WHO Prequalification of Diagnostics Programme
PUBLIC REPORT

Product: Abbott RealTime HIV-1 (Manual)
Number: PQDx 0146-027-00

The Abbott RealTime HIV-1 (Manual) assay with product code 2G31, which includes 2G31-90, 2G31-80, 2G31-70, manufactured by Abbott Molecular Inc., 1300 East Touhy Avenue, Des Plaines, IL 60018, United States of America, CE-marked regulatory version, was accepted for the WHO list of prequalified diagnostics and was listed on 17 October 2011. This version of the product is intended to be used in conjunction with the following instruments/reagents: 9K15-01, 2G31-66, 1L68-14 (or higher), 04J70-24 and 4J71-93.

Summary of prequalification status for Abbott RealTime HIV-1 (Manual)

<table>
<thead>
<tr>
<th>Status on PQ list</th>
<th>Date</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dossier assessment</td>
<td>28-Sep-2011</td>
<td>MR</td>
</tr>
<tr>
<td>Inspection status</td>
<td>26-27-Sep-2017</td>
<td>MR</td>
</tr>
<tr>
<td>Laboratory evaluation</td>
<td>FT</td>
<td>MR</td>
</tr>
</tbody>
</table>

MR: Meets Requirements, FT: Fast-tracked

Report amendments and/or product changes

This public report has since been amended. Amendments may have arisen because of changes to the prequalified product for which WHO has been notified and has undertaken a review. Amendments to the report are summarized in the following table, and details of each amendment are provided below.

<table>
<thead>
<tr>
<th>Version</th>
<th>Summary of amendment</th>
<th>Date of report amendment</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0-3.0</td>
<td>Modified specimen processing protocol, which resulted in labelling and Instructions for Use. In November 2016, Abbott Molecular identified an issue with the mSample Preparation System RNA (4 X 24 Preps) (product code 04J70-24) reagent when used in conjunction with DBS specimens.</td>
<td>23-Apr-2018</td>
</tr>
</tbody>
</table>
After Abbott Molecular confirmed root cause for the inhibition of results, they implemented a change that resulted in a modified version of the DBS processing protocol that had been WHO prequalified/CE marked. A change notification was submitted by Abbott Molecular in May 2017 relating to this change. This change notification was assessed, and the protocol was found to meet WHO prequalification requirements.

### 4.0

1. The Notified Body number on the Abbott RealTime HIV-1 Quantitative and Qualitative kit labels and package inserts has been updated to reflect the new notified body Polskie Centrum Badan i Certyfikacji S.A. (PCBC) Notified Body number of 1434.
2. The word "abbott" has been aligned to the center of the Abbott logo (where applicable). Labelling (labels and IFU) have been revised and version numbers updated.

### 5.0

Updated Abbott’s European Authorized Representative (EC Rep) legal entity name from Abbott GmbH & Co. KG to Abbott GmbH. Labeling changes to comply with the labeling requirements for product registered under IVDR.

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**Intended use:**

According to the claim of Abbott Molecular Inc, “*The Abbott RealTime HIV-1 (Manual) assay is an in vitro reverse transcription-polymerase chain reaction (RT-PCR) assay for the quantitation of Human Immunodeficiency Virus type 1 (HIV-1) in human plasma from HIV-1 infected individuals. The Abbott RealTime HIV-1 (Manual) 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 screening test for HIV-1 or as a diagnostic test to confirm the presence of HIV-1 infection.*

*The intended users for the Abbott RealTime HIV-1 assay are laboratory and healthcare professionals*. 
Assay principle:

According to the claim of Abbott Molecular Inc, “Abbott RealTime HIV-1 (Manual) assay uses RT-PCR 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 sample 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 instrument. The amplification cycle at which an increase in fluorescent signal is detected by the Abbott m2000rt is proportional to the log of the HIV-1 RNA concentration present in the original sample”.

<table>
<thead>
<tr>
<th>Component</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott RealTime HIV-1 Amplification Reagent Kit (2G31-90)-for plasma only</td>
<td>Abbott RealTime HIV-1 Internal Control (2G31Y) 4 vials x 1.2 mL. Abbott RealTime HIV-1 Amplification Reagent Pack (2G31) (4 packs x 24 tests/pack)</td>
</tr>
<tr>
<td>Abbott RealTime HIV-1 Controls (2G31-80)</td>
<td>Negative Control (2G31Z) 8 vials x 1.8 mL. Low Positive Control (2G31W) 8 vials x 1.8 mL. High Positive Control (2G31X) 8 vials, 1.8 mL</td>
</tr>
<tr>
<td>Abbott RealTime HIV-1 Calibrator Kit (2G31-70)</td>
<td>Abbott RealTime HIV-1 Calibrator A 12 vials x 1.8 mL. Abbott RealTime HIV-1 Calibrator B 12 vials, 1.8 mL.</td>
</tr>
</tbody>
</table>

Materials required but not provided:

<table>
<thead>
<tr>
<th>Instrumentation:</th>
<th>Product code(s)</th>
<th>Unit(s) (if applicable)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Abbott m2000rt Instrument</td>
<td>9K15-01</td>
<td>n/a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reagents:</th>
<th>Product code(s)</th>
<th>Unit(s) (if applicable)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. m Sample Preparation SystemRNA</td>
<td>04J70-24</td>
<td>4 × 24 Preps</td>
</tr>
<tr>
<td>2. Abbott RealTime HIV-1 2G31 list which includes the following kits:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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### Abbott RealTime HIV-1 Amplification Reagent Kit
- **Abbott RealTime HIV-1 Control Kit**: 2G31-80, n/a
- **Abbott RealTime HIV-1 Calibrator Kit**: 2G31-70, n/a

### Abbott m2000rt Optical Calibration kit
- 4J71-93

### Software:
1. Abbott RealTime HIV-1 m2000 ROW System Combined Application CD-ROM 1L68-09 or higher

### Optional:
1. Abbott RealTime HIV-1 UNG Protocol: 2G31-66, n/a

### Consumables:
1. 5 mL Reaction Vessels: 4J7120, n/a
2. 96-Well Optical Reaction Plates: 4J7170, n/a
3. Optical Adhesive Covers: 4J7175, n/a
4. Adhesive Cover Applicator: 9K3201, n/a
5. Splash-Free Support Base: 9K3101, n/a
6. m2000 mSample Preparation System Start Up Kit:
   - Eppendorf Cooler: n/a, n/a
   - Magnetic stands:
     - Magnetic Stands for 1 x 5 mL Reaction Vessels and 1 x 1.5 mL tubes: 2N2803, n/a
     - Magnetic Stands for 1 x 5 mL Reaction Vessels and 1 x 1.5 mL tubes: 2N2802, n/a
     - 1.5 mL Screw Top Microfuge Tubes and Caps: 4J7150, n/a

### Storage:
<table>
<thead>
<tr>
<th>Item(s)</th>
<th>Storage temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott RealTime HIV-1 Calibrator A and Calibrator B</td>
<td>-10°C or colder</td>
</tr>
<tr>
<td>Abbott RealTime HIV-1 Negative and Positive Controls</td>
<td>-10°C or colder</td>
</tr>
<tr>
<td>Abbott RealTime HIV-1 Amplification Reagent Pack and Internal Control vials</td>
<td>-10°C or colder when not in use</td>
</tr>
<tr>
<td>Abbott mSample Preparation System RNA (4 X 24 Preps)</td>
<td>15-30°C</td>
</tr>
</tbody>
</table>

1 2N2802 is part of 2N2803
Maximum shelf-life upon manufacture:

<table>
<thead>
<tr>
<th>Item(s)</th>
<th>Product code(s)</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott RealTime HIV-1 Amplification Reagent Kit</td>
<td>2G31-90</td>
<td>n/a</td>
</tr>
<tr>
<td>1. Abbott RealTime HIV-1 Internal Control</td>
<td>2G31Y</td>
<td>18 months</td>
</tr>
<tr>
<td>2. Thermostable rTth Polymerase Enzyme</td>
<td>56685</td>
<td>Per control date on vendor certificate of analysis</td>
</tr>
<tr>
<td>3. HIV-1 Oligonucleotide Reagent</td>
<td>2G31L</td>
<td>18 months</td>
</tr>
<tr>
<td>4. Activation Reagent</td>
<td>93591</td>
<td>18 months</td>
</tr>
<tr>
<td>Abbott RealTime HIV-1 Control Kit</td>
<td>2G31-80</td>
<td>n/a</td>
</tr>
<tr>
<td>1. Negative Control</td>
<td>2G31Z</td>
<td>18 months</td>
</tr>
<tr>
<td>2. Low Positive Control</td>
<td>2G31W</td>
<td>18 months</td>
</tr>
<tr>
<td>3. High Positive Control</td>
<td>2G31X</td>
<td>18 months</td>
</tr>
<tr>
<td>Abbott RealTime HIV-1 Calibrator Kit</td>
<td>2G31-70</td>
<td>n/a</td>
</tr>
<tr>
<td>1. Calibrator A</td>
<td>2G31A</td>
<td>18 months</td>
</tr>
<tr>
<td>2. Calibrator B</td>
<td>2G31B</td>
<td>18 months</td>
</tr>
<tr>
<td>Abbott mSample Preparation SystemRNA Kit</td>
<td>04J70-24</td>
<td>18 months</td>
</tr>
</tbody>
</table>

Prioritization for prequalification

Abbott Molecular Inc. submitted an application for prequalification of Abbott RealTime HIV-1 (Manual). Based on the established eligibility criteria, Abbott RealTime HIV-1 (Manual) was given priority for prequalification assessment.

Product dossier assessment

In 2011, Abbott Molecular Inc. submitted a product dossier for Abbott RealTime HIV-1 (Manual) as per the ‘Instructions for compilation of a product dossier’ (PQDx_018 v1). The information submitted in the product dossier was reviewed in accordance with the ‘Internal report on the screening and assessment of a product dossier’ (PQDx_009 v2) by WHO staff and external experts (assessors) appointed by WHO. Based on the product dossier screening and assessment findings, a recommendation was made to accept the product dossier for Abbott RealTime HIV-1 (Manual) for prequalification.
Commitments for prequalification:
The manufacturer has amended and submitted additional documentation as per the product dossier assessment findings. No further amendments are required.

Manufacturing site inspection

An inspection was conducted at the site of manufacture (1300 East Touhy Avenue, 60018 Des Plaines, IL, USA) of the Abbott RealTime HIV-1 (Manual) on 26 and 27 September 2017 as described in ‘Information for manufacturers on WHO prequalification inspection procedures for the sites of manufacture of diagnostics (PQDx_014 v1)’.

The inspection found that the manufacturer had a well-established quality management system and manufacturing practices in place that would ensure the manufacture of a product of consistent quality. The manufacturer’s responses to the nonconformities noted at the time of the inspection were accepted on 23 March 2018.

Product performance evaluation

Given the regulatory version of the product submitted for prequalification and the quality of the data submitted as part of the product dossier to support the claims for its intended use, the Abbott RealTime HIV-1 (Manual) assay has been found eligible to undergo the WHO fast track procedure. Subsequently, the product was not required to undergo a laboratory evaluation for its use with human plasma².

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² Product performance evaluation went through WHO’s Fast Track procedure at the date of prioritization for assessment. Fast Track procedure was phased out end of 2013.
Labelling

1. Labels
2. Instructions for use
1.1 Abbott RealTime HIV-1 Calibrator Kit (List No. 2G31-70)
Abbott RealTime HIV-1

**Calibrator Kit**

For In Vitro Diagnostic Use. The Abbott RealTime HIV-1 Calibrators are for calibration of the Abbott RealTime HIV-1 assay when used for the quantitative determination of human immunodeficiency virus type 1 (HIV-1) RNA in HIV-1 infected individuals.

**Content:**

1. **CALB** Abbott RealTime HIV-1 Calibrator A (12 vials, 1.8 ml per vial), fluorescently labeled Armored RNA® with HIV-1 sequences in negative human plasma. Negative human plasma spiked and found to be non-reactive for HBsAg, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, HBV DNA, and anti-HCV. Preservatives: 0.1% ProClin® 300 and 0.15% ProClin 950.

2. **CALR** Abbott RealTime HIV-1 Calibrator B (12 vials, 1.8 ml per vial), fluorescently labeled Armored RNA with HIV-1 sequences in negative human plasma. Negative human plasma spiked and found to be non-reactive for HBsAg, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, HBV DNA, and anti-HCV. Preservatives: 0.1% ProClin® 300 and 0.15% ProClin 950.

ProClin® is a registered trademark of Rohm and Haas.

Armored RNA® is a trademark of Abbott Laboratories.

**CAUTION:** Human sourced materials are potentially infectious. Consult the instructions for use. / ATTENTION : les produits d’origine humaine peuvent être potentiellement infectieux. Consulter les instructions d’utilisation.

Abbott Molecular Inc.
100 Abbott Park Drive
Deerfield, IL 60018 USA

Abbott GmbH
Maximilianstraße 2
65285 Weinbaden, Germany

Abbott RealTime HIV-1

**Calibrator Kit**

For In Vitro Diagnostic Use. The Abbott RealTime HIV-1 Calibrators are for calibration of the Abbott RealTime HIV-1 assay when used for the quantitative determination of human immunodeficiency virus type 1 (HIV-1) RNA in HIV-1 infected individuals.

**Content:**

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2. **CALR** Abbott RealTime HIV-1 Calibrator B (12 vials, 1.8 ml per vial), Armored RNA® with HIV-1 sequences in negative human plasma. Negative human plasma spiked and found to be non-reactive for HBsAg, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, HBV DNA, and anti-HCV. Preservatives: 0.1% ProClin® 300 and 0.15% ProClin 950.

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2. **CALR** Abbott RealTime HIV-1 Calibrator B (12 vials, 1.8 ml per vial), Armored RNA® with HIV-1 sequences in negative human plasma. Negative human plasma spiked and found to be non-reactive for HBsAg, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, HBV DNA, and anti-HCV. Preservatives: 0.1% ProClin® 300 and 0.15% ProClin 950.

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Abbott GmbH
Maximilianstraße 2
65285 Weinbaden, Germany

Abbott RealTime HIV-1

**Calibrator Kit**

For In Vitro Diagnostic Use. The Abbott RealTime HIV-1 Calibrators are for calibration of the Abbott RealTime HIV-1 assay when used for the quantitative determination of human immunodeficiency virus type 1 (HIV-1) RNA in HIV-1 infected individuals.

**Content:**

1. **CALB** Abbott RealTime HIV-1 Calibrator A (12 vials, 1.8 ml per vial), fluorescently labeled Armored RNA® with HIV-1 sequences in negative human plasma. Negative human plasma spiked and found to be non-reactive for HBsAg, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, HBV DNA, and anti-HCV. Preservatives: 0.1% ProClin® 300 and 0.15% ProClin 950.

2. **CALR** Abbott RealTime HIV-1 Calibrator B (12 vials, 1.8 ml per vial), Armored RNA® with HIV-1 sequences in negative human plasma. Negative human plasma spiked and found to be non-reactive for HBsAg, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, HBV DNA, and anti-HCV. Preservatives: 0.1% ProClin® 300 and 0.15% ProClin 950.

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Abbott Molecular Inc.
100 Abbott Park Drive
Deerfield, IL 60018 USA

Abbott GmbH
Maximilianstraße 2
65285 Weinbaden, Germany
1.2 Label for Abbott RealTime HIV-1 Calibrator A (List No. 2G31A)
1.3 Label for the Abbott RealTime HIV-1 Calibrator B (List No. 2G31B)
Abbott RealTime HIV-1

1.8mL

Colors: PMS 299 C  PMS 185 C  BLACK

Labeling: Duan

Store at ≤ -10°C

Infection Risk

Abbott

51-602101/R6

8/25/2014 4:01:52 PM
1.4 Abbott RealTime HIV-1 Control Kit (List No. 2G31-80)
Abbott RealTime
HIV-1

Control Kit

just for in vitro Diagnostic Use. The Abbott RealTime HIV-1 Controls are used to establish run validity of the Abbott RealTime HIV-1 assay when used for the quantitative determination of human immunodeficiency virus type 1 (HIV-1) RNA in human plasma from HIV-1 infected individuals.

Contents:
1. Control RealTime HIV-1 Negative Control (8 vials, 1.8 ml per vial). Negative human plasma tested and found to be nonreactive for HBsAg, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, HBV DNA, and anti-HCV Preservatives: 0.1% ProClin® 300 and 0.15% ProClin® 950
2. Control RealTime HIV-1 Low Positive Control (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, anti-HIV-1/HIV-2, HBV DNA, and anti-HCV Preservatives: 0.1% ProClin® 300 and 0.15% ProClin® 950
3. Control RealTime HIV-1 High Positive Control (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, anti-HIV-1/HIV-2, HBV DNA, and anti-HCV Preservatives: 0.1% ProClin® 300 and 0.15% ProClin® 950

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Armored RNA is a registered trademark of Ambion.

Abbott RealTime is a trademark of Abbott Laboratories.

CAUTION: Handle human sourced materials as potentially infectious. Consult instructions for use. 

Abbott RealTime is a trademark of Abbott Laboratories.

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Armored RNA è un marchio commerciale registrato di Ambion.

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ProClin® è un marchio registrato di Rohm and Haas.
Armored RNA® è un marchio registrato di Ambion.
1.5 Abbott RealTime HIV-1 High Positive Control (List No. 2G31X)
Abbott RealTime
HIV-1 CONTROL H

Store at ≤ -10°C
Infection Risk

1.8mL

Colors: PMS 299 C
PMS 185 C
BLACK

Labeling: Duan
1.6 Abbott RealTime HIV-1 Low Positive Control (List No. 2G31W)
1.7 Abbott RealTime HIV-1 Negative Control (List No. 2G31Z)
Abbott RealTime HIV-1 Test

Store at ≤ -10°C

Infection Risk

Colors: PMS 299 C, PMS 185 C, BLACK

Labeling: Duan

Abbott

Lot Number: 51-602106/R6

Expiration Date: 51-602106/R6

2G31Z

1.8mL
1.8 Abbott RealTime HIV-1 Amplification Reagent Kit (List No. 2G31-90)
1 Bottle (0.141 mL) Thermostable rTth Polymerase Enzyme (2.9 to 3.5 Units/µL) in buffered solution.

Contenido:

1 Frasco (1,10 ml) de reagente de oligonucleótidos del HIV-1. <0,1% de oligonucleótidos sintéticos (4 sonde e 1 oligonucleotide primer, 2 e 1 frasco (1,10 ml) de HIV-1 Oligonucleotide Reagent (reactivo de oligonucleótidos VIH-1). <0,1% oligonucleótidos sintéticos (4

Abbott RealTime HIV-1 Amplification Reagent Kit (4x24 Tests)

Amplification Reagent Kit

Product of USA / Produit aux USA / Produit des États-Unis / Product de los Estados Unidos / Produit aux États-Unis

6026 Wiesbaden, Germany / AbbVie GmbH Tax-Parking 2

Abbott Molecular Inc., 1300 East Thirty Avenue Des Plaines, IL 60018 USA

Abbott RealTime HIV-1

just for the diagnostic lab. This Abbott RealTime HIV-1 assay is an in-vitro reverse transcription polymerase chain reaction (RT-PCR) assay for the quantification of Human Immunodeficiency Virus type 1 (HIV-1) in human plasma from HIV-1 infected individuals. The Abbott RealTime HIV-1 assay is intended for use in conjunction with clinical presentation and other laboratory markers as an indicator of disease progression and to use as an aid in assessing and responding to antiretroviral treatment as necessary by changes in plasma HIV RNA levels. 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.

Abbott RealTime HIV-1 Internal Control (4 vials, 1,2 ml each).

Abbott RealTime HIV-1 Amplification Reagent Pack (4 packs, 1 frasco (1,10 ml) de HIV-1 Oligonucleotide Reagent). <0,1% de oligonucleótidos sintéticos (4 sonde e 1 oligonucleotide primer, 2 e 1 frasco (1,10 ml) de HIV-1 Oligonucleotide Reagent (reactivo de oligonucleótidos VIH-1). <0,1% oligonucleótidos sintéticos (4

Abbott RealTime HIV-1 Amplification Reagent Pack (4 packs, 1 frasco (1,10 ml) de HIV-1 Oligonucleotide Reagent). <0,1% de oligonucleótidos sintéticos (4 sonde e 1 oligonucleotide primer, 2 e 1 frasco (1,10 ml) de HIV-1 Oligonucleotide Reagent (reactivo de oligonucleótidos VIH-1). <0,1% oligonucleótidos sintéticos (4

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1.9 Abbott RealTime HIV-1 Internal Control (List No. 2G31Y)
2. Instructions for use

3 English version of the IFU was the one that was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.
HIV-1 Calibrators

Key to symbols used

- REF List Number
- IVD In Vitro Diagnostic Medical Device
- LOT Lot Number
- CAL A Expiration Date
- T-10°C Calibrator (A - B) Store at ≤ -10°C
- Warning Consult instructions for use
- CAUTION: Handle human sourced materials as potentially infectious. Consult instructions for use. (Infection Risk)
- EC REP Authorized Representative
- Manufacturer

Notice to User

If a serious incident occurs in relation to this device, the incident should be reported to the manufacturer and to the appropriate competent authority of the member state in which the user and/or the patient is established. To report to the manufacturer, see the contact information provided in the Customer service section or Technical assistance section of these instructions.

Intended Use

The Abbott RealTime HIV-1 Calibrators are for calibration of the Abbott RealTime HIV-1 assay when used for the quantitative determination of human immunodeficiency virus type 1 (HIV-1) RNA in human plasma from HIV-1 infected individuals.

Intended User

The intended users for the Abbott RealTime HIV-1 Calibrators are laboratory and healthcare professionals.

Contents

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, anti-HIV-1/HIV-2, HBV DNA, 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, anti-HIV-1/HIV-2, HBV DNA, and anti-HCV. Preservatives: 0.1% ProClin® 300 and 0.15% ProClin® 950.

- Calibrator concentrations are specified in each Abbott RealTime HIV-1 Calibrator Kit Card.
- The Abbott RealTime HIV-1 Calibrator Kit must only be used with the Abbott RealTime HIV-1 assay (List No. 2G31-90).

NOTE: Changes Highlighted

Standardization

Abbott manufactures internal reference standards for the Abbott RealTime HIV-1 assay. These internal standards are referenced to a viral standard from the Virology Quality Assurance (VQA) Laboratory of the AIDS Clinical Trial Group, at each concentration level. The Abbott RealTime HIV-1 Calibrators are manufactured against these internal standards.

Precautions

- IVD In Vitro Diagnostic Medical Device
- For In Vitro Diagnostic Use Only
- Do not use beyond expiration date.

CAUTION: This preparation contains human sourced and/or potentially infectious components. Components sourced from human blood have been tested and found to be nonreactive by FDA-licensed tests for antibody to HCV, antibody to HIV-1, antibody to HIV-2, and HBsAg. The material is also tested and found to be negative by FDA-licensed PCR methods for HIV-1 RNA and HCV RNA. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. These reagents and human specimens should be handled as if infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories, OSHA Standards on Bloodborne Pathogens, CLSI Document M29-A3, and other appropriate biosafety practices. Therefore all human sourced materials should be considered infectious.

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.
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state and federal regulations.

Components of the Abbott RealTime HIV-1 Calibrator Kit (List No. 2G31-70) contain the following components:

- 2-Methyl-2H-isothiazol-3-one
- Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one (EC no. 220-239-6)(3:1)
- Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one (EC no. 220-239-6)(3:1)
- Reaction mass of: 2-methyl-2H-isothiazol-3-one (EC no. 220-239-6)(3:1)
- Reaction mass of: 2-methyl-2H-isothiazol-3-one (EC no. 220-239-6)(3:1)

The following warnings apply:

Warning

- H317 May cause an allergic skin reaction.
- P281 Avoid breathing mist / vapours / spray.
- P280 Wear protective gloves / protective clothing / eye protection.
- P272 Contaminated work clothing should not be allowed out of the workplace.
- P302+P352 IF ON SKIN: Wash with plenty of water.
- P333+P313 If skin irritation or rash occurs: Get medical advice / attention.
- P362+P364 Take off contaminated clothing and wash it before reuse.
- P501 Dispose of contents / container in accordance with local regulations.
Consult instructions for use

Store at ≤ -10°C

Shipping Conditions
Ship on dry ice.

BIBLIOGRAPHY

Technical Assistance
For technical assistance, call Abbott Molecular Technical Services at 1-800-553-7042 (within the US) or +49-6122-580 (outside the US), or visit the Abbott Molecular website at www.molecular.abbott/portal.

Armored RNA is a registered trademark of Ambion.

ProClin is a registered trademark of Rohm and Haas.

Abbott RealTime is a trademark of Abbott Laboratories.

The Abbott RealTime HIV-1 Calibrators Kit is imported into the European Union by Abbott Diagnostics GmbH, located at Max-Planck-Ring 2, 65205 Wiesbaden, Germany.
HIV-1 Controls

Key to symbols used

<table>
<thead>
<tr>
<th>REF</th>
<th>IVD</th>
<th>LOT</th>
<th>CONTROL</th>
<th>CONTROL</th>
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<td></td>
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<td>-10°C</td>
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</table>

HIV-1 Controls are used to establish run validity of the Abbott RealTime HIV-1 assay when used for the quantitative determination human immunodeficiency virus type 1 (HIV-1) RNA in human plasma from HIV-1 infected individuals.

Contents

1. **CONTROL** Abbott RealTime HIV-1 Negative Control (List No. 2G312) (8 vials, 1.8 mL per vial). Negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, HBV DNA, and anti-HCV. Preservatives: 0.1% ProClin® 300 and 0.15% ProClin 950.

2. **CONTROL** 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, anti-HIV-1/HIV-2, HBV DNA, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

3. **CONTROL** 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, anti-HIV-1/HIV-2, HBV DNA, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

**NOTE:** Changes Highlighted

- Control concentrations are specified in each Abbott RealTime HIV-1 Control Kit Card.
- The Abbott RealTime HIV-1 Control Kit must only be used with the Abbott RealTime HIV-1 assay (List No. 2G31-90).

**Precautions**

- **IVD** In Vitro Diagnostic Medical Device
- For In Vitro Diagnostic Use Only
- Do not use beyond expiration date.

**CAUTION:** This preparation contains human sourced and/or potentially infectious components. Components sourced from human blood have been tested and found to be nonreactive by FDA-licensed tests for antibody to HCV, antibody to HIV-1, antibody to HIV-2, and HBsAg. The material is also tested and found to be negative by FDA-licensed PCR methods for HIV-1 RNA and HCV RNA. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. These reagents and human specimens should be handled as if infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories. Therefore all human sourced material should be considered infectious.

- 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. Therefore all human sourced material should be considered infectious.

**Components of the Abbott RealTime HIV-1 Control Kit (List No. 2G31-80)**

- 2-Methyl-2H-isothiazol-3-one
- 5-chloro-2-methyl-4-isothiazolin-3-one
- 5-chloro-2-methyl-4-isothiazolin-3-one

The following warnings apply:

**Warning**

- **H317** May cause an allergic skin reaction.
- **P261** Avoid breathing mist / vapours / spray.
- **P280** Wear protective gloves / protective clothing / eye protection.
- **P272** Contaminated work clothing should not be allowed out of the workplace.
- **P302+P352** IF ON SKIN: Wash with plenty of water.
- **P333+P313** If skin irritation or rash occurs: Get medical advice / attention.
- **P362+P364** Take off contaminated clothing and wash it before reuse.
- **P501** Dispose of contents / container in accordance with local regulations.
Consult instructions for use

Store at ≤ -10°C

Shipping Conditions
Ship on dry ice.

BIBLIOGRAPHY

Technical Assistance
For technical assistance, call Abbott Molecular Technical Services at 1-800-553-7042 (within the US) or +49-6122-580 (outside the US), or visit the Abbott Molecular website at www.molecular.abbott portal.

Armored RNA is a registered trademark of Ambion.
ProClin is a registered trademark of Rohm and Haas.
Abbott RealTime is a trademark of Abbott Laboratories.
The Abbott RealTime HIV-1 Controls Kit is imported into the European Union by Abbott Diagnostics GmbH, located at Max-Planck-Ring 2, 65205 Wiesbaden, Germany.

Abbott Molecular Inc.
Des Plaines, IL 60018 USA

Abbott GmbH
Max-Planck-Ring 2
65205 Wiesbaden, Germany

www.abbottmolecular.com

May 2020
NOTE: Changes Highlighted

Key to Symbols Used

| REF | Reference Number |
|LOT | Lot Number |
|IVD | In Vitro Diagnostic Medical Device |
| Use By |
| CONTROL | Negative Control |
| CONTROL | Low Positive Control |
| CONTROL | High Positive Control |
| CAL A | Calibrator A |
| CAL B | Calibrator B |

INTERNAL CONTROL

Internal Control

AMPLIFICATION REAGENT PACK

Amplification Reagent Pack

Upper Limit of Temperature

Consult instructions for use

Caution

Warning

Manufacturer

Authorized Representative in the European Community

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

NOTICE TO USER

If a serious incident occurs in relation to this device, the incident should be reported to the manufacturer and to the appropriate competent authority of the member state in which the user and/or the patient is established. To report to the manufacturer, see the contact information provided in the Customer service section or Technical assistance section of these instructions.

CUSTOMER SERVICE

INTERNATIONAL: CALL YOUR ABBOTT REPRESENTATIVE

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.

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) in human plasma from HIV-1 infected individuals. The Abbott RealTime 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 screening test for HIV-1 or as a diagnostic test to confirm the presence of HIV-1 infection.

INTENDED USER

The intended users for the Abbott RealTime HIV-1 assay are laboratory and healthcare professionals.

SUMMARY AND EXPLANATION OF THE TEST

Human Immunodeficiency Virus (HIV) is the etiologic agent of Acquired Immunodeficiency Syndrome (AIDS). It can be transmitted through sexual contact, exposure to infected blood or blood products, or from an infected mother to the fetus. Acute HIV syndrome, characterized by flu-like symptoms, develops 3 to 5 weeks after initial infection and is associated with high levels of viremia. Within 4 to 6 weeks of the onset of symptoms, HIV specific immune response is detectable. After seroconversion, viral load in peripheral blood declines and most patients enter an asymptomatic phase that can last for years. 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. HIV RNA levels in plasma can be quantitated by nucleic acid amplification or signal amplification technologies. 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 and against World Health Organization (WHO) 1st International Standard for HIV-1 RNA (97/656). 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 consists of 3 reagent kits:

- Abbott RealTime HIV-1 Amplification Reagent Kit
- Abbott RealTime HIV-1 Control Kit
- Abbott RealTime HIV-1 Calibrator Kit

The Abbott RealTime HIV-1 assay uses RT-PCR 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 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 mSample Preparation System (4 x 24 Preps) 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 output tubes or 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.

Two automated instrument systems, the Abbott m2000sp or the Abbott m1000 System can be used to prepare samples for the Abbott RealTime HIV-1 assay. The Abbott m2000sp provides automated sample elute transfer and reaction assembly in the Abbott 96-Well Optical Reaction Plate, while the Abbott m1000 System requires manual sample elute transfer and reaction assembly. Alternatively, samples can be prepared manually using the Abbott mSample Preparation System, followed by manual reaction assembly.

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 Reference Dye). 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.

The Abbott m1000 System users and manual sample preparation method users manually combine the Abbott RealTime HIV-1 amplification reagent components to create the amplification master mix and transfer aliquots of the master mix and sample eluates to the reaction plate. The plate is ready, after manual application of the optical seal and centrifugation, 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 point 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. Primers are designed to hybridize to the pol-integrase region with the fewest possible mismatches among various subtypes.

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.

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 Abbott 96-Well Optical Reaction Plate.
- Detection is carried out automatically without the need to open the Abbott 96-Well Optical Reaction Plate.
- Pipettes with aerosol barrier tips or disposable transfer pipettes are used for all pipetting. The disposable pipettes or 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
Abbott RealTime HIV-1 Amplification Reagent Kit (List No. 2G31-90)
1. Internal Control (List No. 2G33Y) (4 vials, 5 mL per vial)
   - < 0.01% 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.
   - 1 bottle (0.141 mL) Thermostable rTth Polymerase Enzyme (2.9 to 3.5 Units/L) in buffered solution.
   - 1 bottle (1.10 mL) HIV-1 Oligonucleotide Reagent. < 0.1% synthetic oligonucleotides (4 primers, 2 probes, and 1 quencher oligonucleotide), and < 0.3% 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. 2G31-80)
1. Control 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 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 HIV-1 High Positive Control (List No. 2G31X) (8 vials, 1.8 mL per vial) Infectious 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. 2G31-70)
1. CalA 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. CalB Abbott RealTime HIV-1 Calibrator B (List No. 2G31B) (12 vials, 1.8 mL per vial) Infectious 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.
WARNINGS AND PRECAUTIONS

In Vitro 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.

Safety Precautions

Refer to the Abbott m1000 Operating Manual, Safety Section, the Manual Sample Preparation for Abbott RealTime RNA Assays Procedure, Handling Precaution Section, or Abbott m2000sp and Abbott m2000rt Operations Manuals, Hazard Section, for instructions on safety precautions.

CAUTION: This preparation contains human sourced and/or potentially infectious components. Components sourced from human blood have been tested and found to be nonreactive by FDA-licensed tests for antibody to HIV, antibody to HIV-1, antibody to HIV-2, and HBsAg. The material is also tested and found to be negative by FDA-licensed PCR methods for HIV-1 RNA and HCV RNA. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. These reagents and human specimens should be handled as if infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories. OSHA Standards on Bloodborne Pathogens, CLSI Document M29-A3, and other appropriate biosafety practices. Therefore all human sourced materials should be considered infectious.

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 biosafety practices.
- handle all manual pipetting. The pipette tips must be used only 1 time. Clean and disinfect spills of specimens and reagents as stated in 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 either the Reagent Preparation Area or 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 either the Reagent Preparation Area or the Sample Preparation Area.

Only 2 dedicated areas, Sample Preparation Area and Amplification Area, are recommended when the Abbott m2000sp and Abbott m2000rt are used.

Components contained within a kit are intended to be used together. Do not mix 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.

Do not use kits or reagents after the dates shown on kit labels. 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 m1000 Operating Manual and the Abbott m2000sp and Abbott m2000rt Operations Manuals for instrument cleaning procedures.

If the Abbott m1000 System or Abbott m2000sp instrument run is aborted, dispose of all commodities and reagents according to the Abbott m1000 Operating Manual or 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 Abbott 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. See handling unopened reagents, negative control, positive controls, calibrators, or specimens. Refer to the Abbott m1000 Operating Manual and the Abbott m2000sp and Abbott m2000rt Operations Manuals for instrument cleaning procedures.

If the Abbott m1000 System or Abbott m2000sp instrument run is aborted, dispose of all commodities and reagents according to the Abbott m1000 Operating Manual or 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 Abbott 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. See handling unopened reagents, negative control, positive controls, calibrators, or specimens. Refer to the Abbott m1000 Operating Manual and the Abbott m2000sp and Abbott m2000rt Operations Manuals for instrument cleaning procedures.

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 1 time. Clean and disinfect spills of specimens and reagents as stated in the Abbott m1000 Operating Manual or the Abbott m2000sp and Abbott m2000rt Operations Manuals.

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.
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 mSample Preparation System (4 x 24 Preps) reagents are single use only. Use new reagent troughs or 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 m1000 Operating Manual or the Abbott m2000sp Operations Manual and the Abbott mSample Preparation System (4 x 24 Preps) product information sheet.

STORAGE INSTRUCTIONS
Abbott RealTime HIV-1 Amplification Reagent Kit (List No. 2G31-90)
- 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. 2G31-80)
- 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. 2G31-70)
- 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 nucleic acid testing (NAT) software must be installed on the Abbott m1000 System prior to performing the assay. For detailed information on NAT software installation, refer to the Abbott m1000 Operating Manual, Putting into Operation section.

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 ROW 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 to 30°C for up to 6 hours or at 2 to 8°C for up to 24 hours, prior to centrifugation. Separate plasma from cells by centrifugation.

After centrifugation, plasma may be removed from cells. Plasma specimens may be stored at 15 to 30°C for up to 24 hours or at 2 to 8°C for up to 5 days. Plasma specimens may be stored at -20 +/- 10°C for up to 6 months.

If longer storage is required, plasma specimens must be kept at –70°C or lower.12,33 Multiple freeze-thaw cycles should be avoided. If frozen, thaw plasma specimens at 15 to 30°C or at 2 to 8°C. Once thawed, if plasma specimens are not being processed immediately, they can be stored at 2 to 8°C for up to 6 hours.

NOTE: Plasma specimens should not be frozen in non-gel blood collection tubes.

Specimen Transport
Ship specimens according to the recommended storage temperature and time listed in the Specimen Collection and Storage section above. For domestic and international shipments, specimens should be packaged and labeled in compliance with applicable state, federal, and international regulations covering the transport of clinical, diagnostic, or biological specimens.

ABBOTT REALTIME HIV-1 ASSAY PROCEDURE
This Abbott RealTime HIV-1 package insert contains 2 assay protocols:
- Samples prepared for amplification using the Abbott m1000 System or the manual sample preparation method follow ASSAY PROTOCOL I.
- Samples prepared for amplification using the Abbott m2000sp instrument follow ASSAY PROTOCOL II.

The Abbott RealTime HIV-1 assay provides up to 4 sample volume options (0.2 mL, 0.5 mL, 0.6 mL, and 1.0 mL) (See assay protocol step 6 and INTERPRETATION OF RESULTS section).

Materials Provided
- Abbott RealTime HIV-1 Amplification Reagent Kit (List No. 2G31-90)

Materials Required But Not Provided
- Abbott RealTime HIV-1 Calibrator Kit (List No. 2G31-70)
- Abbott RealTime HIV-1 Control Kit (List No. 2G31-80)

For manual sample preparation method refer to the Materials and Equipment Required Section of the Manual Sample Preparation for Abbott RealTime RNA Assays Procedure (List No. 06L73).

For Abbott m1000 System Sample Preparation Area
- Abbott m1000 System
- Abbott mSample Preparation System (4 x 24 Preps)
- Reaction Vessels
- Calibrated precision pipettes capable of delivering 20 to 1000 µL
- 20 µL to 1000 µL aerosol barrier pipette tips for precision pipettes
- 11.6 to 16 mm Sample Tubes
- 200 µL and 1000 µL disposable tips
- Abbott 96 Deep-Well Plate (List No. 04J71-30)
- Vortex Mixer
- Abbott Optical Adhesive Covers (List No. 04J71-75)
- Abbott Adhesive Cover Applicators
- Abbott Splash-Free Support Base (List No. 09K31-01)
- Reagent Troughs
- 1.5 mL Output Tubes
- Centrifuge capable of 5000g

For Abbott m2000sp Instrument Sample Preparation Area
- Abbott m2000sp instrument
- Abbott mSample Preparation System (4 x 24 Preps)
- 5 mL Reaction Vessels
- Calibrated precision pipettes capable of delivering 20 to 1000 µL
- 20 µL to 1000 µL aerosol barrier pipette tips for precision pipettes
- 11.5 to 16 mm Sample Tubes
- 200 µL and 1000 µL disposable tips
- Vortex Mixer
- Abbott Optical Adhesive Covers (List No. 04J71-75)
- Abbott Adhesive Cover Applicators
- Abbott Splash-Free Support Base (List No. 09K31-01)
- Master Mix Vial
- 200 mL Reagent Vessels
- Abbott 96-Deep-Well Plate (List No. 04J71-30)
- Abbott RealTime HIV-1 m2000 ROW System Combined Application CD-ROM (List No. 1L88)
- Abbott 96-Well Optical Reaction Plate (List No. 04J71-70)
- Centrifuge capable of 2000g
For Abbott m1000 System Reagent Preparation Area:
- PCR cooler, either StrataCooler® 96 Benchtop Cooler or Eppendorf® PCR-Cooler
- Abbott 96-Well Optical Reaction Plate (List No. 04J71-70)
- Calibrated precision pipettes capable of delivering 20 to 1000 µL
- 20 µL to 1000 µL aerosol barrier pipette tips for precision pipettes
- Single-use RNase/DNase-free tube or container
- Vortex Mixer

Other Materials:
- Biological safety cabinet approved for working with infectious materials
- Sealable plastic bags
- RNase-free water (Eppendorf or equivalent)
- 1.7 mL molecular biology grade microcentrifuge tubes (Dot Scientific, Inc. or equivalent)
- Cotton Tip Applicators (Puritan or equivalent)

†Note: These 3 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.

Use aerosol barrier pipette tips or disposable pipettes only one time when pipetting specimens, IC, or amplification reagents. 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 of 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 I: ABBOTT m1000 SYSTEM OR THE MANUAL SAMPLE PREPARATION METHOD AND ABBOTT m2000rt INSTRUMENT


Laboratory personnel must be trained to operate the Abbott m1000 System and the Abbott m2000rt instrument. The operator must have a thorough knowledge of the software applications and must follow good laboratory practices.

1. Thaw assay controls and IC at 15 to 30°C or at 2 to 8°C. Thaw calibrators at 15 to 30°C or at 2 to 8°C only if performing a calibration run; see QUALITY CONTROL PROCEDURES section of this package insert.
   - Once thawed, assay controls, IC, and calibrators can be stored at 2 to 8°C for up to 24 hours before use.
   - Vortex each assay calibrator and each control 3 times for 2 to 3 seconds before use. 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.
2. Thaw amplification reagents at 15 to 30°C or at 2 to 8°C and store at 2 to 8°C until required for the amplification master mix procedure.

For Abbott m2000rt Instrument Amplification Area:
- Abbott m2000rt instrument
- Abbott RealTime HIV-1 m2000 ROW System Combined Application CD-ROM (List No. 1L68)
- Abbott m2000rt Optical Calibration Kit (List No. 04J71-93)

• Once thawed, the amplification reagents can be stored at 2 to 8°C for up to 24 hours if not used immediately.

NOTE: Use 1 bottle of Lysis Buffer, 1 vial of IC, and 1 Abbott 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 using an Abbott m1000 instrument.

Sample Preparation Area
For sample preparation using the Abbott m1000 System, follow steps 3 through 10. For the manual sample preparation method refer to the Extraction Protocol Section of the Manual Sample Preparation for Abbott RealTime RNA Assays Procedure (List No. 06L73).

3. Gently invert the Abbott Sample Preparation bottles to ensure a homogeneous solution. 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.
4. Vortex each IC 3 times for 2 to 3 seconds before use.
5. Use a calibrated precision PIPETTE DEDICATED FOR INTERNAL CONTROL USE ONLY to add 500 µL of IC to each bottle of Lysis Buffer. Mix by gently inverting the container 5 to 10 times to minimize foaming.

6. A total of 48 samples can be processed in 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.

- The Abbott RealTime HIV-1 assay minimum sample volume and associated rack requirements on the Abbott m1000 System are:

<table>
<thead>
<tr>
<th>Rack Diameter</th>
<th>Tube Diameter</th>
<th>0.2 mL</th>
<th>0.5 mL</th>
<th>1.0 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>13 mm</td>
<td>11.6 mm - 14.0 mm</td>
<td>0.7 mL</td>
<td>1.0 mL</td>
<td>1.5 mL</td>
</tr>
<tr>
<td>16 mm</td>
<td>15.0 mm - 16.0 mm</td>
<td>1.0 mL</td>
<td>1.3 mL</td>
<td>1.8 mL</td>
</tr>
</tbody>
</table>

6 Refers to sample tube outer diameter

- If frozen, thaw specimens at 15 to 30°C or at 2 to 8°C. Once thawed, specimens can be stored at 2 to 8°C for up to 6 hours if not processed immediately.

NOTE: For every stored specimen, the following actions must be done in the order described: vortex the specimen first and follow with centrifugation. If these actions are not performed in this order, then invalid results may occur.

- Vortex each specimen 3 times for 2 to 3 seconds.
- Centrifuge specimens at 2000g for 5 minutes before loading onto the Abbott m1000 worktable. Aliquot each specimen into clean tubes or vials if necessary. Refer to the Abbott m1000 Operating Manual for tube sizes. Avoid touching the inside of the cap when opening tubes.

7. Place the calibrators (if applicable), low and high positive controls, the negative control, and the patient specimens into the Abbott m1000 sample rack. Follow directions for performing a user-defined protocol, as described in the Abbott m1000 Operating Manual, Operation section.

8. Place the Reaction Vessels into the Abbott m1000 1 mL subsystem carrier.

9. Load the Abbott mSample Preparation System reagents and the 1.5 mL Output Tubes on the Abbott m1000 System worktable as described in the Abbott m1000 Operating Manual, Operation section.

10. Initiate the Abbott m1000 protocol as described in the Abbott m1000 Operating Manual, Operation section. From the Protocol screen, select the appropriate application file corresponding to the sample volume being tested.

- The assembly of the amplification master mix and sample eluates into the Abbott 96-Well Optical Reaction Plate (step 17) must be initiated within 1 hour after completion of Sample Preparation.

Amplification Area
11. Switch on and initialize the Abbott m2000rt instrument.

NOTE: The Abbott m2000rt instrument requires 15 minutes to warm up.
12. Create the Abbott m2000rt test order. Refer to the Operating Instructions section of the Abbott m2000rt Operations Manual. From the Protocol screen, select the appropriate application file corresponding to the sample volume being tested.

- Enter calibrator (needed if a calibration curve has not been stored on the Abbott m2000rt) and control lot specific values in the test order for accurate calibration and control evaluation. Lot-specific values are specified in each Abbott RealTime HIV-1 Calibrator and Control Kit Card.

Reagent Preparation Area

All reagent preparation must take place in the dedicated Reagent Preparation Area. Refer to the Handling Precautions section of this package insert before preparing reagents.

NOTE: Change gloves before handling the amplification reagents.

13. Prepare the amplification master mix.

- Each Amplification Reagent Pack supports up to 24 reactions.
- Prior to opening the amplification reagents, ensure that the contents of the vials are at the bottom by tapping the vials in an upright position on the bench to bring the liquid to the bottom of the vials.
- Prepare the master mix by using a pipette dedicated for reagent use only to add 271 μL of the HIV-1 Activation Reagent (Reagent 1) and 949 μL of the HIV-1 Oligonucleotide Reagent (Reagent 2) together in the Thermostable rTth DNA Polymerase Enzyme bottle (Reagent 3).
- If performing 25 to 48 reactions, prepare a second amplification master mix with a second Amplification Reagent Pack.
- The Abbott m2000rt protocol (step 20) must be initiated within 40 minutes of the addition of Activation Reagent into the first rTth Enzyme Reagent bottle (step 13).

14. Pipette the contents of the master mix from the enzyme bottle(s) into a single-use RNase/DNase-free tube and vortex to mix.

15. Place an Abbott 96-Well Optical Reaction Plate in a PCR cooler stored as indicated in the PCR cooler instruction manual. Using a dedicated pipette, dispense 50 μL aliquots of the amplification master mix into the Abbott 96-Well Optical Reaction Plate. A calibrated repeat pipettor may be used. Visually verify that 50 μL has been dispensed into each well.

16. Transfer the Abbott 96-Well Optical Reaction Plate on the PCR cooler to the Sample Preparation Area.

Sample Preparation Area

17. In the Sample Preparation Area, transfer 50 μL of sample eluate to the Abbott 96-Well Optical Reaction Plate on the PCR cooler. Use a separate pipette tip for each sample eluate transfer. During the transfer of each sample, mix the reaction by pipetting up and down 3 to 5 times. Visually verify that 100 μL has been dispensed into each well.


19. Remove the Abbott 96-Well Optical Reaction Plate from the PCR cooler and place in the Abbott Splash-Free Support Base. Centrifuge the Abbott 96-Well Optical Reaction Plate in the Abbott Splash-Free Support Base at 5,000g for 5 minutes. Transfer to the Amplification Area.

NOTE: Do not transfer the PCR cooler to the Amplification Area.

Amplification Area

20. Place the Abbott 96-Well Optical Reaction Plate in the Abbott m2000rt instrument. From the Protocol screen, select the appropriate application file corresponding to the sample volume being tested. Initiate the Abbott RealTime HIV-1 protocol, as described in the Abbott m2000rt Operations Manual, Operating Instructions section.

POST PROCESSING PROCEDURES

1. Clean the PCR cooler as described in the PCR cooler instruction manual and return to the Reagent Preparation Area.

2. Remove the 1.5 mL Output Tubes from the worktable and dispose of according to the Abbott m1000 Operating Manual.

3. Place the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose of according to the Abbott m2000rt Operations Manual along with the gloves used to handle the plate.


5. For manual sample preparation method users, refer to the Clean Up Section of the Manual Sample Preparation for Abbott RealTime RNA Assays Procedure (List No. 06L73).

ASSAY PROTOCOL II: ABBOTT m2000sp INSTRUMENT AND ABBOTT m2000rt INSTRUMENT

For a detailed description of how to perform an Abbott m2000sp instrument and Abbott m2000rt instrument protocol, refer to the Abbott m2000sp and Abbott m2000rt Operations Manuals. Operating Instructions sections. The 96-sample capability requires Abbott m2000sp Software Version 2.0 or higher. Please follow Abbott m2000sp Operations Manual (List 09K20-02) and addendum or addenda. Laboratory personnel must be trained to operate the Abbott m2000sp and Abbott 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 to 30°C or at 2 to 8°C. Thaw calibrators at 15 to 30°C or at 2 to 8°C only if performing a calibration run; see QUALITY CONTROL PROCEDURES section of this package insert.

- Once thawed, assay controls, IC, and calibrators can be stored at 2 to 8°C for up to 24 hours before use.
- Vortex each assay calibrator and each control 3 times for 2 to 3 seconds before use. 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.

2. Thaw amplification reagents at 15 to 30°C or at 2 to 8°C and store at 2 to 8°C.

- If performing 25 to 48 reactions, prepare a second amplification master mix with a second Amplification Reagent Pack.

- Once thawed, the amplification reagents can be stored at 2 to 8°C for up to 24 hours if not used immediately.

NOTE: Use 1 bottle of mLysis Buffer, 1 vial of IC, and 1 Abbott 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 third set of reagents to support 49 to 72 reactions, and a fourth set of reagents to support 73 to 96 reactions with the exception of nmICROPARTICLES. USE ONLY 2 BOTTLES OF nmICROPARTICLES WHEN PROCESSING 25 TO 96 SAMPLES.

3. Gently invert the Abbott Sample Preparation bottles to ensure a homogeneous solution. 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.

4. Vortex each IC 3 times for 2 to 3 seconds before use.

5. Use a calibrated precision pipette dedicated for internal control use only to add 500 μL of IC to each bottle of mLysis Buffer. Mix by gently inverting the container 5 to 10 times to minimize foaming.

6. A total of 96 samples can be processed in each run, with the exception of the 1.0 ml Assay Application. A negative control, a low positive control, and a high positive control are included in each run, therefore allowing a maximum of 93 specimens to be processed per run. For the 1.0 ml Assay Application, a total of 48 samples can be processed in each run, allowing a maximum of 45 specimens to be processed per run.

- The Abbott RealTime HIV-1 assay minimum sample volume and associated rack requirements on the Abbott m2000sp are:

<table>
<thead>
<tr>
<th>Rack</th>
<th>Tube Diametera</th>
<th>0.2 mL</th>
<th>0.5 mL</th>
<th>0.6 mL</th>
<th>1.0 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>13 mm</td>
<td>11.5-14.0 mm</td>
<td>0.4 mL</td>
<td>0.7 mL</td>
<td>0.8 mL</td>
<td>1.2 mL</td>
</tr>
<tr>
<td>16 mm</td>
<td>14.5-16.0 mm</td>
<td>0.4 mL</td>
<td>0.8 mL</td>
<td>0.9 mL</td>
<td>1.3 mL</td>
</tr>
</tbody>
</table>

a Refers to sample tube outer diameter. Minimum sample volume varies with tube geometry and size. Refer to the Abbott m2000sp Operations Manual and QUICK REFERENCE GUIDE FOR SAMPLE TUBE SIZES AND VOLUMES for recommended sample input volume.

- If frozen, thaw specimens at 15 to 30°C or at 2 to 8°C. Once thawed, specimens can be stored at 2 to 8°C for up to 6 hours if not processed immediately.

NOTE: For every stored specimen, the following actions must be done in the order described: vortex the specimen first and follow with centrifugation. If these actions are not performed in this order, invalid results may occur.
Vortex each specimen 3 times for 2 to 3 seconds.
Centrifuge specimens at 2000g for 5 minutes before loading onto the Abbott m2000sp worktable. 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.
Place the low and high positive controls, the negative control, the calibrators, if applicable, and the patient specimens into the Abbott m2000sp sample rack.
Place the 5 mL Reaction Vessels into the Abbott m2000sp 1 mL subsystem carrier.
Load the amplification reagents and the master mix vial on the Abbott 96-Well Optical Reaction Plate in the Abbott m2000sp Operations Manual, Operating Instructions section.
If prompted by the instrument, Reagent Carrier 2 should remain in place. A new Abbott RealTi 2000 instrument is required to perform 73 to 96 reactions.
Centrifuge specimens at 2000g for 5 minutes before loading onto the Abbott m2000sp worktable. 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.
NOTE: Change gloves before handling the amplification reagents.
Load the amplification reagents and the master mix vial on the Abbott m2000sp worktable after sample preparation is completed.
Each Amplification Reagent Pack supports up to 24 reactions.
Prior to opening the amplification reagents, ensure that the contents at the bottom of the vials by tapping the vials in an upright position on the bench.
Remove and discard the amplification vial caps.
A second Amplification Reagent Pack is required if performing 25 to 48 reactions.
A third Amplification Reagent Pack is required if performing 49 to 72 reactions.
A fourth Amplification Reagent Pack is required if performing 73 to 96 reactions.
Select the appropriate deep-well plate 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.
NOTE: The operator should not manually fill any empty/unfilled wells in the Abbott 96-Well Optical Reaction Plate.
After sample extraction is complete, the Abbott m2000sp automatically fills any empty wells in the Abbott 96-Well Optical Reaction Plate when there are greater than 48 samples processed within a run. Plate fill is not performed for runs containing 48 samples or fewer.
If prompted by the instrument, Reagent Carrier 2 should remain in place, minimally containing the reagent vessel for mEulsion Buffer (Reagent Carrier 2, location 6). If this reagent vessel has been unloaded, place a new reagent vessel with the mEulsion Buffer label into Reagent Carrier 2, location 6. System fluid will be added to the reagent vessel and used to fill empty wells. Once this process is complete, the system will continue with the master mix addition.
NOTE: System instructions for use of the automated plate-filling feature are found in the Abbott m2000sp Operations Manual (List No. 9K20-04 or higher), section 5, Operating Instructions, Sample Extraction—Closed Mode.
The Abbott m2000rt protocol (step 16) must be started within 50 minutes of the initiation of the Master Mix Addition protocol (step 12).
NOTE: If the run is aborted for any reason subsequent to step 12, a new 96-well PCR plate must be used if the Abbott m2000sp Master Mix Addition Protocol (step 12) will be repeated.
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.
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.
Place the sealed optical reaction plate into the Abbott Splash-Free Support Base for transfer to the Abbott m2000rt instrument.
NOTE: If creating the Abbott m2000rt test order manually, enter sample IDs in the corresponding PCR tray locations according to the “Wells for Selected Plate” grid, found on the detail screen of the “PCR Plate Results” on the Abbott m2000sp. See Section 5 of the Abbott m2000sp Operations Manual.
POST PROCESSING PROCEDURES
1. Remove the Abbott 96-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.
QUALITY CONTROL PROCEDURES
Abbott m2000rt Optical Calibration
Once an Abbott RealTi HIV-1 protocol, as described in the Abbott Operations Manual, and the HIV-1 application file for a different sample volume is used.
A calibration curve is required to quantitate the HIV-1 RNA concentration for specimens and controls. Two assay calibrators are run in replicates of 3 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 Abbott m2000rt workstation.
Follow the procedure for sample extraction, master mix addition, amplification and detection protocols as stated in the Abbott m1000 Operating Manual or Abbott m2000sp Operations Manual, and the Abbott m2000rt Operations Manual.
A calibration curve is required to quantitate the HIV-1 RNA concentration for specimens and controls. Two assay calibrators are run in replicates of 3 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 Abbott m2000rt workstation.
Follow the procedure for sample extraction, master mix addition, amplification and detection protocols as stated in the Abbott m1000 Operating Manual or Abbott m2000sp Operations Manual, and the Abbott m2000rt Operations Manual.
A calibration curve is required to quantitate the HIV-1 RNA concentration for specimens and controls. Two assay calibrators are run in replicates of 3 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 Abbott m2000rt workstation.
Pure Dye optical re-calibration of the Abbott RealTime HIV-1 assay-specific dyes (FAM, VIC, or ROX) is performed per the Calibration Procedures section of the Abbott m2000rt Operations Manual.

Detection of Inhibition
An IC threshold cycle [CT] 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 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 CT validity range to be met by all subsequent processed specimens. An error control flag is displayed when a specimen or control fails to meet this specification. Refer to the Abbott m2000rt Operations Manual for an explanation of the corrective actions for the error control flag. Specimens whose IC CT 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 control flag 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 control flag. 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.

Specimens whose IC CT value exceeds the established range must be retested starting with sample preparation.

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 m1000 System, 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 molecular biology grade microcentrifuge tube.
2. Saturate the cotton tip of an applicator (Puritan or equivalent) in the RNase-free water from the microcentrifuge tube.
3. Place the applicator into the microcentrifuge tube.
4. Saturate the cotton tip of the applicator, wipe the area to be monitored using a sweeping motion. Place the applicator into the microcentrifuge tube.
5. Swirl the cotton tip in RNase-free water 10 times, and then press the applicator along the side of the tube so that the liquid drains back into the solution at the bottom of the microcentrifuge tube. Discard the applicator.
6. Pipette 0.5 mL of mWash 1 buffer to a clean tube using the pipette dedicated for Internal Control use.
7. Add 20 µL of the mWash 1 buffer to each microcentrifuge tube.
8. Cap the microcentrifuge tube.
9. Test this sample 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 1.5 mL with RNase-free water.

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 10% (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], and dilutions of [IU/mL] or [ng/mL]; (1 IU = 0.98 copies, 1 copy = 1.74 IU).

INTERPRETATION OF RESULTS

<table>
<thead>
<tr>
<th>Sample Volume</th>
<th>Result</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 mL</td>
<td>Not Detected</td>
<td>Target not detected</td>
</tr>
<tr>
<td>0.6 mL</td>
<td>Not Detected</td>
<td>Target not detected</td>
</tr>
<tr>
<td>0.5 mL</td>
<td>Not Detected</td>
<td>Target not detected</td>
</tr>
<tr>
<td>0.2 mL</td>
<td>Not Detected</td>
<td>Target not detected</td>
</tr>
</tbody>
</table>

- 40 Copies/mL
- 75 Copies/mL
- 150 Copies/mL
- ULQ = upper limit of quantitation

LIMITATIONS OF THE PROCEDURE

- FOR IN VITRO DIAGNOSTIC USE
- Optimal performance of this test requires appropriate specimen collection, storage, and transport to the test site (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. The use of other anticoagulants has not been validated with the Abbott RealTime HIV-1 assay.
- The Abbott RealTime HIV-1 assay is limited to personnel who have been trained in the procedures of a molecular diagnostic assay and/or the Abbott m1000 System, 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 practices and careful adherence to the procedures specified in this package insert.
- As with any diagnostic test, results from the Abbott RealTime HIV-1 assay should be interpreted in conjunction with other clinical and laboratory findings. A specimen with a result of “Not Detected” cannot be presumed to be negative for HIV-1 RNA.

SPECIFIC PERFORMANCE CHARACTERISTICS

The performance characteristics were determined using the Abbott RealTime HIV-1 assay with Abbott m2000sp sample preparation 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 of 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 3 lots of amplification reagents on 3 Abbott m2000 Systems. The results, representative of the analytical sensitivity performance of the Abbott RealTime HIV-1 assay, are summarized in Table 1.

Table 1.

<table>
<thead>
<tr>
<th>Conc. (Copies/mL)</th>
<th>Number Tested</th>
<th>Number Detected</th>
<th>Percent Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>57</td>
<td>57</td>
<td>100</td>
</tr>
<tr>
<td>75</td>
<td>57</td>
<td>57</td>
<td>100</td>
</tr>
<tr>
<td>60</td>
<td>57</td>
<td>57</td>
<td>100</td>
</tr>
<tr>
<td>50</td>
<td>57</td>
<td>57</td>
<td>100</td>
</tr>
<tr>
<td>40</td>
<td>57</td>
<td>57</td>
<td>100</td>
</tr>
<tr>
<td>30</td>
<td>57</td>
<td>57</td>
<td>100</td>
</tr>
<tr>
<td>20</td>
<td>57</td>
<td>57</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>57</td>
<td>57</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>57</td>
<td>57</td>
<td>100</td>
</tr>
</tbody>
</table>

* One 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 to 33).

Limit of Detection, 0.6 mL Sample Volume

The LOD of the Abbott RealTime HIV-1 assay is 40 copies/mL with the 0.6 mL sample volume procedure. The LOD for the 0.6 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 Abbott RealTime HIV-1 assay, are summarized in Table 2.

Table 2.

<table>
<thead>
<tr>
<th>Conc. (Copies/mL)</th>
<th>Number Tested</th>
<th>Number Detected</th>
<th>Percent Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>57</td>
<td>57</td>
<td>100</td>
</tr>
<tr>
<td>75</td>
<td>57</td>
<td>57</td>
<td>100</td>
</tr>
<tr>
<td>60</td>
<td>57</td>
<td>57</td>
<td>100</td>
</tr>
<tr>
<td>50</td>
<td>57</td>
<td>57</td>
<td>100</td>
</tr>
<tr>
<td>40</td>
<td>57</td>
<td>57</td>
<td>100</td>
</tr>
<tr>
<td>30</td>
<td>57</td>
<td>57</td>
<td>100</td>
</tr>
<tr>
<td>20</td>
<td>57</td>
<td>57</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>57</td>
<td>57</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>57</td>
<td>57</td>
<td>100</td>
</tr>
</tbody>
</table>

Probit analysis of the data determined that the concentration of HIV-1 RNA detected with 95% probability was 39 copies/mL (95% CI 33 to 49).

Limit of Detection, 0.5 mL Sample Volume

The LOD of 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 and 0.6 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 7-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. The results, representative of the Abbott RealTime HIV-1 assay linearity, are shown in Figure 1.

Figure 1.

The Abbott 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 precision of the Abbott RealTime HIV-1 assay was evaluated for the 1.0 mL sample volume procedure using the Abbott m1000 and Abbott m2000sp sample preparation systems and the manual sample preparation method. The Abbott RealTime HIV-1 assay is designed to achieve an inter-assay standard deviation (SD) of less than or equal to 0.25 log copies of HIV-1 RNA per mL for samples containing HIV-1 concentrations from 500 to 5 million copies/mL. A 7-member HIV-1 RNA panel was prepared by diluting an HIV-1 viral stock (panel members 1 through 3) and armored HIV-1 RNA (panel members 4 through 7) in negative human plasma. For the precision studies with the Abbott m1000 and the Abbott m2000sp, the panel members were tested in replicates.
Hemoglobin 500 mg/dL
Protein 9 g/dL
Triglycerides 3000 mg/dL
Bilirubin 20 mg/dL

Table 5. Precision with the Abbott m1000 System

<table>
<thead>
<tr>
<th>Panel Member</th>
<th>Conc. Mean (copies/mL)</th>
<th>Conc. Mean (log copies/mL)</th>
<th>Within-Run SD Component</th>
<th>Between-Run SD Component</th>
<th>Inter-Assay SDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>75</td>
<td>57</td>
<td>1.75</td>
<td>0.21</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>75</td>
<td>573</td>
<td>2.76</td>
<td>0.08</td>
<td>0.00</td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>5,000</td>
<td>3.70</td>
<td>0.05</td>
<td>0.02</td>
</tr>
<tr>
<td>4</td>
<td>75</td>
<td>35,751</td>
<td>4.55</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>5</td>
<td>75</td>
<td>315,065</td>
<td>5.50</td>
<td>0.07</td>
<td>0.03</td>
</tr>
<tr>
<td>6</td>
<td>75</td>
<td>2,947,538</td>
<td>6.47</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>7</td>
<td>75</td>
<td>5,347,285</td>
<td>6.73</td>
<td>0.04</td>
<td>0.05</td>
</tr>
</tbody>
</table>

a Inter-assay contains within-run and between-run components.

Table 6. Precision with the Abbott m2000 System

<table>
<thead>
<tr>
<th>Panel Member</th>
<th>Conc. Mean (copies/mL)</th>
<th>Conc. Mean (log copies/mL)</th>
<th>Within-Run SD Component</th>
<th>Between-Run SD Component</th>
<th>Inter-Assay SDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>75</td>
<td>72</td>
<td>1.86</td>
<td>0.18</td>
<td>0.07</td>
</tr>
<tr>
<td>2</td>
<td>75</td>
<td>652</td>
<td>2.81</td>
<td>0.08</td>
<td>0.00</td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>5,417</td>
<td>3.73</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>4</td>
<td>75</td>
<td>39,468</td>
<td>4.60</td>
<td>0.04</td>
<td>0.03</td>
</tr>
<tr>
<td>5</td>
<td>75</td>
<td>358,587</td>
<td>5.55</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>6</td>
<td>75</td>
<td>3,102,654</td>
<td>6.49</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>7</td>
<td>75</td>
<td>5,953,879</td>
<td>6.77</td>
<td>0.04</td>
<td>0.04</td>
</tr>
</tbody>
</table>

a Inter-assay contains within-run and between-run components.

Table 7. Precision with Manual Sample Preparation Method

<table>
<thead>
<tr>
<th>Panel Member</th>
<th>Conc. Mean (copies/mL)</th>
<th>Conc. Mean (log copies/mL)</th>
<th>Within-Run SD Component</th>
<th>Between-Run SD Component</th>
<th>Inter-Assay SDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40b</td>
<td>46</td>
<td>1.66</td>
<td>0.21</td>
<td>0.07</td>
</tr>
<tr>
<td>2</td>
<td>41c</td>
<td>471</td>
<td>2.67</td>
<td>0.11</td>
<td>0.09</td>
</tr>
<tr>
<td>3</td>
<td>42</td>
<td>4,474</td>
<td>3.65</td>
<td>0.05</td>
<td>0.10</td>
</tr>
<tr>
<td>4</td>
<td>42</td>
<td>34,503</td>
<td>4.54</td>
<td>0.02</td>
<td>0.06</td>
</tr>
<tr>
<td>5</td>
<td>42</td>
<td>362,283</td>
<td>5.56</td>
<td>0.04</td>
<td>0.08</td>
</tr>
<tr>
<td>6</td>
<td>42</td>
<td>3,597,099</td>
<td>6.66</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>7</td>
<td>42</td>
<td>4,652,825</td>
<td>6.82</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

a Inter-assay contains within-run and between-run components.

Table 8. Detection of HIV-1 Subtypes and Groups

<table>
<thead>
<tr>
<th>Drug Pool</th>
<th>Drugs Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Zidovudine, Saquinavir, Ritonavir, Clarithromycin, Interferon 2a, Interferon 2b</td>
</tr>
<tr>
<td>2</td>
<td>Abacavir sulfate, Amprenavir, Peginterferon 2a, Peginterferon 2b, Ribavirin</td>
</tr>
<tr>
<td>3</td>
<td>Tenofovir disoproxil fumarate, Lamivudine, Indinavir sulfate, Ganciclovir, Valganciclovir hydrochloride, Acyclovir</td>
</tr>
<tr>
<td>4</td>
<td>Stavudine, Efavirenz, Lopinavir, Enfuvirtide, Ciprifloxacin</td>
</tr>
<tr>
<td>5</td>
<td>Zalcitabine, Nevirapine, Nelfinavir, Azithromycin, Valacyclovir</td>
</tr>
</tbody>
</table>

Drugs at concentrations in excess of the peak plasma or serum levels were tested in 5 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:

- Human Immunodeficiency virus 2
- HIV-1 RNA was not detected

Specificity

The target specificity of the Abbott RealTime HIV-1 assay is greater than or equal to 99.6% after resolution.

The specificity of the Abbott RealTime HIV-1 assay was evaluated by testing 187 HIV-1 seronegative plasma specimens. The specimens were tested on 3 Abbott m2000 instrument systems with 3 lots of amplification reagents. HIV-1 RNA was not detected, resulting in 100% (187/187) specificity (95% CI 98.05 to 100.00) in this representative study.

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 Abbott RealTime HIV-1 assay. Purified nucleic acid or viral lysate from each microorganism or virus was added to 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-reagents for all positive and negative samples tested.

Detection of HIV-1 Subtypes and Groups

The performance of the Abbott 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, and H), Group O, and Group N, and by testing 10 clinical specimens of each Group M subtype (A, B, C, CRF01-AE, F, CRF02-AG, and G), and 10 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.

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, 10 of each Group M subtype (A, B, C, D, CRF01-AE, F, CRF02-AG, G) and Group O, were tested with the Abbott RealTime HIV-1 assay and by 2 other HIV-1 quantitative assays referred to as Comparator 1 and Comparator 2. The results are summarized in Table 8.

Table 8.

<table>
<thead>
<tr>
<th>Group/Subtypes</th>
<th>n</th>
<th>RealTime Detected</th>
<th>Comparator 1 Detected</th>
<th>Comparator 2 Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/Subtype A</td>
<td>10</td>
<td>10</td>
<td>10 (1)</td>
<td>10 (1)</td>
</tr>
<tr>
<td>M/Subtype B</td>
<td>10</td>
<td>10</td>
<td>10 (0)</td>
<td>10 (0)</td>
</tr>
<tr>
<td>M/Subtype C</td>
<td>10</td>
<td>10</td>
<td>10 (0)</td>
<td>10 (0)</td>
</tr>
<tr>
<td>M/Subtype D</td>
<td>10</td>
<td>10</td>
<td>10 (0)</td>
<td>10 (0)</td>
</tr>
<tr>
<td>M/Subtype AE</td>
<td>10</td>
<td>10</td>
<td>10 (0)</td>
<td>10 (0)</td>
</tr>
<tr>
<td>M/Subtype F</td>
<td>10</td>
<td>10</td>
<td>10 (0)</td>
<td>10 (0)</td>
</tr>
<tr>
<td>M/Subtype AG</td>
<td>10</td>
<td>10</td>
<td>10 (3)</td>
<td>10 (1)</td>
</tr>
<tr>
<td>M/Subtype G</td>
<td>10</td>
<td>10</td>
<td>10 (2)</td>
<td>10 (1)</td>
</tr>
<tr>
<td>Group O</td>
<td>10</td>
<td>10</td>
<td>0 (NA)</td>
<td>7 (7)</td>
</tr>
</tbody>
</table>

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

- The Abbott RealTime HIV-1 assay detected all subtypes and groups tested.
- Comparator 1 detected all Group M subtypes tested and did not detect the 10 Group O samples.
- Comparator 2 detected all Group M subtypes tested and 7 out of 10 Group O samples.
- There were no samples that had Abbott RealTime assay quantitation values lower than Comparator 1 or Comparator 2 values by more than 1.00 log copies/mL.
- There were 6 Group M samples that had lower quantitation values with Comparator 1 by more than 1.00 log copies/mL when compared to Abbott RealTime HIV-1 assay.
- There were 3 Group M samples and 7 Group O samples that had lower quantitation values with Comparator 2 by more than 1.00 log copies/mL when compared to Abbott RealTime HIV-1 assay.

**BIBLIOGRAPHY**