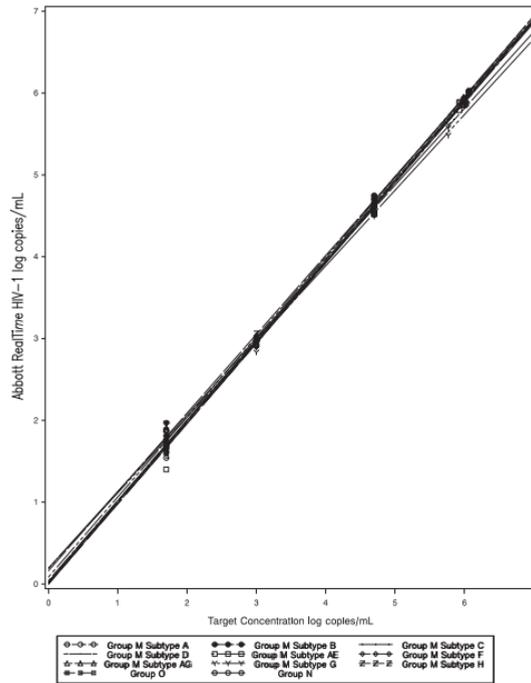
No interference in the performance of the Abbott RealTime HIV-1 assay was observed in the presence of the potential cross-reactants for all positive and negative samples tested.

Detection of HIV-1 Subtypes and Groups

The performance of the RealTime HIV-1 assay with HIV-1 subtypes/groups was evaluated by analysis of purified RNA transcripts from Group M (subtypes A, B, C, D, CRF01-AE, F, CRF02-AG, G, and H), Group O, and Group N, and by testing ten clinical specimens of each Group M subtype (A, B, C, D, CRF01-AE, F, CRF02-AG, G), and ten specimens from Group O.

RNA transcripts of Group M (subtypes A, B, C, D, CRF01-AE, F, CRF02-AG, G, and H), Group O, and Group N with concentrations targeted to approximately 6.0 log copies/mL, 4.7 log copies/mL, 3.0 log copies/mL, and 1.7 log copies/mL were tested. Three replicates were tested at each concentration for each transcript. The results, representative of the dilution linearity for the 11 subtypes/groups tested, are shown in Figure 2.

Figure 2



The results showed that all subtypes and groups tested were detected, and dilution linearity was demonstrated for all groups and subtypes tested (correlation coefficients ranged from 0.997 to 1.000).

A total of 90 clinical specimens, ten of each Group M subtype (A, B, C, D, CRF01-AE, F, CRF02-AG, G) and Group O, were tested with the RealTime HIV-1 assay and by two other approved HIV-1 quantitative assays referred to as Comparator 1 (FDA-approved version used) and Comparator 2 (CE-marked version used). The results are summarized in Table 5.

Table 5

| Group/ Subtypes | n | RealTime Detected | Comparator 1 Detected^a | Comparator 2 Detected^a |
|----------------------------|----------|------------------------------|--|--|
| M/Subtype A | 10 | 10 | 10 (1) | 10 (1) |
| M/Subtype B | 10 | 10 | 10 (0) | 10 (0) |
| M/Subtype C | 10 | 10 | 10 (0) | 10 (0) |
| M/Subtype D | 10 | 10 | 10 (0) | 10 (0) |
| M/Subtype AE | 10 | 10 | 10 (0) | 10 (0) |
| M/Subtype F | 10 | 10 | 10 (0) | 10 (0) |
| M/Subtype AG | 10 | 10 | 10 (3) | 10 (1) |
| M/Subtype G | 10 | 10 | 10 (2) | 10 (1) |
| Group O | 10 | 10 | 0 (NA) | 7 (7) |

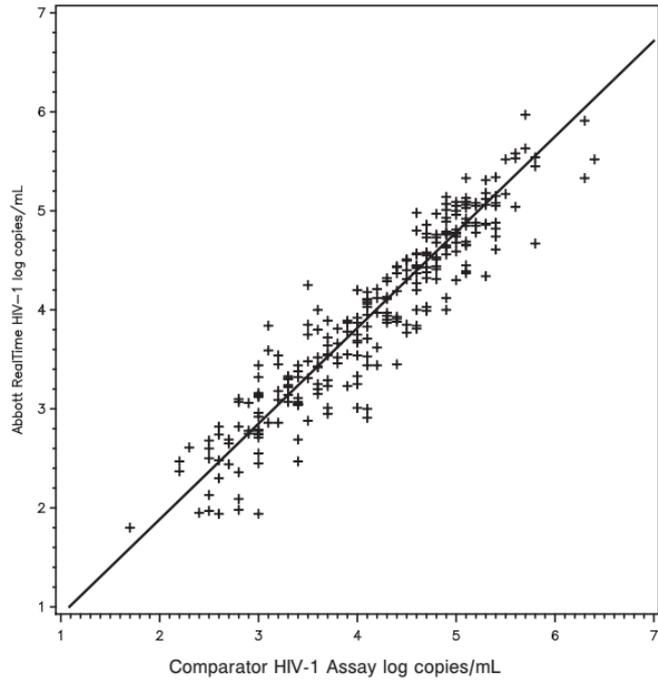
^aThe numbers in parentheses are the number of specimens that had lower quantitation values by more than 1.00 log copies/mL when compared to RealTime HIV-1 assay.

- The RealTime HIV-1 assay detected all subtypes and groups tested (quantitation range was 2.56 to 6.14 log copies/mL).
- Comparator 1 detected all Group M subtypes tested and did not detect the ten Group O samples (quantitation range of those detected was 2.01 to 5.54 log copies/mL, and three samples were above the upper limit of quantitation [ULQ]).
- Comparator 2 detected all Group M subtypes tested and seven out of ten Group O samples (quantitation range of those detected was 1.75 to 5.41 log copies/mL).
- There were no samples that had RealTime assay quantitation values lower than Comparator 1 or Comparator 2 values by more than 1.00 log copies/mL.
- There were six Group M samples that had lower quantitation values with Comparator 1 by more than 1.00 log/copies/mL when compared to RealTime HIV-1 assay.
- There were three Group M samples and seven Group O samples that had lower quantitation values with Comparator 2 by more than 1.00 log copies/mL when compared to RealTime HIV-1 assay.

Correlation

HIV-1 RNA quantitation was compared between the Abbott RealTime HIV-1 assay and an FDA-approved comparator HIV-1 RNA quantitative assay. A total of 301 specimens collected from HIV-1 infected patients were tested with the RealTime HIV-1 assay at three external sites and with the comparator method at a central laboratory site. The results from a total of 259 specimens that fell within the common assay dynamic range were analyzed by the Passing-Bablok linear regression method (Figure 3).⁴⁰ The correlation coefficient was 0.936, the slope was 0.97 (95% CI 0.92 to 1.01), and the intercept was -0.05 log copies/mL (95% CI -0.22 to 0.14).

Figure 3



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May 2007