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I. General Information

A. Device Trade Name

Abbott RealTi*m*e HIV-1 Amplification Reagent Kit, Abbott RealTi*m*e HIV-1 Calibrator Kit, Abbott RealTi*m*e HIV-1 Control Kit.

B. Device Generic Name

In vitro reverse transcription-polymerase chain reaction (RT-PCR) for HIV-1 viral load.

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F. Date of Panel recommendation:

G. Date of notice of approval:

II. Indications for Use

The Abbott RealTi*me* 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 *m*2000 System in human plasma from HIV-1 infected individuals over the range of 40 to 10,000,000 copies/mL. The Abbott RealTi*me* 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.

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]

III. Device Description

The Abbott RealTi*m*e HIV-1 assay is an in vitro reverse transcriptase-polymerase chain reaction assay for the quantitative detection of HIV-1 RNA in human plasma from HIV-1 infected individuals on the *m*2000 System. The Abbott RealTi*m*e HIV-1 assay uses the Abbott *m*2000*sp* instrument for processing samples and the Abbott *m*2000*rt* instrument for amplification and detection. The Abbott RealTi*m*e HIV-1 assay uses 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).

The Abbott RealTi*m*e HIV-1 assay consists of three reagent kits:

- Abbott RealTi*m*e HIV-1 Amplification Reagent Kit

- Abbott RealTi*m*e HIV-1 Control Kit

- Abbott RealTi*m*e HIV-1 Calibrator Kit

The Abbott RealTi*m*e HIV-1 assay uses RT-PCR[26] 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 *m*2000*rt*™ 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 *m*2000*rt* is proportional to the log of the HIV-1 RNA concentration present in the original sample.

A. 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 *m*2000*sp* instrument prepares samples for the Abbott RealTi*m*e HIV-1 assay using the Abbott *m*Sample Preparation System (4 x 24 Preps) reagents. The Abbott *m*2000*sp* 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.

B. Reagent Preparation and Reaction Plate Assembly

The Abbott *m*2000*sp* combines the Abbott RealTi*m*e HIV-1 amplification reagent components (HIV-1 Oligonucleotide Reagent, Thermostable rTth Polymerase Enzyme, and Activation Reagent). The Abbott *m*2000*sp* dispenses the resulting master mix to the Abbott 96-Well Optical Reaction Plate and aliquots of the nucleic acid samples prepared by the Abbott *m*2000*sp*. The plate is ready, after manual application of the optical seal, for transfer to the Abbott *m*2000*rt*.

C. Amplification

Two sets of oligonucleotide primers and probes are used in the Abbott RealTi*m*e HIV-1 amplification, one specific for amplifying and detecting HIV-1 RNA, and the other specific for amplifying and detecting Internal Control RNA. The target sequence for HIV-1 is in the *pol* integrase region of the HIV-1 genome. The target sequence for Internal Control is derived from the hydroxypyruvate reductase gene from the pumpkin plant, *Cucurbita pepo*.

During the amplification reaction on the Abbott *m*2000*rt*, 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.

D. Detection

During the read cycles of amplification on the Abbott *m*2000*rt*, 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 *m*2000*rt* is proportional to the log of the HIV-1 RNA concentration present in the original sample.

E. 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 stored on the instrument. The concentration of the HIV-1 RNA in specimens and controls is calculated from the stored calibration curve, and the results are automatically reported on the *m*2000*rt* workstation. The Abbott RealTi*m*e HIV-1 Negative Control, Low Positive Control, and High Positive Control must be included in each run to verify run validity. The *m*2000 System verifies that the controls are within the assigned ranges.

F. Abbott RealTi*m*e HIV-1 Amplification Reagent Kit

The Abbott RealTi*m*e HIV-1 Amplification Reagent Kit contains four vials of Abbott RealTi*m*e HIV-1 Internal Control and four Abbott RealTi*m*e HIV-1 Amplification Reagent Packs. The Abbott RealTi*m*e reagents are intended for single-use only and unused reagent should be discarded.

1. Abbott RealTi*m*e HIV-1 Internal Control

The Abbott RealTi*m*e HIV-1 Internal Control consists of Armored RNA$^{®}$ with a RNA sequence unrelated to HIV-1 in negative human plasma containing the antimicrobial compounds ProClin$^{®}$ 300 and ProClin 950. The negative human plasma used in the Abbott RealTi*m*e HIV-1 Internal Control component is tested and found to be nonreactive for HBsAg, HBV DNA, HIV RNA, HCV RNA,

anti-HIV-1/HIV-2, and anti-HCV and contains the antimicrobial compounds ProClin 300 and ProClin 950.

2. **Abbott RealTi*m*e HIV-1 Amplification Reagent Pack**

The Abbott RealTi*m*e HIV-1 Amplification Reagent Pack consists of the Thermostable rTth Polymerase Enzyme, the HIV-1 Oligonucleotide Reagent, and the Activation Reagent.

a. **Thermostable rTth Polymerase Enzyme**

Each vial contains recombinant Thermostable rTth Polymerase Enzyme in a buffered solution. The recombinant *Thermus thermophilus* thermostable DNA polymerase enzyme has a dual function as a reverse transcriptase transcribing cDNA from the RNA target, and as a DNA polymerase in PCR amplification.

b. **HIV-1 Oligonucleotide Reagent**

Each vial of HIV-1 Oligonucleotide Reagent contains two sets of oligonucleotide primers and probes, one specific for amplifying and detecting HIV-1 RNA, and the other specific for amplifying and detecting Internal Control RNA. The reagent also contains dNTPs, ROX™ passive reference dye and an aptamer oligonucleotide. The reagent is formulated in a potassium acetate-bicine buffer with the antimicrobial compounds ProClin 300 and ProClin 950.

c. **Activation Reagent**

Each vial of Activation Reagent contains a 30 mM manganese chloride solution, and the antimicrobial compounds ProClin 300 and ProClin 950. Manganese chloride is a co-factor of recombinant *Thermus thermophilus* thermostable DNA polymerase.

G. Abbott RealTi*m*e HIV-1 Control Kit

The Abbott RealTi*m*e HIV-1 Control Kit contains three controls (8 vials of Abbott RealTi*m*e HIV-1 Negative Control, 8 vials of Abbott RealTi*m*e HIV-1 Low Positive Control, and 8 vials of Abbott RealTi*m*e HIV-1 High Positive Control) that are used to establish the run validity of the Abbott RealTi*m*e HIV-1 assay.

The Abbott RealTi*m*e HIV-1 Negative Control contains negative human plasma that is tested and found to be nonreactive for HBsAg, HBV DNA, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, and anti-HCV and contains the antimicrobial compounds ProClin 300 and ProClin 950.

The Abbott RealTi*m*e HIV-1 Low Positive Control and High Positive Control contain Armored RNA with HIV-1 sequences in negative human plasma. The negative human plasma used in the Abbott RealTi*m*e HIV-1 Low Positive Control and High Positive Control is tested and found to be nonreactive for HBsAg, HBV DNA, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, and anti-HCV and contains the antimicrobial compounds ProClin 300 and ProClin 950.

H. Abbott RealTi*m*e HIV-1 Calibrator Kit

The Abbott RealTi*m*e HIV-1 Calibrator Kit contains two calibrators (12 vials of Abbott RealTi*m*e HIV-1 Calibrator A and 12 vials of Abbott RealTi*m*e HIV-1 Calibrator B) that are used to generate a calibration curve for the quantitative determination of HIV-1 in human plasma.

The Abbott RealTi*m*e HIV-1 Calibrator A and Calibrator B contain Armored RNA with HIV-1 sequences in negative human plasma. The negative human plasma used in the Abbott RealTi*m*e HIV-1 Calibrator Kit is tested and found to be nonreactive for HBsAg, HBV DNA, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, and anti-HCV and contains the antimicrobial compounds ProClin 300 and ProClin 950.

I. Assay Calibration

A calibration curve is required to quantitate the HIV-1 RNA concentration of specimens and controls. The lot specific values for the Calibrator A and Calibrator B are specified on each Abbott RealTi*m*e HIV-1 Calibrator Kit Card and must be entered when an assay calibration is performed. The calibrators are run in replicates of three to generate a calibration curve (HIV-1 concentration versus the threshold cycle [Ct]) 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 *m*2000*rt* workstation. 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:

- a new lot of the Abbott RealTi*m*e HIV-1 Amplification Reagent Kit is used;
- an Abbott *m*Sample Preparation System (4 × 24 Preps) with a new lot number is used;
- an Abbott RealTi*m*e HIV-1 application file for a different sample volume is used;
- a new Abbott RealTi*m*e HIV-1 application specification file is used.

J. Quality Control Procedures

1. 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 measured on the Abbott *m*2000*rt* 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. Specimens whose IC C_t value exceeds the established range must be retested starting with sample preparation.

2. Negative and Positive Controls

A negative control, a low positive control, and a high positive control are included in each run to evaluate run validity. The lot specific values for the low positive control and high positive control are specified on each Abbott RealTi*me* HIV-1 Control Kit Card and must be entered into the assay test order when a run is performed. 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.

IV. Warnings and Precautions

- For in vitro diagnostic use.

- 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 RealTi*m*e HIV-1 reagents are intended to be used only on the Abbott *m*2000 System consisting of the *m*2000*sp* for sample processing and the *m*2000*rt* for amplification and detection.

- Do not use expired reagents.

- The Abbott *m*2000*sp* Master Mix Addition protocol must be initiated within one hour after completion of Sample Preparation. If the Abbott *m*2000*sp* master mix addition protocol is aborted, seal the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the *m*2000*sp* Operations Manual, hazards section, along with the gloves used to handle the plate. Do not import the test order onto the *m*2000*rt*.

- The appropriate PCR plate must be selected when samples are loaded into the *m*2000*rt* instrument.

- The *m*2000*rt* protocol must be started within 40 minutes of the initiation of the Master Mix Addition protocol. If the Abbott *m*2000*rt* 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 *m*2000*rt* Operations Manual, hazards section, along with the gloves used to handle the plate.

- The Abbott RealTi*m*e HIV-1 assay was evaluated using frozen plasma samples. Fresh plasma samples were not evaluated.

- This product contains human sourced and/or potentially infectious components. 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.[27] Biosafety Level 2[28] or other appropriate biosafety practices [29,30] should be used for materials that contain or are suspected of containing infectious agents.

- The Abbott RealTi*m*e 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 Abbott RealTi*m*e HIV-1 assay is only for use with plasma specimens that have been handled and stored in capped tubes as described by the manufacturer.

- 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.

- 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.

- Decontaminate and dispose of all specimens, reagents, and other potentially biohazardous materials in accordance with local, state, and federal regulations.[31,32] All materials should be handled in a manner that minimizes the chance of potential contamination of the work area. 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.

- 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.

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

V. Alternate Practices and Procedures

Other commercially available devices are available including RNA viral load assays, HIV antigen assays, and CD4 and CD8 cell surface receptors.

VI. Potential Adverse Effects of the Device on Health

An erroneous low test result may lead to inappropriate treatment, or instill a false sense of security in a patient, which could lead to deterioration to the patient's condition. The possibility of incorrect results can happen with assignable causes such as a technician's error in following the procedures in the package insert or a device malfunction. An erroneous high test result on the other hand may contribute to unnecessary treatment or create anxiety or trauma to the patient. However, if the appropriate direction is followed as stated in the package insert, the likelihood of erroneous results are minimal from the use of this device.

The performance of the product in the clinical studies indicates that the benefit to the patient far outweighs any potential risk of adverse effect to the patient as a result of its use.

VII. Marketing History

This product is intended to be marketed in US and other countries requiring FDA approval.

The Abbott RealTi*m*e HIV-1 assay received CE certification and was launched in June 2005 outside of the United States, under the list code of 2G31. The following countries receive the Abbott RealTi*m*e HIV-1 assay:

Algeria, Austria, Belgium, Bulgaria, Central Africa, China, Commonwealth of Independent States, Colombia, Costa Rica, Croatia, Denmark, Finland, France, Germany, Honduras, Israel, Italy, Kuwait, Latvia, Lebanon, Mexico, Netherlands, Poland, Portugal, Rumania, Saudi Arabia, South Africa, South Korea, Spain, Sweden, Switzerland, Taiwan, United Arab Emirates, Ukraine, United Kingdom.

The CE-marked version of the assay has identical reagent components in the three kits in this submission, namely,

- Abbott RealTi*m*e HIV-1 Amplification Reagent Kit
- Abbott RealTi*m*e HIV-1 Control Kit
- Abbott RealTi*m*e HIV-1 Calibrator Kit

The sample preparation methods introduced with the CE-marked version includes *m*1000 and *m*2000*sp* for automated sample preparation, as well as a manual sample preparation procedure. Amplification and detection is performed on *m*2000*rt*.

This product has not been withdrawn from the market from any country related to safety or effectiveness, or for any other reasons.

VIII. Summary of Preclinical Studies

A. Limit of Detection

The LOD claim of the Abbott RealTi*m*e HIV-1 assay is 40 copies/mL with the 1.0 mL sample volume procedure, 75 copies/mL with the 0.5 mL sample volume procedure, and 150 copies/mL with the 0.2 mL sample volume procedure. The LOD is defined as the HIV-1 RNA concentration detected with a probability of 95% or greater. 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 RealTi*m*e HIV-1 amplification reagents on three *m*2000 Systems. Probit analysis of the data determined that the concentration of HIV-1 RNA detected with 95% probability was 25 copies/mL (95% CI 20 to 33) with the 1.0 mL sample volume procedure, 65 copies/mL (95% CI 51 to 88) with the 0.5 mL sample volume procedure, and 119 copies/mL (95% CI 102 to 150) with the 0.2 mL sample volume procedure.

B. Linear Range

The upper limit of quantitation (ULQ) for the Abbott RealTi*m*e 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 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-A guideline.[33] The RealTi*m*e HIV-1 assay was shown to be linear (n=99, r=0.999, slope=0.93, and intercept=0.26) within the range tested of 7.44 log copies/mL through 1.16 log copies/mL.

C. Specimen Type

The performance of the RealTi*m*e HIV-1 assay with plasma specimens collected in potassium EDTA, sodium EDTA, and ACD-A collection tubes was evaluated and supports the use of these anticoagulants.

D. Cross-Reactivity

The following viruses and microorganisms were evaluated for potential cross-reactivity in the RealTi*m*e 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 to samples that contained 10,000 copies/mL HIV-1 RNA. No interference in the performance of the Abbott RealTi*m*e HIV-1 assay was observed in the presence of the potential cross-reactants for all positive and negative samples tested.

Microorganism / Virus
Human immunodeficiency virus 2 (HIV-2)
Human T-lymphotropic virus 1 (HTLV-1)
Hepatitis C virus (HCV)
Hepatitis B virus (HBV)
Epstein-Barr virus (EBV)
Herpes simplex virus 1 (HSV-1)
Herpes simplex virus 2 (HSV-2)
Cytomegalovirus (CMV)
Human herpesvirus 6B (HHV-6B)
Human herpesvirus 8 (HHV-8)
Varicella-zoster virus (VZV)
Vaccinia virus (VACV)
BK human polyomavirus
Human papilloma virus 16 (HPV-16)
Human papilloma virus 18 (HPV-18)
Neisseria gonorrhoeae
Chlamydia trachomatis
Candida albicans
Staphylococcus aureus
Staphylococcus epidermidis
Mycobacterium gordonae
Mycobacterium smegmatis

E. Analytical Specificity

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 RealTi*me* HIV-1 assay.

F. Detection of HIV-1 Groups and Subtypes

The performance of the RealTi*me* HIV-1 assay with HIV-1 groups/subtypes 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 Group O.

A total of 11 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. 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 RealTi*me* 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 RealTi*me* HIV-1 assay detected and quantitated all 90 specimens (quantitation range was 2.56 to 6.14 log copies/mL). Comparator 1 detected all Group M

subtypes tested and did not detect 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).

G. **Potentially Interfering Substances**

The susceptibility of the Abbott RealTi*m*e 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 RealTi*m*e HIV-1 assay was observed in the presence of high levels of hemoglobin (500 mg/dL), bilirubin (20 mg/dL), protein (9 g/dL), and triglycerides (3,000 mg/dL).

Drugs at concentrations in excess of the peak plasma or serum levels were combined and tested in five pools. No interference in the performance of the Abbott RealTi*m*e HIV-1 assay was observed in the presence of the following drug pools:

Pool	Drug Name
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

IX. Summary of Clinical Studies

A. Clinical Study Objectives

The Abbott RealTi*m*e HIV-1 clinical study was conducted as a multi-center analytical evaluation with the following study objectives:

- evaluate the assay precision by testing an HIV-1 RNA panel

- calculate the assay specificity by testing specimens from HIV-1 RNA negative blood donors

- correlate the results obtained by the Abbott RealTi*m*e HIV-1 assay to those obtained by an FDA approved HIV-1 RNA quantitative assay by testing specimens from HIV-1 infected patients

B. Clinical Sites

Abbott RealTi*m*e HIV-1 assay testing was performed on the *m*2000 System at three US external sites.

C. Study Population

The HIV-1 negative samples (636) were sourced as surplus, unlinked specimens and tested. The average age was 46 years and 57.7% of the donors were male. HIV-1 positive specimens (359) were collected and tested from HIV-1 infected patients. The average age was 43 years and 71.6% were male. The Abbott RealTi*m*e HIV-1 results were not used in the management of the clinical study patients.

D. Study Period

May to October 2006.

E. **Clinical Study Results and Statistical Analyses**

1. **Precision**

   The Abbott RealTi*m*e HIV-1 assay precision was demonstrated by testing a coded 45-member panel consisting of nine unique members repeated five times within the panel. The mean RNA concentrations of the panel members ranged from 6.51 to 1.46 log copies/mL. 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.

   The total SD (includes within-run, between-run, between-lot, and between-site components) ranged from 0.09 to 0.18 log copies/mL in panel members that were within the dynamic range of the assay. For the member whose overall mean value was 1.46 log copies/mL (29 copies/mL) and was below the limit of detection of 1.60 log copies/mL (40 copies/mL), the total SD was 0.30 log copies/mL.

2. **Specificity**

   The specificity was evaluated at the three external sites by testing HIV-1 sero-negative plasma specimens collected from volunteer whole blood donors. All 514 specimens were HIV-1 RNA negative. In this study, the RealTi*m*e HIV-1 assay specificity was estimated to be 100.00% (514/514; 95% CI, 99.28%-100.00%).

3. **Correlation**

   HIV-1 RNA quantitation was compared between the Abbott RealTi*m*e 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 RealTi*m*e 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.[34] 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).

X. Conclusions Drawn from the Studies

The studies demonstrate that the Abbott RealTi*m*e HIV-1 assay is safe and effective for use in measuring HIV-1 RNA within a range between 40 copies/mL and 10 million copies/mL.

XI. Benefit Analysis

The Abbott RealTi*m*e 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 *m*2000 System in human plasma from HIV-1 infected individuals over the range of 40 to 10,000,000 copies/mL. The Abbott RealTi*m*e HIV-1 assay is intended for use in conjunction with clinical presentation and other laboratory markers as an indicator of 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.

Quantitative measurement of HIV levels in peripheral blood has greatly contributed to the understanding of the pathogenesis of HIV infection and has been shown to be an essential parameter in prognosis and management of HIV infected individuals. 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. The goal of antiretroviral therapy is to reduce the HIV virus in plasma to below detectable levels of available viral load tests. With a wide dynamic range, the RealTi*m*e HIV-1 assay allows for quantitation of viral load samples ranging from
40 copies/mL to 10 million copies/mL. Due to the selection of the primers and probes from highly conserved regions of the *pol* integrase region, and the novel partially double stranded fluorescent probe design, the assay quantitates the diverse Group M

subtypes A through H and Group O samples. The *m*2000 System automates critical assay steps including sample preparation to minimize user hands-on time.

The studies presented in this PMA show that the Abbott RealTi*m*e HIV-1 assay is a sensitive, reproducible, and automated test for the quantitation of HIV-1 RNA and should be made available to the medical community.

XII. References

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XIII. Panel Recommendations

XIV. CDRH Decision

XV. Approval Spec