WHO Prequalification of Diagnostics Programme
PUBLIC REPORT

Product: Abbott RealTime HIV-1 Qualitative (Manual)
Number: PQDx 0151-027-00

The Abbott RealTime HIV-1 Qualitative (Manual) with product code 4N66-90, 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 30 May 2013. This version of the product is intended to be used in conjunction with the following instruments/reagents: 9K15-01, 4N66-80, 4N66-03 or higher, 6K12-24 and 4N66-66 (optional).

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

<table>
<thead>
<tr>
<th>Date</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Status on PQ list</td>
<td>30-May-2013</td>
</tr>
<tr>
<td>Dossier assessment</td>
<td>20-May-2013</td>
</tr>
<tr>
<td>Site inspection(s) of quality management system</td>
<td>FT</td>
</tr>
<tr>
<td>Product performance evaluation</td>
<td>FT</td>
</tr>
</tbody>
</table>

MR: Meets Requirements
FT: Fast-Track

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</td>
<td>Implemented software change</td>
<td>1-Jun-2018</td>
</tr>
<tr>
<td>3.0</td>
<td>1. The Notified Body number on the Abbott RealTime HIV-1 Quantitative and Qualitative kit labels and package inserts has</td>
<td>24-Jul-2019</td>
</tr>
</tbody>
</table>
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.

3. Update on manufacturing site inspection commitments.

| 4.0 | 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. | 1-Nov-2021 |

**Intended use:**

According to the claim of Abbott Molecular Inc, “*Abbott RealTime HIV-1 Qualitative is an in vitro amplification assay for the qualitative detection of Human Immunodeficiency Virus Type 1 (HIV-1) nucleic acids from human plasma and dried blood spots (DBS). The Abbott RealTime HIV-1 Qualitative is intended to be used as an aid in the diagnosis of HIV-1 infection in pediatric and adult subjects. The Abbott RealTime HIV-1 Qualitative is not intended to be used as a donor screening test for HIV-1.*”

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

**Assay principle:**

According to the claim of Abbott Molecular Inc, “*Abbott RealTime HIV-1 Qualitative assay uses PCR to generate amplified product from the HIV-1 nucleic acids in clinical specimens. The presence of HIV-1 target sequence is indicated by the fluorescent signal generated through the use of fluorescent-labeled oligonucleotide probes on the Abbott m2000rt instrument. The probes do not generate a signal unless they are specifically bound to the amplified product. 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, and serves as an internal control (IC) to demonstrate that the process has proceeded correctly for each sample.*”

**Test kit contents:**

<table>
<thead>
<tr>
<th>Item</th>
<th>Product code(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott RealTime HIV-1 Qualitative Amplification Reagent Kit (to reference the new assay CD-ROM version 3)</td>
<td>4N66-90 (4 packs x 24 tests/pack)</td>
</tr>
</tbody>
</table>
**Materials required but not provided:**

<table>
<thead>
<tr>
<th>Item</th>
<th>Product code(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Instrumentation:</strong></td>
<td></td>
</tr>
<tr>
<td>Abbott <em>m</em>2000rt Instrument with software version 3.0 or higher</td>
<td>9K15-01</td>
</tr>
<tr>
<td><strong>Reagents:</strong></td>
<td></td>
</tr>
<tr>
<td>Abbott <em>m</em>Sample Preparation SystemDNA</td>
<td>6K12-24</td>
</tr>
<tr>
<td>Abbott RealTime HIV-1 Internal Control Kit</td>
<td>2G31Y</td>
</tr>
<tr>
<td>Abbott RealTime HIV-1 Qualitative Control Kit</td>
<td>4N66-80</td>
</tr>
<tr>
<td><strong>Software:</strong></td>
<td></td>
</tr>
<tr>
<td>Abbott RealTime HIV-1 Qualitative m2000 System ROW Combined Application CD-ROM, version 3.0</td>
<td>04N66-003 or higher</td>
</tr>
<tr>
<td><strong>Optional:</strong></td>
<td></td>
</tr>
<tr>
<td>Abbott RealTime HIV-1 UNG Protocol</td>
<td>4N66-66</td>
</tr>
<tr>
<td>Repeat pipette capable of delivering 40-50 μL volumes</td>
<td>n/a</td>
</tr>
<tr>
<td>Sterile repeat pipettor tips capable of delivering 40-50 μL volumes</td>
<td>n/a</td>
</tr>
<tr>
<td>Disposable transfer pipettes</td>
<td>n/a</td>
</tr>
<tr>
<td>96 well polypropylene plate</td>
<td>n/a</td>
</tr>
<tr>
<td><strong>Consumables required:</strong></td>
<td></td>
</tr>
<tr>
<td>Abbott <em>m</em>2000rt Optical Calibration kit</td>
<td>4J71-93</td>
</tr>
<tr>
<td>5 mL Reaction Vessels</td>
<td>4J71-20</td>
</tr>
<tr>
<td>1.5 mL screw top microfuge tubes and caps</td>
<td>04J71-50 or equivalent</td>
</tr>
<tr>
<td>Abbott Optical Adhesive Covers</td>
<td>4J71-75</td>
</tr>
<tr>
<td>Abbott Adhesive Cover Applicator</td>
<td>9K32-01</td>
</tr>
<tr>
<td>Abbott Splash-Free Support Base</td>
<td>9K31-01</td>
</tr>
<tr>
<td>Abbott 96-Well Optical Reaction Plate</td>
<td>4J71-70</td>
</tr>
<tr>
<td>Calibrated Pipettes capable of delivering 20-1000 μL</td>
<td>n/a</td>
</tr>
<tr>
<td>Aerosol Barrier Pipette Tips for 20-1000 μL Pipettes</td>
<td>n/a</td>
</tr>
<tr>
<td>Magnetic stands for 5 mL Reaction Vessels</td>
<td>n/a</td>
</tr>
<tr>
<td>Non-magnetic stands for 5 mL Reaction Vessels</td>
<td>n/a</td>
</tr>
<tr>
<td>Magnetic stands for 1.5 mL tubes</td>
<td>n/a</td>
</tr>
<tr>
<td>Non-magnetic stands for 1.5 mL tubes</td>
<td>n/a</td>
</tr>
<tr>
<td>Dry heating block for 5 mL Reaction Vessels (capable of achieving 50°C)</td>
<td>n/a</td>
</tr>
<tr>
<td>Dry heating block for 1.5 mL tubes (capable of achieving 75°C)</td>
<td>n/a</td>
</tr>
<tr>
<td>Thermometer</td>
<td>n/a</td>
</tr>
<tr>
<td>Timer</td>
<td>n/a</td>
</tr>
<tr>
<td>Vortex Mixer</td>
<td>n/a</td>
</tr>
</tbody>
</table>
USP Grade 190-200 Proof Ethanol (95%-100% Ethanol). Do not use ethanol that contains denaturants | n/a

Liquid Container for waste (no bleach should be allowed to contact liquid waste) | n/a

Centrifuge capable of 2000g | n/a

Centrifuge capable of 5,000g (for centrifuging the Abbott 96-Well Optical Reaction Plate) | n/a

Bulk mLysisDNA Buffer (for DBS processing only) | 2N77-01

50 mL Tubes (NUNC or equivalent) (optional) (for DBS processing only) | n/a

Eppendorf PCR Cooler | n/a

Single-use Rnase/Dnase-free tube or container | n/a

### Storage:

<table>
<thead>
<tr>
<th>Item</th>
<th>Temperature ( °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott RealTime HIV-1 Qualitative Control Kit</td>
<td>-10°C or colder</td>
</tr>
<tr>
<td>Abbott RealTime HIV-1 Qualitative Amplification Reagent Kit</td>
<td>-10°C or colder</td>
</tr>
<tr>
<td>Abbott mSample Preparation System DNA</td>
<td>15 – 30 °C</td>
</tr>
<tr>
<td>Uracil-N-glycosylase (UNG) required for the Abbott RealTime HIV-1 UNG Protocol (4N66-66)</td>
<td>-15 to -25°C</td>
</tr>
</tbody>
</table>

### Maximum shelf-life upon manufacture:

<table>
<thead>
<tr>
<th>Item</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott RealTime HIV-1 Qualitative Amplification Reagent Kit 4N66-90&gt;</td>
<td>18 months</td>
</tr>
<tr>
<td>Abbott RealTime HIV-1 Internal Control 2G31Y</td>
<td>18 months</td>
</tr>
<tr>
<td>Thermostable rTth Polymerase Enzyme 56685</td>
<td>per control date on vendor certificate of analysis</td>
</tr>
<tr>
<td>HIV-1 Oligonucleotide Reagent 2G31L</td>
<td>18 months</td>
</tr>
<tr>
<td>Activation Reagent 93591</td>
<td>18 months</td>
</tr>
</tbody>
</table>

### Prioritization for prequalification

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

Abbott Molecular Inc. submitted a product dossier for Abbott RealTi
time HIV-1 Qualitative (Manual) as per the Instructions for compilation of a product dossier (PQDx_018 v1). The information submitted in the product dossier was reviewed by WHO staff and external experts (assessors) appointed by WHO in accordance with the internal report on the screening and assessment of a product dossier (PQDx_009 v2). Based on the product dossier screening and assessment findings, a recommendation was made to accept the product dossier for Abbott RealTime HIV-1 Qualitative (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 performed at the site of manufacture (1300 East Touhy Avenue, 60018 Des Plaines, IL, USA) of the Abbott RealTime HIV-1 Qualitative (manual) between 12 -14 September 2018 as described in ‘Information for manufacturers on WHO prequalification inspection procedures for the sites of manufacture of diagnostics (PQDx_014 v4)’.

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 12 February 2019.

**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 Qualitative (Manual) assay was found eligible to undergo the WHO fast track procedure. Subsequently, the product was not required to undergo a laboratory evaluation.
Labelling

1. Labels
2. Instructions for use
1. Labels
Abbott RealTime HIV-1 Qualitative Amplification Reagent Kit label(04N66-90)
1 Bottle (0.141 mL) Thermostable rTth Polymerase Enzyme (2.9 to 3.5 Units/µL) in buffered solution.

1 Bottle (0.40 mL) Activation Reagent. 30 mM manganese chloride solution. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

2. The Abbott RealTime HIV-1 Qualitative is an in vitro amplification assay for the qualitative detection of Human Immunodeficiency Virus Type 1 (HIV-1) nucleic acids from human plasma and dried blood spots (DBS). The Abbott RealTime HIV-1 Qualitative is intended to be used as an aid in the diagnosis of HIV-1 infection in pediatric and adult subjects.

Abbott RealTime HIV-1 Qualitative is not intended to be used as a donor screening tool for HIV-1.

Contenido:
1. INTENSO CONTROL. Abbott RealTime HIV-1 Internal Control (2 vials, 1.2 ml each).

Reactivos. Abbott RealTime HIV-1 Qualitative incluye reactivo de activación, enzima rTth, oligonucleótidos desactivador y dNTPs. Preseresores: 0.1% ProClin 300 y 0.15% ProClin 950.

2. AMPLIFICACIÓN REAGENTE. Abbott RealTime HIV-1 Qualitative Amplification Reagent Pack (4 packs, 24 tests/Pack).

Inhalte:
1 Fläschchen (0,40 ml) Aktivierungsreagenz. 30 mM de solución de cloruro de manganeso. Conservantes: ProClin 300 al 0,1% y ProClin 950 al 0,15%.
1 Fläschchen (1,10 ml) de réactif d'oligonucléotides VIH-1. < 0,1 % d'oligonucléotides synthétiques (4 amorces, 2 sondes et 1 oligonucléotide "quencher") e dNTP < 0,3% in una soluzione di tampone con un colorante di riferimento. Conservanti: 0,1% de ProClin 300 and 0,15% ProClin 950.

1 Frasco (0.40 ml) de reagente de activación. 30 mM de solución de cloruro de manganeso. Conservantes: ProClin 300 al 0,1% y ProClin 950 al 0,15%.

Amplification Reagent Kit

Abbott RealTime HIV-1 Qualitative

Abbott RealTime HIV-1 Qualitative is an in vitro amplification assay for the qualitative detection of Human Immunodeficiency Virus Type 1 (HIV-1) nucleic acids from human plasma and dried blood spots (DBS). The Abbott RealTime HIV-1 Qualitative is intended to be used as an aid in the diagnosis of HIV-1 infection in pediatric and adult subjects.
HIV-1 Qualitative Control Kit label(04N66-80)
Contents:

1. Abbott RealTime HIV-1 Qualitative Control Kit
2. Abbott RealTime HIV-1 High Positive Control
3. Abbott RealTime HIV-1 Negative Control

Abbott RealTime is a trademark of Abbott Laboratories.

Armored RNA is a registered trademark of Ambion.

ProClin is a registered trademark of Rohm and Haas.

Molecular identifiers: H317, 2 e anti-HCV, HIV RNA, HCV RNA and HBV DNA.

Conservation: 0.1% ProClin 300 and 0.15% ProClin 950.

Abbott RealTime HIV-1 Qualitative is a test for the qualitative detection of HIV-1 RNA from human plasma and dried blood spots (DBS).

Abbott RealTime HIV-1 Qualitative Controls are used to establish the validity of the Abbott RealTime HIV-1 Qualitative assay when used for the qualitative detection of Human Immunodeficiency Virus Type 1 (HIV-1) nucleic acids from human plasma and dried blood spots (DBS).

Abbott RealTime is a trademark of Abbott Laboratories.

Armored RNA is a registered trademark of Ambion.

ProClin is a registered trademark of Rohm and Haas.

Molecular identifiers: H317, 2 e anti-HCV, HIV RNA, HCV RNA and HBV DNA.

Conservation: 0.1% ProClin 300 and 0.15% ProClin 950.

Abbott RealTime HIV-1 Qualitative is a test for the qualitative detection of HIV-1 RNA from human plasma and dried blood spots (DBS).

Abbott RealTime HIV-1 Qualitative Controls are used to establish the validity of the Abbott RealTime HIV-1 Qualitative assay when used for the qualitative detection of Human Immunodeficiency Virus Type 1 (HIV-1) nucleic acids from human plasma and dried blood spots (DBS).

Abbott RealTime is a trademark of Abbott Laboratories.

Armored RNA is a registered trademark of Ambion.

ProClin is a registered trademark of Rohm and Haas.

Molecular identifiers: H317, 2 e anti-HCV, HIV RNA, HCV RNA and HBV DNA.

Conservation: 0.1% ProClin 300 and 0.15% ProClin 950.

Abbott RealTime HIV-1 Qualitative is a test for the qualitative detection of HIV-1 RNA from human plasma and dried blood spots (DBS).

Abbott RealTime HIV-1 Qualitative Controls are used to establish the validity of the Abbott RealTime HIV-1 Qualitative assay when used for the qualitative detection of Human Immunodeficiency Virus Type 1 (HIV-1) nucleic acids from human plasma and dried blood spots (DBS).

Abbott RealTime is a trademark of Abbott Laboratories.

Armored RNA is a registered trademark of Ambion.

ProClin is a registered trademark of Rohm and Haas.

Molecular identifiers: H317, 2 e anti-HCV, HIV RNA, HCV RNA and HBV DNA.

Conservation: 0.1% ProClin 300 and 0.15% ProClin 950.

Abbott RealTime HIV-1 Qualitative is a test for the qualitative detection of HIV-1 RNA from human plasma and dried blood spots (DBS).

Abbott RealTime HIV-1 Qualitative Controls are used to establish the validity of the Abbott RealTime HIV-1 Qualitative assay when used for the qualitative detection of Human Immunodeficiency Virus Type 1 (HIV-1) nucleic acids from human plasma and dried blood spots (DBS).

Abbott RealTime is a trademark of Abbott Laboratories.

Armored RNA is a registered trademark of Ambion.

ProClin is a registered trademark of Rohm and Haas.

Molecular identifiers: H317, 2 e anti-HCV, HIV RNA, HCV RNA and HBV DNA.

Conservation: 0.1% ProClin 300 and 0.15% ProClin 950.

Abbott RealTime HIV-1 Qualitative is a test for the qualitative detection of HIV-1 RNA from human plasma and dried blood spots (DBS).

Abbott RealTime HIV-1 Qualitative Controls are used to establish the validity of the Abbott RealTime HIV-1 Qualitative assay when used for the qualitative detection of Human Immunodeficiency Virus Type 1 (HIV-1) nucleic acids from human plasma and dried blood spots (DBS).

Abbott RealTime is a trademark of Abbott Laboratories.

Armored RNA is a registered trademark of Ambion.

ProClin is a registered trademark of Rohm and Haas.

Molecular identifiers: H317, 2 e anti-HCV, HIV RNA, HCV RNA and HBV DNA.

Conservation: 0.1% ProClin 300 and 0.15% ProClin 950.

Abbott RealTime HIV-1 Qualitative is a test for the qualitative detection of HIV-1 RNA from human plasma and dried blood spots (DBS).

Abbott RealTime HIV-1 Qualitative Controls are used to establish the validity of the Abbott RealTime HIV-1 Qualitative assay when used for the qualitative detection of Human Immunodeficiency Virus Type 1 (HIV-1) nucleic acids from human plasma and dried blood spots (DBS).

Abbott RealTime is a trademark of Abbott Laboratories.

Armored RNA is a registered trademark of Ambion.

ProClin is a registered trademark of Rohm and Haas.

Molecular identifiers: H317, 2 e anti-HCV, HIV RNA, HCV RNA and HBV DNA.

Conservation: 0.1% ProClin 300 and 0.15% ProClin 950.

Abbott RealTime HIV-1 Qualitative is a test for the qualitative detection of HIV-1 RNA from human plasma and dried blood spots (DBS).

Abbott RealTime HIV-1 Qualitative Controls are used to establish the validity of the Abbott RealTime HIV-1 Qualitative assay when used for the qualitative detection of Human Immunodeficiency Virus Type 1 (HIV-1) nucleic acids from human plasma and dried blood spots (DBS).

Abbott RealTime is a trademark of Abbott Laboratories.

Armored RNA is a registered trademark of Ambion.

ProClin is a registered trademark of Rohm and Haas.

Molecular identifiers: H317, 2 e anti-HCV, HIV RNA, HCV RNA and HBV DNA.

Conservation: 0.1% ProClin 300 and 0.15% ProClin 950.
High Positive Control Vial label (2G31X)
HI V-1 PO S

Exp.

Store at ≤ -10°C

2G31X 1.8mL

Colors: PMS 185 C

BLACK

PMS 299 C

Abbott RealTime HIV-1

HIV-1 CONTROL

Abbott RealTime

1.8mL

REF 2631X

Top Edge
Negative Control Vial label (2G31Z)
Abbott RealTime HIV-1 Internal Control Vial label (2G31Y)
Abbott RealTime HIV-1

INTERNAL CONTROL

1.2 mL

Abbott Molecular Inc.
Des Plaines, IL 60018 USA

Colors: PMS 299 C
PMS 185 C
BLACK

Infection Risk

Store at ≤ -10°C

Exp.

LOT

51-602110/R6

Abbott RealTime

Ref: 2G31Y
2. Instructions for use

\[1\] 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.
INTENDED USE

Abbott RealTime HIV-1 Qualitative

Key to Symbols Used

<table>
<thead>
<tr>
<th>REF</th>
<th>Reference Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVD</td>
<td>In Vitro Diagnostic Medical Device</td>
</tr>
<tr>
<td>LOT</td>
<td>Lot Number</td>
</tr>
<tr>
<td>Use By</td>
<td></td>
</tr>
<tr>
<td>Upper Limit of Temperature</td>
<td></td>
</tr>
<tr>
<td>CONTROL</td>
<td>Negative Control</td>
</tr>
<tr>
<td>CONTROL</td>
<td>High Positive Control</td>
</tr>
<tr>
<td>Consult instructions for use</td>
<td></td>
</tr>
<tr>
<td>Caution</td>
<td></td>
</tr>
<tr>
<td>Warning</td>
<td></td>
</tr>
</tbody>
</table>

INTERNAL CONTROL

Internal Control

AMPLIFICATION REAGENT PACK

Amplification Reagent Pack

EC REP

Authorized Representative in the European Community

Manufacturer

NOTE: Changes highlighted FOR IN VITRO DIAGNOSTIC USE IN VITRO TEST

The Abbott RealTime HIV-1 Qualitative is intended to be used as an aid in the diagnosis of HIV-1 infection in pediatric and adult subjects. The Abbott RealTime HIV-1 Qualitative is not intended to be used as a donor screening test for HIV-1.

INTENDED USER

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

SUMMARY AND EXPLANATION OF THE TEST

HIV-1 is the etiologic agent of Acquired Immunodeficiency Syndrome (AIDS). 1,2,3 It can be transmitted through sexual contact, exposure to infected blood or blood products, perinatal or postnatal infection of a newborn. 4,5,6 Acute HIV-1 infection usually presents signs and symptoms such as acute febrile illness that start within days to weeks after initial exposure and typically last for less than 14 days. 7 Acute HIV-1 infection is associated with high levels of viremia prior to a detectable immune response. 8,9,10,11 Therefore, HIV-1 nucleic acid testing can be more sensitive than standard serologic testing in detection of acute infection. 7 For pediatric HIV-1 infection, maternal antibody can be transferred passively to infants and may be detectable for up to 18 months; 12,13 therefore, early diagnosis of HIV-1 in infants requires direct detection of the virus or its components. 14 As a result, HIV-1 nucleic acid testing has been recommended for testing infection in pediatric patients 18 months of age or younger. 13,15,16,17 Human plasma has been widely used as the specimen type for HIV-1 nucleic acid testing. The use of DBS as an alternative specimen type can potentially facilitate the implementation of HIV-1 nucleic acid testing by simplifying sample collection and transportation to the testing laboratory. 16,17 The Abbott RealTime HIV-1 Qualitative assay detects HIV-1 nucleic acids by using Polymerase Chain Reaction (PCR) technology with homogeneous real-time fluorescent detection. Partially double-stranded fluorescent probe design allows detection of diverse HIV-1 groups and subtypes. The assay uses either human plasma or DBS specimen type and reports a qualitative result.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The Abbott RealTime HIV-1 Qualitative assay consists of 2 reagent kits:

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

The Abbott RealTime HIV-1 Qualitative assay uses PCR 18 to generate amplified product from the HIV-1 nucleic acids in clinical specimens. The presence of HIV-1 target sequence is indicated by the fluorescent signal generated through the use of fluorescent-labeled oligonucleotide probes on the Abbott m2000rt instrument. The probes do not generate a signal unless they are specifically bound to the amplified product. 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, and serves as an internal control (IC) to demonstrate that the process has proceeded correctly for each sample.

Sample Preparation

The purpose of sample preparation is to extract, concentrate, and purify the target nucleic acids for amplification. The Abbott mSample Preparation System 19 uses magnetic particle technology to capture target nucleic acids and washes the particles to remove unbound sample components. The bound nucleic acids are eluted and are then ready for amplification. The IC is taken through the entire sample preparation procedure along with the controls and specimens. The Abbott m2000sp automated sample preparation system can be used to prepare samples for the Abbott RealTime HIV-1 Qualitative assay. Alternatively, samples can be prepared manually.

NOTE: One Abbott mSample Preparation System 19 kit is sufficient to complete 4 × 24 (96) sample preparations.

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

NAME

Abbott RealTime HIV-1 Qualitative

INTENDED USE

The Abbott RealTime HIV-1 Qualitative is an in vitro amplification assay for the qualitative detection of Human Immunodeficiency Virus Type 1 (HIV-1) nucleic acids from human plasma and dried blood spots (DBS).
The Abbott RealTime HIV-1 Qualitative Amplification Reagent components (HIV-1 Oligonucleotide Reagent, Thermostable rTth Polymerase Enzyme, and Activation Reagent) are each labeled with a different fluorophore, which allows for fluorescent emission and detection by the Abbott RealTime Amplification detection system. The IC probe binds to its complementary sequence in the target, the fluorophore and the quencher are held apart, allowing fluorescent emission. When the IC probe binds to its complementary sequence in the target, the fluorophore and the quencher are held apart, allowing fluorescent emission. During the amplification reaction on the Abbott RealTime Amplification Detection system, the target RNA is reverse transcribed to cDNA by the reverse transcriptase activity of the enzyme and subsequently amplified. Amplification of HIV-1 and IC targets takes place simultaneously in the same reaction. The target sequence for the Abbott RealTime Amplification detection system is in the pol integrase region of the HIV-1 genome. This region is highly conserved. The IC sequence is derived from the retroviral enzyme region of the HIV-1 genome.

Detection
During the read cycles of amplification on the Abbott RealTime Amplification Detection system, the temperature is lowered to allow fluorescent detection of amplification products as the HIV-1 and IC probes anneal to their respective targets (real-time fluorescent detection). The HIV-1 probe has a fluorescent moiety that is covalently linked to the 5' end. A short quencher oligonucleotide is complementary to the 5' end of the HIV-1 probe and has a quencher moiety 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 and 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 sequences, the IC probe adopts a series of random conformations, some of which bring the quencher close enough to the excited fluorophore to absorb its energy before it can be fluorescently emitted. When the IC probe binds to its complementary sequence in the target, the fluorophore and the quencher are held apart, allowing fluorescent emission and detection by the Abbott RealTime Amplification Detection system. The HIV-1 and IC specific probes are each labeled with a different fluorophore, thus allowing simultaneous detection of both amplified products.

Assay Results
The Abbott RealTime HIV-1 Qualitative assay results are reported as either “HIV-1 Detected” or “Not Detected.” Refer to the RESULTS section of this package insert for further details.

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 Qualitative assay. Refer to the Contamination Precautions section of this package insert.

REAGENTS
Abbott RealTime HIV-1 Qualitative Amplification Reagent Kit (List No. 04N66-90)

1. INTERNAL CONTROL Abbott RealTime HIV-1 Internal Control (List No. 2G31Y) (4 vials, 1.2 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, anti-HIV-1/HIV-2, anti-HCV, HIV RNA, HCV RNA, and HBV DNA. Preservatives: 0.1% ProClin® 300 and 0.15% ProClin 950.

2. [AMPLIFICATION REAGENT PACK] Abbott RealTime HIV-1 Qualitative Amplification Reagent Pack (List No. 4N66E-80) (4 packs, 24 tests/pack)
- 1 bottle (0.141 mL) Thermostable rTth Polymerase Enzyme (2.9 to 3.5 Units/mL) 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 Qualitative Control Kit (List No. 4N66-80)

1. [CONTROL | Abbott RealTime HIV-1 Negative Control (List No. 2G31Z) (12 vials, 1.8 mL per vial)]
Negative human plasma tested and found to be nonreactive for HBsAg, anti-HIV-1/HIV-2, anti-HCV, HIV RNA, HCV RNA, and HBV DNA. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

2. [CONTROL | Abbott RealTime HIV-1 High Positive Control (List No. 2G31X) (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, anti-HIV-1/HIV-2, anti-HCV, HIV RNA, HCV RNA, and HBV DNA. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

WARNINGS AND PRECAUTIONS

**In Vitro Diagnostic Medical Device**
This assay is not intended to be used as a screening test for HIV-1.

Safety Precautions
Refer to the Abbott m2000sp and Abbott m2000rt Operations Manuals, Hazards 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 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, CLSI Document M29-A2, 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% (v/v) 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 Qualitative Control Kit (List No. 4N66-80) and the Abbott RealTime HIV-1 Qualitative Amplification Reagent Kit (List No. 4N66-90) contain the following components:

- 2-Methyl-2H-isothiazol-3-one-3-one
- Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one (EC no. 247-500-7) and 2-methyl-2H-isothiazol-3-one (EC no. 220-239-6) (3:1)
- 2-Methyl-2H-isothiazol-3-one (EC no. 223-239-6)(3:1)
- Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one (EC no. 247-500-7) and 2-methyl-4-isothiazolin-3-one (EC no. 223-239-6)(3:1)
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.

**Specimen Collection and Handling Precautions**

The Abbott RealTime HIV-1 Qualitative assay is only for use with plasma and DBS specimens that have been collected and handled as described in the **SPECIMEN COLLECTION AND HANDLING INSTRUCTIONS** section.

**Laboratory Precautions**

- During preparation of samples, compliance with good laboratory practices is essential to minimize the risk of cross-contamination between samples as well as the inadvertent introduction of nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with RNA or DNA.
- Work area and instrument platforms must be considered potential sources of contamination. Change gloves after having contact with potential contaminants (such as RNases, DNases, specimens, eluates, and/or amplified product) before handling unopened reagents, negative control, positive control, or specimens. Refer to the Abbott m2000sp and m2000rt Operations Manuals and the instructions in the **POST PROCESSING PROCEDURES** section of this package insert for cleaning procedures.
- Wear appropriate personal protective equipment at all times.
- Use powder-free gloves.
- To reduce the risk of nucleic acid contamination due to aerosols formed during pipetting, pipettes with aerosol barrier tips or disposable transfer pipettes 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.
- Clean and disinfect spills of specimens and reagents as stated in the Abbott m2000sp and m2000rt Operations Manuals and the instructions in the **POST PROCESSING PROCEDURES** section of this package insert.

**Amplification Precautions**

- Amplification reactions such as PCR are sensitive to accidental introduction of product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the reagents used became contaminated by accidental introduction of even a few molecules of amplification product. Measures to reduce the risk of contamination in the laboratory include physically separating the activities involved in performing PCR in compliance with good laboratory practices.
- The use of 2 dedicated areas within the laboratory is recommended for performing the Abbott RealTime HIV-1 Qualitative assay with the automated Abbott m2000sp and m2000rt.
- The **Sample Preparation Area** is dedicated to processing samples (specimens, Abbott RealTime HIV-1 Qualitative Controls) and to adding processed specimens and controls to the Abbott 96-Well Optical Reaction Plate. All reagents used in the Sample Preparation Area must remain in this area and not be moved to the Amplification Area.
- The Amplification Area is dedicated to the amplification and the detection of amplified product. Laboratory coats and equipment used in the Amplification Area must remain in this area and not be moved to the Sample Preparation Area.
- The use of 3 dedicated areas within the laboratory is recommended for performing the Abbott RealTime HIV-1 Qualitative assay with the manual sample preparation method and the Abbott m2000rt.
- The **Reagent Preparation Area** is dedicated to combining the Abbott RealTime HIV-1 Qualitative amplification reagent components to create the amplification master mix and transferring aliquots of the master mix to the Abbott 96-Well Optical Reaction Plate. Laboratory coats, pipettes, and pipette tips used in the Reagent Preparation Area must remain in this area and not be moved to either the Sample Preparation Area or the Amplification Area. Do not bring target or amplification product into the Reagent Preparation Area.
- The **Sample Preparation Area** is dedicated to processing samples (specimens, Abbott RealTime HIV-1 Qualitative Controls) and to adding processed specimens and controls to the Abbott 96-Well Optical Reaction Plate. All reagents used in the Sample Preparation Area should remain in this dedicated area at all times. Laboratory coats, pipettes, pipette tips, and vortex mixers 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.
- The **Amplification Area** is dedicated to the amplification and the detection of amplified product. Laboratory coats and equipment used in the Amplification Area should remain in this area and not be moved to either the Reagent Preparation Area or the Sample Preparation Area.
- If the Abbott m2000sp run is aborted, dispose of all commodities and reagents according to the Abbott m2000sp Operations Manual.
- If the manual sample preparation procedure is incorrectly performed or is interrupted at any point so that the timing of the steps exceeds the recommended timing per the protocol instructions, dispose of all commodities and reagents according to the instructions in the **POST PROCESSING PROCEDURES** section of this package insert.
- If the Abbott m2000sp master mix addition protocol is aborted after amplification reagents are added to the Abbott 96-Well Optical Reaction Plate, seal the Abbott 96-Well Optical Reaction Plate and put in a sealable plastic bag and dispose of according to the Abbott m2000sp Operations Manual, Hazards section, along with the gloves used to handle the plate.
- If manual preparation of the PCR reaction mix is aborted after amplification reagents are added to the Abbott 96-Well Optical Reaction Plate, seal the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose of according to laboratory guidelines, along with the gloves used to handle the plate.
- For all completed, interrupted or aborted Abbott m2000rt runs, dispose of the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag according to the Abbott m2000rt Operations Manual along with the gloves used to handle the plate.
- Autoclaving the sealed Abbott 96-Well Optical Reaction Plate will not degrade the amplified product and may contribute to the release of the amplified product by opening the sealed plate. The laboratory area can become contaminated with amplified product if the waste materials are not carefully handled and contained.
- Decontaminate and dispose of all specimens, reagents, and other potentially biohazardous or contaminated materials in accordance with local, state, and federal regulations. All materials should be handled in a manner that minimizes the chance of potential contamination of the work area.

**Contamination from External Deoxy-URacil (dU)-Containing Amplified Product**

HIV-1 amplification assays containing dU may cause contamination and inaccurate results in the Abbott RealTime HIV-1 Qualitative assay. When negative controls are persistently reactive or where contamination with dU-containing HIV-1 amplified product is likely to have occurred, it is recommended that the laboratory use an additional contamination control procedure. This procedure (List No. 04N66-66) is available through your Abbott representative.

**REAGENT STORAGE AND HANDLING INSTRUCTIONS**

Abbott RealTime HIV-1 Qualitative Amplification Reagent Kit (List No. 4N66-90)

![Image](image-url)
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 regarded as invalid and specimens must be retested.

INSTRUMENT METHODS
The Abbott RealTime HIV-1 Qualitative assay is performed using the Abbott m2000sp or the manual sample preparation method for sample processing, and the Abbott m2000r for amplification and detection. Refer to the appropriate Assay Protocol instructions in this package insert or to the Abbott m2000sp or m2000r Operations Manuals for detailed operating procedures. The Abbott RealTime HIV-1 Qualitative application files must be installed on the Abbott m2000sp and Abbott m2000r systems from the Abbott RealTime HIV-1 Qualitative m2000 System Combined Application CD-ROM (List No. 4N66-003 or higher) prior to performing the assay. For detailed information on application file installation, refer to the Abbott m2000sp and Abbott m2000r Operations Manuals, Operating Instructions section.

SPECIMEN COLLECTION AND HANDLING INSTRUCTIONS

Plasma Specimen Collection and Storage
Human plasma (ACD-A and EDTA) specimens may be used with the Abbott RealTime HIV-1 Qualitative 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 preparing plasma specimens through centrifugation or preparing DBS specimens. After centrifugation, remove plasma from cells and pipette plasma into a separate tube for immediate testing or storage. 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. If longer storage is required, plasma specimens may be stored at −10 to −30°C for up to 30 days, or at −70°C or lower,24,25 Multiple freeze-thaw cycles should be avoided and should not exceed 3 freeze-thaw cycles. 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.

DBS Specimen Collection and Storage
DBS may be made on a Whatman 903 card (or equivalent) using blood obtained from a heel- or finger-stick or collected in a blood collection tube. 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. DBS are made by following these steps:
- Spot whole blood onto a minimum of 2 one-half-inch (12-millimeter) circles on a Whatman 903 filter paper card (or equivalent), ensuring that the entire circle is covered (approximately 50 μL). If the whole blood has been collected in a blood collection tube, the blood should be mixed prior to spotting using a pipette.
- Air dry the card at room temperature.
- Package each card in a bag with desiccant packs. The cards may be stored at 15 to 30°C for up to 12 weeks. Alternatively, cards may be stored at 2 to 8°C or −10°C or colder for up to 12 weeks.

Specimen Transport
Ship plasma specimens frozen on dry ice. Ship DBS specimens under ambient conditions, packaged in bags with desiccant packs. For DBS specimens, total time under ambient conditions during transport and storage should not exceed 12 weeks. 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.

ABSTRACT REALTIME HIV-1 QUALITATIVE ASSAY PROCEDURE
This Abbott RealTime HIV-1 Qualitative package insert contains 4 assay protocols:

ASSAY PROTOCOL I: PLASMA SPECIMENS USING ABBOTT m2000sp AUTOMATED SAMPLE PREPARATION SYSTEM

ASSAY PROTOCOL II: DBS SPECIMENS USING ABBOTT m2000sp AUTOMATED SAMPLE PREPARATION SYSTEM.

ASSAY PROTOCOL III: PLASMA SPECIMENS USING MANUAL SAMPLE PREPARATION METHOD

ASSAY PROTOCOL IV: DBS SPECIMENS USING MANUAL SAMPLE PREPARATION METHOD

Materials Provided
- Abbott RealTime HIV-1 Qualitative Amplification Reagent Kit (List No. 4N66-90)

Materials Required But Not Provided
- Abbott RealTime HIV-1 Qualitative Control Kit (List No. 4N66-80)
- Abbott RealTime HIV-1 Qualitative m2000 System Combined Application CD-ROM (List No. 4N66-003 or higher)

SAMPLE PREPARATION AREA

Materials for Abbott m2000sp (ASSAY PROTOCOLS I and II)
- Abbott m2000sp instrument with software version 4.0 or higher
- Abbott mSample Preparation System® (List No. 6K12-24)

NOTE: One kit is sufficient to complete 96 sample preparations.
- 5 mL Reaction Vessels (List No. 4J71-20)
- Calibrated Pipettes capable of delivering 20 to 1000 μL
- Aerosol Barrier Pipette Tips for 20 to 1000 μL Pipettes
- 1000 μL Disposable Tips (List No. 4J71–10)
- 200 μL Disposable Tips (List No. 4J71-17)
- Vortex Mixer
- USP Grade 190 to 200 Proof Ethanol (95 to 100% Ethanol). Do not use ethanol that contains denaturants.
- Abbott Optical Adhesive Covers (List No. 4J71-75)
- Abbott Adhesive Cover Applicator (List No. 9K32-01)
- Abbott Splash-Free Support Base (List No. 9K31-01)
- Master Mix Tube (List No. 4J71-80)
- 13 mm Sample Racks (List No. 4J72-82)
- 200 mL Reagent Vessels (List No. 4J71-60)
- Abbott 96-Deep-Well Plate (List No. 4J71–30)
- Abbott 96-Well Optical Reaction Plate (List No. 4J71–70)
- Centrifuge capable of 2000g
- Bulk mLysing Buffer (List No. 2N77-01) (for DBS processing only)
- 50 mL Tubes (NUNC or equivalent) (optional) (for DBS processing only)

Materials for Manual Sample Preparation (ASSAY PROTOCOLS III and IV)
- Abbott mSample Preparation System® (List No. 6K12-24)

NOTE: One kit is sufficient to complete 96 sample preparations.
- Calibrated Pipettes capable of delivering 20 to 1000 μL
- Aerosol Barrier Pipette Tips for 20 to 1000 μL Pipettes
- 5 mL Reaction Vessels (List No. 4J71-20)
- 1.5 mL screw top microfuge tubes and caps (List No. 04J71-50 or equivalent)
- Magnetic stands for 5 mL Reaction Vessels
- Non-magnetic stands for 5 mL Reaction Vessels
- Magnetic stands for 1.5 mL tubes
- Non-magnetic stands for 1.5 mL tubes
- Dry heating block for 5 mL Reaction Vessels (capable of achieving 50°C)
- Dry heating block for 1.5 mL tubes (capable of achieving 75°C)
- Thermometer
- Timer
- Vortex Mixer
- USP Grade 190 to 200 Proof Ethanol (95 to 100% Ethanol). Do not use ethanol that contains denaturants.
- Liquid Container for waste (no bleach should be allowed to contact liquid waste).

See the instructions in the POST PROCESSING PROCEDURES section of this package insert for warning and precaution information.
- Abbott Optical Adhesive Covers (List No. 4J71-75)
- Abbott Adhesive Cover Applicator (List No. 9K32-01)
- Abbott Splash-Free Support Base (List No. 9K31-01)
- Centrifuge capable of 2000g
- Centrifuge capable of 5000g (for centrifuging the Abbott 96-Well Optical Reaction Plate)
- Repeat pipettor capable of delivering 40 to 50 μL volumes (optional)
- Sterile repeat pipettor tips capable of delivering 40 to 50 μL volumes (optional)
- Disposable transfer pipettes (optional)
- 96-well polystyrene plate (optional)
- Bulk mLysing Buffer (List No. 2N77-01) (for DBS processing only)
- 50 mL Tubes (NUNC or equivalent) (optional) (for DBS processing only)
REAGENT PREPARATION AREA
Materials for Manual Sample Preparation (ASSAY PROTOCOLS III and IV)
- StrataCooler™ 96 Benchtop Cooler or Eppendorf PCR Cooler
- Abbott 96-Well Optical Reaction Plate (List No. 4J71–70)
- Calibrated Pipettes capable of delivering 20 to 1000 μL
- Aerosol Barrier Pipette Tips for 20 to 1000 μL Pipettes
- Single-use RNase/DNase-free tube or container

NOTE: Magnetic stands for 5 mL Reaction Vessels, magnetic stands for 1.5 mL tubes, and Eppendorf PCR Cooler can be ordered as m2000 mSample Preparation System Start Up Kit (List No. 02N28-03).

AMPLIFICATION AREA
Materials for Abbott m2000sp and Manual Sample Preparation (ASSAY PROTOCOLS I, II, III and IV)
- Abbott m2000rt instrument with software version 3.0 or higher
- Abbott m2000rt Optical Calibration Kit (List No. 4J71-93)

Other Materials (ASSAY PROTOCOLS I, II, III and IV)
- Biological safety cabinet approved for working with infectious materials
- Lab coat
- Powder-free disposable gloves
- Protective eyewear
- Solid waste container
- Sealable plastic bags
- RNase/DNase-free water (Eppendorf or equivalent)†
- Cotton Tip Applicators (Puritan or equivalent)
- Sealable plastic bags

†These items are used in the procedure in the section Monitoring the Laboratory for the Presence of Contamination section.

Procedural Precautions
- Read the instructions in the package insert carefully before processing samples.
- The Abbott RealTime HIV-1 Qualitative reagents are intended to be used on the Abbott m2000sp or with the manual sample preparation method for sample processing and the Abbott m2000rt for amplification and detection.
- Do not use kits or reagents beyond expiration date.
- Control kit lots and amplification reagent kit lots can be used interchangeably. However, components contained within a kit are intended to be used together. For example, do not use the negative control from control kit lot X with the positive control from control kit lot Y.
- Amplification reagent components (enzyme, oligonucleotide reagent and activation reagent), Controls, and mSample Preparation System reagents are for single-use only and should be discarded after use. Use new reagent vessels and new reaction vessels, for every new Abbott RealTime HIV-1 Qualitative assay run. At the end of each run, discard all these remaining reagents as stated in the Abbott m2000sp Operations Manual and the POST PROCESSING PROCEDURES section of this package insert.
- The amplification reagent kit and control kit can be thawed and refrozen up to 3 times before use.
- The Abbott RealTime HIV-1 Qualitative Controls must be processed with the specimens to be tested. The use of the Abbott RealTime HIV-1 Qualitative Controls is integral to the performance of the Abbott RealTime HIV-1 Qualitative assay. Refer to the QUALITY CONTROL PROCEDURES section in the package insert for details.
- Do not use ethanol that contains denaturants.
- Replace any empty or partially used 200 μL and 1000 μL disposable tips on the Abbott m2000sp with full trays before every run. Refer to the Abbott m2000sp Operations Manual, Operating Instructions section.
- Monitoring procedures for the presence of amplification product can be found in the QUALITY CONTROL PROCEDURES section in the package insert.
- To reduce the risk of nucleic acid contamination, clean and disinfect spills of specimens, reagents and controls by using a detergent solution followed by a tuberculocidal disinfectant such as 1.0% (v/v) sodium hypochlorite or other suitable disinfectant.

ASSAY PROTOCOL I: PLASMA SPECIMENS USING ABBOTT m2000sp AUTOMATED SAMPLE PREPARATION SYSTEM
For a detailed description of how to operate the Abbott m2000sp and m2000rt instruments, refer to the Abbott m2000sp and m2000rt Operations Manuals, Operating Instructions sections. Laboratory personnel must be trained to operate the Abbott m2000sp and m2000rt instruments. The operator must have a thorough knowledge of the applications run on the instruments and must follow good laboratory practices.

Refer to the WARNINGS AND PRECAUTIONS section of the package insert before preparing samples.

1. Thaw assay controls and IC at 15 to 30°C or at 2 to 8°C; see QUALITY CONTROL PROCEDURES section of the package insert.

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

3. Open the mSample Preparation System reagent pack(s). 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. Add USP Grade 190 to 200 Proof Ethanol (95 to 100% Ethanol) to the mLysisDNA, mWash 1DNA, and mWash 2DNA bottles as indicated below. Do not use ethanol that contains denaturants.

4. Vortex each IC vial 3 times for 2 to 3 seconds before use. Use a calibrated PRECISION PIPETTE DEDICATED FOR INTERNAL CONTROL USE ONLY to add 750 μL of IC to each bottle of mLysisDNA Buffer.

5. Gently invert all the reagent bottles except mMicroparticlessDNA to 10 times to ensure a homogeneous solution and pour the contents into the appropriate reagent vessels as indicated in the table below. Ensure bubbles or foam are not generated in the reagent vessels; if present, remove with a sterile pipette tip, using a new tip for each reagent vessel. For each bottle of mLysisDNA Buffer being used, measure and gently transfer 95 mL of the mixture of mLysisDNA Buffer, Ethanol and IC into the appropriate reagent vessel(s) as indicated in the table.

Sample extraction reagents are distributed as follows:

<table>
<thead>
<tr>
<th>Samples</th>
<th>1st Vessel</th>
<th>2nd Vessel</th>
<th>3rd Vessel</th>
<th>4th Vessel</th>
<th>5th Vessel</th>
<th>6th Vessel</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 24</td>
<td>1 mLysisDNA NA</td>
<td>1 mMicroparticlessDNA</td>
<td>1 mLysisDNA</td>
<td>1 mLysisDNA</td>
<td>1 mLysisDNA</td>
<td>1 mLysisDNA</td>
</tr>
<tr>
<td>25 to 48</td>
<td>1 mLysisDNA NA</td>
<td>1 mMicroparticlessDNA</td>
<td>1 mLysisDNA</td>
<td>1 mLysisDNA</td>
<td>1 mLysisDNA</td>
<td>1 mLysisDNA</td>
</tr>
<tr>
<td>49 to 72</td>
<td>1 mLysisDNA</td>
<td>1 mLysisDNA</td>
<td>2 mLysisDNA</td>
<td>2 mLysisDNA</td>
<td>2 mLysisDNA</td>
<td>2 mLysisDNA</td>
</tr>
<tr>
<td>73 to 96</td>
<td>2 mLysisDNA</td>
<td>2 mLysisDNA</td>
<td>2 mLysisDNA</td>
<td>2 mLysisDNA</td>
<td>2 mLysisDNA</td>
<td>2 mLysisDNA</td>
</tr>
</tbody>
</table>

NOTE: Immediately prior to initiation of the sample extraction protocol, vigorously mix or vortex the mMicroparticlessDNA until they are fully resuspended and pour the mMicroparticlessDNA into the appropriate 200 mL reagent vessel as indicated in the table.
6. A maximum of 96 samples can be processed per run. A negative control and a positive control are included in each run, therefore allowing a maximum of 94 specimens to be processed per run. Prepare the plasma specimens for processing by following these steps:
   • 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.
   • Vortex each specimen 3 times for 2 to 3 seconds.
   • Centrifuge specimens at 2000g for 5 minutes before loading on the Abbott m2000sp worktable. Aliquot each specimen into a 5 mL Reaction Vessel. Ensure that each newly aliquotted specimen has a minimal volume of 0.3 mL. Avoid touching the inside of the cap when opening tubes. Take care not to disturb contents of the tube while removing the tube from the centrifuge and that the bottom of the tube is not touched by the pipette tip.
   7. Place the negative control, positive control, and the patient specimens into the Abbott m2000sp sample rack.
   NOTE: Use only 13 mm sample racks. Load specimens and controls into the 13 mm sample racks in consecutive positions.
   • Insert specimen and control tubes (uncapped) into sample racks carefully to avoid splashing. If used, bar codes on tube labels must face right for scanning. Ensure that each tube is placed securely in the sample rack so that the bottom of the tube reaches the inside bottom of the rack.
   • Load filled sample racks onto the Abbott m2000sp in consecutive sample rack positions, with the first rack farthest to the right on the worktable, and any additional rack progressively to the left of the first rack.
   8. Place the 5 mL Reaction Vessels into the Abbott m2000sp 1 mL subsystem carrier.


10. From the Protocol screen, select the appropriate application file. Initiate the sample extraction protocol as described in the Abbott m2000sp Operations Manual, Operating Instruction section.
   NOTE: The Abbott m2000sp Master Mix Addition protocol (step 12) must be initiated within 1 hour after completion of sample preparation.
   NOTE: Change gloves before handling the amplification reagents.
   NOTE: The plate-fill setup will automatically be enabled for batch sizes of 49 or greater. In such case, the reagent vessel for mElutionDNA Buffer (Reagent Carrier 2, location 6) should remain in place.

11. Load the amplification reagents and the master mix tube 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 are at the bottom of the vials by tapping the vials in an upright position on the bench.
   • Remove and discard the amplification vial caps.

12. From the Protocol screen, select the appropriate deep well plate from the Run Master Mix Addition screen that matches the corresponding sample preparation extraction. Initiate the Abbott m2000sp Master Mix Addition protocol. Follow the instructions as described in the Abbott m2000sp Operations Manual, Operating Instructions section.
   NOTE: The Abbott m2000rt protocol (step 16) must be started within 50 minutes of the initiation of the Master Mix Addition protocol (step 12).

13. 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.

14. 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.

15. Place the sealed Abbott 96-well Optical Reaction Plate into the Abbott Splash-Free Support Base for transfer to the Abbott m2000rt instrument.

16. Place the Abbott 96-Well Optical Reaction Plate in the Abbott m2000rt instrument and initiate the Abbott RealTime HIV-1 Qualitative assay protocol, as described in the Abbott m2000rt Operations Manual, Operating Instructions section. At the completion of the run, assay results are reported on the Abbott m2000rt. Refer to the RESULTS section of the package insert for further details.

ASSAY PROTOCOL II: DBS SPECIMENS USING ABBOTT m2000sp AUTOMATED SAMPLE PREPARATION SYSTEM

For a detailed description of how to operate the Abbott m2000sp and m2000rt instruments, refer to the Abbott m2000sp and m2000rt Operations Manuals, Operating Instructions sections. Laboratory personnel must be trained to operate the Abbott m2000sp and m2000rt instruments. The operator must have a thorough knowledge of the applications run on the instruments and must follow good laboratory practices.

Refer to the WARNINGS AND PRECAUTIONS section of the package insert before preparing samples.

1. A maximum of 96 samples can be processed per run. A negative control and a positive control are included in each run, therefore allowing a maximum of 94 DBS specimens to be processed per run. Process the DBS specimens by following these steps:
   • Prepare tubes with 1.7 mL Abbott mLysisDNA Buffer (from the Bulk mLysisDNA Buffer).
   • Cut out 2 entire DBS for each specimen from a Whatman 903 filter paper card (or equivalent). Each DBS should be approximately one-half-inch (12 millimeters) in diameter.
   • Place DBS in the tube containing the Abbott mLysisDNA Buffer. Ensure that the DBS are fully submerged in the mLysisDNA Buffer.
   • Incubate at room temperature for 20 minutes with intermittent gentle mixing.
   • Carefully pipette all the liquid to a 5 mL Reaction Vessel. Avoid transfer of bubbles. DO NOT transfer the filter paper.
   NOTE: The above steps do not apply to controls. Controls should not be spotted on filter paper cards and should be processed directly as liquid samples.

NOTE: Avoid direct contact of the cutting surface with DBS specimens. Clean the instrument used to cut DBS between specimens if necessary according to good laboratory practices.

2. Thaw assay controls and IC at 15 to 30°C or at 2 to 8°C; see QUALITY CONTROL PROCEDURES section of the package insert.
   • Once thawed, assay controls and IC can be stored at 2 to 8°C for up to 24 hours before use.
   • Vortex each assay 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. Ensure bubbles or foam are not generated; if present, remove with a sterile pipette tip, using a new tip for each vial.

3. Thaw amplification reagents at 15 to 30°C or at 2 to 8°C.
   • Once thawed, the amplification reagents can be stored at 2 to 8°C for up to 24 hours until required for the amplification master mix procedure.
   • For up to 24 reactions use: 1 tube of positive control, 1 tube of negative control, 1 amplification reagent pack, 1 vial of IC, and 1 set of mSample Preparation SystemDNA reagents.
   • For 25 to 48 reactions use: 1 tube of positive control, 1 tube of negative control, 2 amplification reagent packs, 2 vials of IC, 2 bottles of mLysisDNA Buffer, and 1 bottle mWash 1DNA Buffer, mWash 2DNA Buffer, mMicroparticleDNA and mLysisDNA Buffer.
   • For 49 to 72 reactions use: 1 tube of positive control, 1 tube of negative control, 3 amplification reagent packs, 3 vials of IC, 3 bottles of mLysisDNA Buffer, 2 bottles of mWash 1DNA Buffer and mWash 2DNA Buffer, and 1 bottle of mMicroparticleDNA and mLysisDNA Buffer.
   • For 73 to 96 reactions use: 1 tube of positive control, 1 tube of negative control, 4 amplification reagent packs, 4 vials of IC, 4 bottles of mLysisDNA Buffer, 2 bottles of mWash 1DNA Buffer and mWash 2DNA Buffer, and 1 bottle of mMicroparticleDNA and mLysisDNA Buffer.

4. Open the mSample Preparation SystemDNA reagent pack(s). 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. Add USP Grade 190 to 200 Proof Ethanol (95 to 100% Ethanol) to the mLysisDNA, mWash 1DNA, and mWash 2DNA bottles as indicated below. Do not use ethanol that contains denaturants.
• Add 35 mL ethanol to each bottle of mLysisDOMA being used.
• Add 23 mL ethanol to each bottle of mWash1DOMA being used.
• Add 70 mL ethanol to each bottle of mWash2DOMA being used.
5. Vortex each IC vial 3 times for 2 to 3 seconds before use. Use a calibrated PRECISION PIPETTE DEDICATED FOR INTERNAL CONTROL USE ONLY to add 750 μL of IC to each bottle of mLysisDOMA Buffer.
6. Gently invert all the reagent bottles except mMicroparticlesDOMA 5 to 10 times to ensure a homogenous solution and pour the contents into the appropriate reagent vessel(s) as indicated in the table below. Ensure bubbles or foam are not generated in the reagent vessels; if present, remove with a sterile pipette tip, using a new tip for each reagent vessel. For each bottle of mLysisDOMA Buffer being used, measure and gently transfer 95 mL of the mixture of mLysisDOMA Buffer, ethanol and IC into the appropriate reagent vessel(s) as indicated in the table.
Sample extraction reagents are distributed as follows:

<table>
<thead>
<tr>
<th>Samples</th>
<th>1st Vessel</th>
<th>2nd Vessel</th>
<th>3rd Vessel</th>
<th>4th Vessel</th>
<th>5th Vessel</th>
<th>6th Vessel</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 24</td>
<td>1 mLysDOMA</td>
<td>NA</td>
<td>1 mMicrompDOMA</td>
<td>1 mWashDOMA</td>
<td>1 mWash2DOMA</td>
<td>mElutionDOMA</td>
</tr>
<tr>
<td>25 to 48</td>
<td>2 mLysDOMA</td>
<td>NA</td>
<td>1 mMicrompDOMA</td>
<td>1 mWashDOMA</td>
<td>1 mWash2DOMA</td>
<td>mElutionDOMA</td>
</tr>
<tr>
<td>49 to 72</td>
<td>2 mLysDOMA</td>
<td>1 mLysDOMA</td>
<td>1 mMicrompDOMA</td>
<td>2 mWashDOMA</td>
<td>2 mWash2DOMA</td>
<td>mElutionDOMA</td>
</tr>
<tr>
<td>73 to 96</td>
<td>2 mLysDOMA</td>
<td>2 mLysDOMA</td>
<td>1 mMicrompDOMA</td>
<td>2 mWashDOMA</td>
<td>2 mWash2DOMA</td>
<td>mElutionDOMA</td>
</tr>
</tbody>
</table>

NOTE: Immediately prior to initiation of the sample extraction protocol, vigorously mix or vortex the mMicroparticlesDOMA until they are fully resuspended and pour the mMicroparticlesDOMA into the appropriate 200 mL Reagent Vessel as indicated in the table.
7. Place the negative control, positive control, and the patient specimens into the Abbott m2000sp sample rack.
NOTE: Use only 13 mm sample racks. Load specimens and controls into the 13 mm sample racks in consecutive positions.
• Insert specimen and control tubes (uncapped) into sample racks carefully to avoid splashing. If used, bar codes on tube labels must face right for scanning. Ensure that each tube is placed securely in the sample rack so that the bottom of the tube reaches the inside bottom of the rack.
• Load filled sample racks onto the Abbott m2000sp in consecutive sample rack positions, with the first rack farthest to the right on the worktable, and any additional rack progressively to the left of the first rack.
• Place the 5 mL Reaction Vessels into the Abbott m2000sp 1 mL subsystem carrier.
• Load the Abbott 96 Deep-Well Plate on the Abbott m2000sp worktable as described in the Abbott m2000sp Operations Manual, Operating Instructions section.
• From the Protocol screen, select the appropriate application file. Initiate the sample extraction protocol as described in the Abbott m2000sp Operations Manual, Operating Instruction section.
NOTE: The Abbott m2000sp Master Mix Addition protocol (step 12) must be initiated within 1 hour after completion of sample preparation.
NOTE: Change gloves before handling the amplification reagents.
NOTE: The plate-fill setup will automatically be enabled for batch sizes of 49 or greater. In such case, the reagent vessel for mElutionDOMA Buffer (Reagent Carrier 2, location 6) should remain in place.

11. Load the amplification reagents and the master mix tube 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 are at the bottom of the vials by tapping the vials in an upright position on the bench.
• Remove and discard the amplification vial caps.
12. From the Protocol screen, select the appropriate deep well plate from the Run Master Mix Addition screen that matches the corresponding sample preparation extraction. Initiate the Abbott m2000sp Master Mix Addition protocol. Follow the instructions as described in the Abbott m2000sp Operations Manual, Operating Instruction section.
NOTE: The Abbott m2000sp protocol (step 16) must be started within 50 minutes of the initiation of the Master Mix Addition protocol (step 12).
13. Switch on and initialize the Abbott m2000sp instrument in the Amplification Area.
NOTE: The Abbott m2000sp requires 15 minutes to warm-up.
NOTE: Remove gloves before returning to the sample preparation area.
14. 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.
15. Place the sealed Abbott 96-Well Optical Reaction Plate into the Abbott Splash-Free Support Base for transfer to the Abbott m2000sp instrument.
16. Place the Abbott 96-Well Optical Reaction Plate in the Abbott m2000sp instrument and initiate the Abbott RealTime HIV-1 Qualitative assay protocol, as described in the Abbott m2000sp Operations Manual, Operating Instructions section. At the completion of the run, assay results are reported on the Abbott m2000sp. Refer to the RESULTS section of the package insert for further details.
ASSAY PROTOCOL III: PLASMA SPECIMENS USING MANUAL SAMPLE PREPARATION METHOD
For a detailed description of how to operate the Abbott m2000sp instrument, refer to the Abbott m2000sp Operations Manual, Operating Instructions section. Laboratory personnel must be trained to operate the Abbott m2000sp instrument. The operator must have a thorough knowledge of the application run on the instrument and must follow good laboratory practices. Refer to the WARNINGS AND PRECAUTIONS section of the package insert before preparing samples.
Reagent Preparation Area
Set Up
1. Thaw amplification reagents at 15 to 30°C or at 2 to 8°C.
• Once thawed, the amplification reagents can be stored at 2 to 8°C for up to 24 hours until required for the amplification master mix procedure.
• For up to 24 reactions use: 1 tube of positive control, 1 tube of negative control, 1 amplification reagent pack, 1 vial of IC, and 1 set of mSample Preparation SystemDOMA reagents.
• For 25 to 48 reactions use: 1 tube of positive control, 1 tube of negative control, 2 amplification reagent packs, 2 vials of IC, 2 bottles of mLysisDOMA Buffer, and 1 bottle mL Wash1DOMA Buffer, mL Wash2DOMA Buffer, mMicroparticleDOMA and mElutionDOMA Buffer.
• For 49 to 72 reactions use: 1 tube of positive control, 1 tube of negative control, 3 amplification reagent packs, 3 vials of IC, 3 bottles of mLysisDOMA Buffer, 2 bottles of mL Wash1DOMA Buffer and mL Wash2DOMA Buffer, and 1 bottle of mMicroparticleDOMA and mElutionDOMA Buffer.
• For 73 to 96 reactions use: 1 tube of positive control, 1 tube of negative control, 4 amplification reagent packs, 4 vials of IC, 4 bottles of mLysisDOMA Buffer, 2 bottles of mL Wash1DOMA Buffer and mL Wash2DOMA Buffer, and 1 bottle of mMicroparticleDOMA and mElutionDOMA Buffer.
NOTE: Refer to step 3 for thawing assay controls and IC.
Sample Preparation Area
Set Up
2. A maximum of 96 samples can be processed per run. A negative control and a positive control are included in each run, therefore allowing a maximum of 96 plasma specimens to be processed per run. Prepare the plasma specimens for processing by following these steps:
• 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.
• Vortex each specimen 3 times for 2 to 3 seconds.
• Centrifuge specimens at 2000g for 5 minutes before adding to the reaction vessels. Avoid touching the inside of the cap when opening tubes. Take care not to disturb contents of the tube while removing the tube from the centrifuge and that the bottom of the tube is not touched by the pipette tip.
3. Thaw assay controls and IC at 15 to 30°C or at 2 to 8°C; see QUALITY CONTROL PROCEDURES section of the package insert.
• Once thawed, assay controls and IC can be stored at 2 to 8°C for up to 24 hours before use.
• Vortex each assay control 3 times for 2 to 3 seconds before use. After vortexing, ensure that the contents of each vial are at the bottom by tapping the vials on the bench to bring liquid to the bottom of the vial. Ensure bubbles or foam are not generated; if present, remove with a sterile pipette tip, using a new tip for each vial.

4. Open the mSample Preparation System mDNA reagent pack(s). 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. Add USP Grade 190 to 200 Proof Ethanol (95 to 100% Ethanol) to the mLysisDNA, mWash 1DNA, and mWash 2DNA bottles as indicated below. Do not use ethanol that contains denaturants.

• Add 35 mL ethanol to each bottle of mLysisDNA being used.
• Add 23 mL ethanol to each bottle of mWash 1DNA being used.
• Add 70 mL ethanol to each bottle of mWash 2DNA being used.

5. Vortex each vial 3 times for 2 to 3 seconds before use. Use a calibrated PRECISION PIPETTE DEDICATED FOR INTERNAL CONTROL USE ONLY to add 750 μL of IC to each bottle of mLysisDNA Buffer.

6. Gently invert all the reagent bottles except mMicroparticlesDNA 5 to 10 times to ensure a homogenous solution prior to use.

7. Turn on the temperature controlled dry heating blocks.

• Set the 5 mL Reaction Vessel block to 50°C.
• Set the 1.5 mL tubes block to 75°C.

NOTE: Check the temperature of the heating blocks.

Do not proceed until the heating blocks are at the correct temperature.

WARNING: To avoid personal injury, follow manufacturer’s instructions for dry heating block. To avoid burns, turn off the power and allow the heating blocks to cool to 35°C or below before handling.

8. Label all necessary tubes.

• One 5 mL Reaction Vessel per sample for the mLysisDNA and mWash 1DNA steps.
• One 1.5 mL screw top microfuge tube per sample for the mWash 2DNA and mElutionDNA Buffer steps.
• One 1.5 mL screw top microfuge tube per sample or a 96-well polypropylene plate for the eluate.

mLysisDNA

9. Resuspend mLymphoparticlesDNA by vortexing or vigorously shaking until particles are in suspension and settled particles are no longer seen on the bottom of the bottle.

10. Place the 5 mL Reaction Vessels in a non-magnetic rack at room temperature. After the particles are resuspended, use a precision pipettor and a sterile 200 μL aerosol barrier pipette tip to add 40 μL of mMicroparticlesDNA to each reaction vessel. A calibrated repeat pipettor may be used.

11. Using a precision pipettor and a sterile 1000 μL aerosol barrier pipette tip, add 2.4 mL of mLysDNA to each reaction vessel in the non-magnetic rack.

12. Using a precision pipettor and a fresh, sterile 200 μL or 1000 μL aerosol barrier pipette tip for each sample, add 0.2 mL of the controls and specimens to the reaction vessels. Mix the sample-mLysDNA mixture by aspiration and dispense until a uniform suspension is obtained.

NOTE: Aspirate and dispense liquid slowly to avoid foaming.

13. Place the reaction vessels in the 50°C heating block, start the timer and incubate for 20 minutes.

14. Remove the reaction vessels from the 50°C heating block. Using a fresh, sterile 1000 μL aerosol barrier pipette tip for each sample, mix the sample-mLysDNA mixture by aspiration and dispense until a uniform suspension is obtained.

15. Place the reaction vessels back in the 50°C heating block, start the timer and incubate for 10 minutes.

16. After the incubation is complete, place the reaction vessels in a magnetic capture stand for 2 minutes to allow the particles to be captured on the side of the reaction vessels.

17. With the reaction vessels in the magnetic capture stand, use a fresh, sterile 1000 μL aerosol barrier pipette tip or disposable transfer pipette for each sample to carefully remove the lysate from each reaction vessel and discard the fluid into a liquid waste container. Remove the fluid as completely as possible. DO NOT disturb or aspirate the captured magnetic particles.

18. Remove the reaction vessels from the magnetic rack and transfer to a non-magnetic rack.

mWash 1DNA

19. Using a precision pipettor and a fresh, sterile 1000 μL aerosol barrier pipette tip for each sample, add 700 μL of mWash 1DNA to the samples and resuspend the magnetic particles in the wash fluid by gentle aspiration and dispense with the pipette tip. Wash the particles from the side of the reaction vessel, if necessary. 

NOTE: When adding wash buffers, dispense liquid slowly to avoid splashing.

20. Place the reaction vessels in the 50°C heating block, start the timer and incubate for 5 minutes.

21. Remove the reaction vessels from the 50°C heating block. Using a fresh, sterile 1000 μL aerosol barrier pipette tip for each sample, mix the sample-mWash 1DNA mixture by aspiration and dispense until a uniform suspension is obtained.

22. Place the reaction vessels in a magnetic capture stand for 1 minute to allow the particles to be captured on the side of the tubes.

23. With the reaction vessels in the magnetic capture stand, use a fresh, sterile 1000 μL aerosol barrier pipette tip or disposable transfer pipette for each sample to carefully remove the mWash 1DNA from each reaction vessel and discard the fluid into a liquid waste container. Remove the fluid as completely as possible. DO NOT disturb or aspirate the captured magnetic particles.

24. Remove the reaction vessels from the magnetic rack and transfer to a non-magnetic rack.

mWash 2DNA First Wash

25. Using a precision pipettor and a fresh, sterile 1000 μL aerosol barrier pipette tip for each sample, add 800 μL of mWash 2DNA to the samples and resuspend the magnetic particles in the wash fluid by gentle aspiration and dispense with the pipette tip. Wash the particles from the side of the reaction vessel, if necessary.

NOTE: When adding wash buffers, dispense liquid slowly to avoid splashing.

26. Transfer the wash fluid and particles to a labeled 1.5 mL screw top microfuge tube.

27. Place the tubes in a magnetic capture stand for 1 minute to allow the particles to be captured on the side of the tubes.

28. With the tubes in the magnetic capture stand, use a fresh, sterile 1000 μL aerosol barrier pipette tip for each sample to carefully remove the mWash 2DNA from each tube and discard fluid into a liquid waste container. Remove the fluid as completely as possible.

DO NOT disturb or aspirate the captured magnetic particles.

29. Remove the tubes from the magnetic rack and transfer to a non-magnetic rack.

mWash 2DNA Second Wash

30. Using a precision pipettor and a fresh, sterile 1000 μL aerosol barrier pipette tip for each sample, add 800 μL of mWash 2DNA to the samples and resuspend the magnetic particles in the wash fluid by gentle aspiration and dispense with the pipette tip. Wash the particles from the side of the tube, if necessary.

NOTE: When adding wash buffers, dispense liquid slowly to avoid splashing.

31. Place the tubes in a magnetic capture stand for 1 minute to allow the particles to be captured on the side of the tubes.

32. With the tubes in the magnetic capture stand, use a fresh, sterile 1000 μL aerosol barrier pipette tip for each sample to carefully remove the mWash 2DNA from each tube and discard fluid into a liquid waste container. Remove the fluid as completely as possible.

DO NOT disturb or aspirate the captured magnetic particles.

33. Remove the tubes from the magnetic rack and transfer to the 75°C heating block and incubate for 10 minutes to allow for the evaporation of the ethanol.

mElutionDNA Buffer

34. Remove the tubes from the 75°C heating block. Using a precision pipettor and a fresh, sterile 200 μL aerosol barrier pipette tip for each sample, add 88 μL of mElutionDNA Buffer to the samples and resuspend the magnetic particles in the fluid by aspiration and dispense with the pipette tip. Wash the particles from the side of the tube, if necessary.

35. Place the tubes in the 75°C heating block, start the timer and incubate for 5 minutes.

36. Remove the tubes from the 75°C heating block. Using a fresh, sterile 200 μL aerosol barrier pipette tip for each sample, mix the sample-mElutionDNA Buffer mixture by aspiration and dispense until a uniform suspension is obtained.
37. Place the tubes back in the 75°C heating block, start the timer and incubate for 5 minutes.
38. Remove the tubes from the 75°C heating block and place in a magnetic capture stand for 1 minute to allow the particles to be captured on the side of the tubes.
39. With the tubes in the magnetic capture stand, use a fresh, sterile 200 μL aerosol barrier pipette tip for each sample to carefully remove the eluted sample from the tubes. DO NOT disturb or aspirate the captured microparticles. The eluted sample(s) can be placed into a fresh, labeled 1.5 mL screw top microfuge tube or a 96-well polypropylene plate.

NOTE: The assembly of the amplification master mix and sample eluates into the Abbott 96-Well Optical Reaction Plate (step 43) must be initiated within 1 hour after completion of sample preparation.

Amplification Area

40. Switch on and initialize the Abbott m2000rt instrument.

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

41. 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.

NOTE: Remove gloves before returning to the Reagent Preparation Area.

Reagent Preparation Area

42. Place a new Abbott 96-Well Optical Reaction Plate in a StratACooler 96 Benchtop Cooler or Eppendorf PCR Cooler stored as indicated in the StratACooler 96 Benchtop Cooler instruction manual or Eppendorf PCR Cooler Instructions for Use, respectively. DO NOT touch the surface or bottom of the plate.

IF APPLICABLE: For batch sizes between 49 and 95 (samples plus controls), prior to preparation of the amplification master mix, fill the wells that will be empty with distilled or deionized water using the following instructions:
- Using a calibrated pipette, add 100 μL of distilled or deionized water to empty wells based on batch size:

<table>
<thead>
<tr>
<th>Batch/Sample Size</th>
<th>Empty Well(s) to fill</th>
</tr>
</thead>
<tbody>
<tr>
<td>49 to 56</td>
<td>Full columns 8 through 12; partial column 7</td>
</tr>
<tr>
<td>57 to 64</td>
<td>Full columns 9 through 12; partial column 8</td>
</tr>
<tr>
<td>65 to 72</td>
<td>Full columns 10 through 12; partial column 9</td>
</tr>
<tr>
<td>73 to 80</td>
<td>Full columns 11 and 12; partial column 10</td>
</tr>
<tr>
<td>81 to 88</td>
<td>Full columns 12; partial column 11</td>
</tr>
<tr>
<td>89 to 95</td>
<td>Partial column 12</td>
</tr>
</tbody>
</table>

NOTE: The order of adding PCR reactions (master mix and sample eluate) to the Abbott 96-Well Optical Reaction Plate starts with column 1 (from top to bottom) and moves to each consecutive column from left to right. Be sure to read the column numbers on the top of the Abbott 96-Well Optical Reaction Plate to ensure that correct well(s) are filled with water.

43. Prepare the amplification master mix.

NOTE: All reagent preparation must take place in the dedicated Reagent Preparation Area. Refer to the Contamination Precautions section of the package insert before preparing reagents. Change gloves before handling the amplification reagents.
- Each amplification reagent pack supports up to 24 reactions.
- Prior to opening the amplification reagents, ensure that the contents of the amplification reagent pack are at the bottom by tapping the amplification reagent pack in an upright position on the bench to bring the liquid to the bottom of the vials.
- Identify the amplification reagents as follows:
  - Activation Reagent (Reagent 1): clear bottle, teal cap
  - Oligonucleotide Reagent (Reagent 2): black bottle, white cap
  - Thermostable rTth DNA Polymerase Enzyme (Reagent 3): clear bottle, white cap
  - Remove and discard caps.
- Prepare the master mix by using a PRECISION 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) to the Thermostable rTth DNA Polymerase Enzyme bottle (Reagent 3). Mix the Enzyme vial containing the reaction mixture (master mix) by gently pipetting up and down 5 to 7 times. Avoid creating foam.
- If performing 25 to 48 reactions, prepare the amplification master mix from 2 amplification reagent packs.
- If performing 49 to 72 reactions, prepare the amplification master mix from 3 amplification reagent packs.
- If performing 73 to 96 reactions, prepare the amplification master mix from 4 amplification reagent packs.

NOTE: The Abbott m2000rt protocol (step 50) must be started within 50 minutes of the addition of the activation reagent into the first enzyme bottle (step 43).

44. Pipette the contents of the master mix from the enzyme bottle(s) into a single-use RNase/DNase-free tube or container. Mix by gently pipetting up and down 5 to 7 times. Avoid creating foam.

45. Using a PRECISION PIPETTE DEDICATED FOR REAGENT USE ONLY, dispense 50 μL aliquots of the amplification master mix into each well of the Abbott 96-Well Optical Reaction Plate that will be used to run the samples and controls. A calibrated repeat pipettor may be used.
- Add the master mix in an order starting with column 1 (from top to bottom), and moving to each consecutive column from left to right.
- Visually verify that 50 μL has been dispensed into each well.
- Transfer the Abbott 96-Well Optical Reaction Plate on the StrataCooler 96 Benchtop Cooler or Eppendorf PCR Cooler to the Sample Preparation Area.

Sample Preparation Area

46. Using a precision pipettor and a fresh, sterile 200 μL aerosol barrier pipette tip for each sample, transfer 50 μL of each eluted sample to the Abbott 96-Well Optical Reaction Plate on the StratACooler 96 Benchtop Cooler or Eppendorf PCR Cooler. During the transfer of each sample, mix the reaction by pipetting up and down 3 to 5 times. Visually verify that a total of 100 μL has been dispensed into each well.

47. Seal the Abbott 96-Well Optical Reaction Plate according to the instructions in the Abbott m2000rt Operations Manual, Operating Instructions section.

48. Remove the Abbott 96-Well Optical Reaction Plate from the StratACooler 96 Benchtop Cooler or Eppendorf 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 5000g for 5 minutes.

49. Transfer the Abbott 96-Well Optical Reaction Plate in the Abbott Splash-Free Support Base to the Amplification Area.

NOTE: Do not transfer the StratACooler 96 Benchtop Cooler or Eppendorf PCR Cooler to the Amplification Area.

Amplification Area

50. Place the Abbott 96-Well Optical Reaction Plate in the Abbott m2000rt instrument, select the test order created (step 41), and initiate the Abbott RealTime HIV-1 Qualitative assay protocol, as described in the Abbott m2000rt Operations Manual, Operating Instructions section. At the completion of the run, assay results are reported on the Abbott m2000rt. Refer to the RESULTS section of the package insert for further details.

ASSAY PROTOCOL IV: DBS SPECIMENS USING MANUAL SAMPLE PREPARATION METHOD

For a detailed description of how to operate the Abbott m2000rt instrument, refer to the Abbott m2000rt Operations Manual, Operating Instructions section. Laboratory personnel must be trained to operate the Abbott m2000rt instrument. The operator must have a thorough knowledge of the application run on the instrument and must follow good laboratory practices.

Refer to the WARNINGS AND PRECAUTIONS section of the package insert before preparing samples.

Reagent Preparation Area

Set Up

1. Thaw amplification reagents at 15 to 30°C or at 2 to 8°C.
   - Once thawed, the amplification reagents can be stored at 2 to 8°C for up to 24 hours until required for the amplification master mix procedure.
   - For up to 24 reactions use: 1 tube of positive control, 1 tube of negative control, 1 amplification reagent pack, 1 vial of IC, and 1 set of mSample Preparation System DNA reagents.
   - For 25 to 48 reactions use: 1 tube of positive control, 1 tube of negative control, 2 amplification reagent packs, 2 vials of IC, 2 bottles of mLysoDNA Buffer, 1 bottle mWash DNA Buffer, mWash 2DNA Buffer, mMicroparticleDNA and mEulsionDNA Buffer.
For 49 to 72 reactions use: 1 tube of positive control, 1 tube of negative control, 3 amplification reagent packs, 3 vials of IC, 4 bottles of mLysDNA Buffer, 2 bottles of mLysDNA Buffer, and 1 bottle of mLysDNA Buffer and mLWash DNA Buffer.

For 73 to 96 reactions use: 1 tube of positive control, 1 tube of negative control, 4 amplification reagent packs, 4 vials of IC, 4 bottles of mLysDNA Buffer, 2 bottles of mLWash DNA Buffer, and 1 bottle of mLysDNA Buffer and mLWash DNA Buffer.

NOTE: Refer to step 3 for thawing assay controls and IC.

Sample Preparation Area

Set Up

1. A maximum of 96 samples can be processed per run. A negative control and a positive control are included in each run, therefore allowing a maximum of 94 DBS specimens to be processed per run.

Prepare the DBS specimens for processing by following these steps:

- Prepare tubes with 1.7 mL Abbott mLysDNA Buffer (from the Bulk mLysDNA Buffer).
- Cut out 2 entire DBS for each specimen from a Whatman 903 filter paper card (or equivalent). Each DBS should be approximately one-half-inch (12 millimeters) in diameter.
- Place DBS in the tube containing the Abbott mLysDNA Buffer. Ensure that DBS are fully submerged in the mLysDNA Buffer.
- Incubate at room temperature for 20 minutes with intermittent gentle mixing.

NOTE: The above steps do not apply to controls. Controls should not be spotted on filter paper cards and should be processed directly as liquid samples.

NOTE: Avoid direct contact of the cutting surface with DBS specimens. Clean the instrument used to cut DBS between specimens if necessary according to good laboratory practices.

3. Thaw assay controls and IC at 15 to 30°C or at 2 to 8°C; see QUALITY CONTROL PROCEDURES section of the package insert.

- Once thawed, assay controls and IC can be stored at 2 to 8°C for up to 24 hours before use.
- Vortex each assay control 3 times for 2 to 3 seconds before use. After vortexing ensure that the contents of each vial are at the bottom by tapping the vials on the bench to bring liquid to the bottom of the vial. Ensure bubbles or foam are not generated; if present, remove with a sterile pipette tip, using a new tip for each vial.

4. Open the mSample Preparation System (G) reagent packs(s). If crystals are observed in any of the reagent bottles upon opening, do not use the reagents until the crystals have dissolved. Add USP Grade 190 to 200 Proof Ethanol (95 to 100% Ethanol) to mLysDNA, mLWash DNA, and mLWash 2 DNA bottles as indicated below. Do not use ethanol that contains denaturants.

- Add 35 mL ethanol to each bottle of mLysDNA being used.
- Add 23 mL ethanol to each bottle of mLWash 1 DNA being used.
- Add 70 mL ethanol to each bottle of mLWash 2 DNA being used.

- Vortex each IC vial 3 times for 2 to 3 seconds before use. Use a calibrated PRECISION PIPETTE DEDICATED FOR INTERNAL CONTROL USE ONLY to add 750 μL of IC to each bottle of mLysDNA Buffer.
- Gently invert all the reagent bottles except mMicroparticlesDNA 5 to 10 times to ensure a homogenous solution prior to use.

7. Turn on the temperature controlled dry heating blocks.

- Set the 5 mL Reaction Vessel block to 50°C.
- Set the 1.5 mL tubes block to 72°C.

NOTE: Check the temperature of the heating blocks. Do not proceed until the heating blocks are at the correct temperature.

WARNING: To avoid personal injury, follow manufacturer’s instructions for dry heating block. To avoid burns, turn off the power and allow the heating blocks to cool to 35°C or below before handling.

8. Label all necessary tubes.

- One 5 mL Reaction Vessel per sample for the mLysDNA and mLWash 1 DNA steps.
- One 1.5 mL screw top microunirage tube per sample for the mLWash 2 DNA and mElution DNA Buffer steps.
- One 1.5 mL screw top microunirage tube per sample or a 96 well polypropylene plate for the eluate.

9. Resuspend mLMicroparticlesDNA by vortexing or vigorously shaking until particles are in suspension and settled particles are no longer seen on the bottom of the bottle.

10. Place the 5 mL Reaction Vessels in a non-magnetic rack at room temperature. After the particles are resuspended, use a precision pipettor and a sterile 200 μL aerosol barrier pipette tip to add 40 μL of mLMicroparticlesDNA to each reaction vessel. A calibrated repeat pipettor may be used.

11. Using a precision pipettor and a sterile 1000 μL aerosol barrier pipette tip, add 24.5 mL of mLWashDNA to each reaction vessel in the non-magnetic rack.

12. Using a precision pipettor and a fresh, sterile 1000 μL aerosol barrier pipette tip for each sample, add 1.0 mL of the controls and specimens to the reaction vessels. DO NOT transfer the filter paper. Mix the sample-mLysDNA mixture by aspiration and dispense until a uniform suspension is obtained.

NOTE: Aspirate and dispense liquid slowly to avoid foaming.

13. Place the reaction vessels in the 50°C heating block, start the timer and incubate for 20 minutes.

14. Remove the reaction vessels from the 50°C heating block. Using a fresh, sterile 1000 μL aerosol barrier pipette tip for each sample, mix the sample-mLysDNA mixture by aspiration and dispense until a uniform suspension is obtained.

15. Place the reaction vessels back in the 50°C heating block, start the timer and incubate for 10 minutes.

16. After the incubation is complete, place the reaction vessels in a magnetic capture stand for 2 minutes to allow the particles to be captured on the side of the reaction vessels.

17. With the reaction vessels in the magnetic capture stand, use a fresh, sterile 1000 μL aerosol barrier pipette tip or disposable transfer pipette for each sample to carefully remove the lysate from each reaction vessel and discard the fluid into a liquid waste container. Remove the fluid as completely as possible. DO NOT disturb or aspirate the captured magnetic particles.

18. Remove the reaction vessels from the magnetic rack and transfer to a non-magnetic rack.

mWash 1 DNA

19. Using a precision pipettor and a fresh, sterile 1000 μL aerosol barrier pipette tip for each sample, add 700 μL of mLWash 1 DNA to the samples and resuspend the magnetic particles in the wash fluid by gentle aspiration and dispense with the pipette tip. Wash the particles from the side of the reaction vessel, if necessary.

NOTE: When adding wash buffers, dispense liquid slowly to avoid splashing.

20. Place the reaction vessels in the 50°C heating block, start the timer and incubate for 5 minutes.

21. Remove the reaction vessels from the 50°C heating block. Using a fresh, sterile 1000 μL aerosol barrier pipette tip for each sample, mix the sample-mLWash 1 DNA mixture by aspiration and dispense until a uniform suspension is obtained.

22. Place the reaction vessels in a magnetic capture stand for 1 minute to allow the particles to be captured on the side of the tubes.

23. With the reaction vessels in the magnetic capture stand, use a fresh, sterile 1000 μL aerosol barrier pipette tip or disposable transfer pipette for each sample to carefully remove the mLWash 1 DNA from each reaction vessel and discard the fluid into a liquid waste container. Remove the fluid as completely as possible. DO NOT disturb or aspirate the captured magnetic particles.

24. Remove the reaction vessels from the magnetic rack and transfer to a non-magnetic rack.

mWash 2 DNA First Wash

25. Using a precision pipettor and a fresh, sterile 1000 μL aerosol barrier pipette tip for each sample, add 800 μL of mLWash 2 DNA to the samples and resuspend the magnetic particles in the wash fluid by gentle aspiration and dispense with the pipette tip. Wash the particles from the side of the reaction vessel, if necessary.

NOTE: When adding wash buffers, dispense liquid slowly to avoid splashing.

26. Transfer the wash fluid and particles to a labeled 1.5 mL screw top microfuge tube.

27. Place the tubes in a magnetic capture stand for 1 minute to allow the particles to be captured on the side of the tubes.
28. With the tubes in the magnetic capture stand, use a fresh, sterile 1000 μL aerosol barrier pipette tip for each sample to carefully remove the mWash 2DNA from each tube and discard fluid into a liquid waste container. Remove the fluid as completely as possible. DO NOT disturb or aspirate the captured magnetic particles.

29. Remove the tubes from the magnetic rack and transfer to a non-magnetic rack.

mWash 2DNA Second Wash

30. Using a precision pipettor and a fresh, sterile 1000 μL aerosol barrier pipette tip for each sample, add 800 μL of mWash 2DNA to the samples and resuspend the magnetic particles in the wash fluid by gentle aspiration and dispense with the pipette tip. Wash the particles from the side of the tube, if necessary. NOTE: When adding wash buffers, dispense liquid slowly to avoid splashing.

31. Place the tubes in a magnetic capture stand for 1 minute to allow the particles to be captured on the side of the tubes.

32. With the tubes in the magnetic capture stand, use a fresh, sterile 1000 μL aerosol barrier pipette tip for each sample to carefully remove the mWash 2DNA from each tube and discard fluid into a liquid waste container. Remove the fluid as completely as possible. DO NOT disturb or aspirate the captured magnetic particles.

33. Remove the tubes from the magnetic rack and transfer to the 75°C heating block and incubate for 10 minutes to allow for the evaporation of the ethanol.

mElutionDNA Buffer

34. Remove the tubes from the 75°C heating block. Using a precision pipettor and a fresh, sterile 200 μL aerosol barrier pipette tip for each sample, add 88 μL of mElutionDNA Buffer to the samples and resuspend the magnetic particles in the fluid by aspiration and dispense with the pipette tip. Wash the particles from the side of the tube, if necessary.

35. Place the tubes in the 75°C heating block, start the timer and incubate for 5 minutes.

36. Remove the tubes from the 75°C heating block. Using a fresh, sterile 200 μL aerosol barrier pipette tip for each sample, mix the sample- mElutionDNA Buffer mixture by aspiration and dispense until a uniform suspension is obtained.

37. Place the tubes back in the 75°C heating block, start the timer and incubate for 5 minutes.

38. Remove the tubes from the 75°C heating block and place in a magnetic capture stand for 1 minute to allow the particles to be captured on the side of the tubes.

39. With the tubes in the magnetic capture stand, use a fresh, sterile 200 μL aerosol barrier pipette tip for each sample to carefully remove the eluted sample from the tubes. DO NOT disturb or aspirate the captured microparticles. The eluted sample(s) can be placed into a fresh, labeled 1.5 mL screw top microfuge tube or a non-magnetic rack.

NOTE: The assembly of the amplification master mix and sample eluates into the Abbott 96-Well Optical Reaction Plate (step 43) must be initiated within 1 hour after completion of sample preparation.

Amplification Area

40. Switch on and initialize the Abbott m2000rt instrument. NOTE: The Abbott m2000rt requires 15 minutes to warm-up.

41. 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. NOTE: Remove gloves before returning to the Reagent Preparation Area.

Reagent Preparation Area

42. Place a new Abbott 96-Well Optical Reaction Plate in a StrataCooler 96 Benchtop Cooler or Eppendorf PCR Cooler stored as indicated in the StrataCooler 96 Benchtop Cooler instruction manual or Eppendorf PCR Cooler Instructions for Use, respectively. DO NOT touch the surface or bottom of the plate.

IF APPLICABLE: For batch sizes between 49 and 95 (samples plus controls), prior to preparation of the amplification master mix, fill the wells that will be empty with distilled or deionized water using the following instructions:

• Using a calibrated pipette, add 100 μL of distilled or deionized water to empty wells based on batch size:

<table>
<thead>
<tr>
<th>Batch/Sample Size</th>
<th>Empty Well(s) to fill</th>
</tr>
</thead>
<tbody>
<tr>
<td>49 to 56</td>
<td>Full columns 8 through 12; partial column 7</td>
</tr>
<tr>
<td>57 to 64</td>
<td>Full columns 9 through 12; partial column 8</td>
</tr>
<tr>
<td>65 to 72</td>
<td>Full columns 10 through 12; partial column 9</td>
</tr>
<tr>
<td>73 to 80</td>
<td>Full columns 11 and 12; partial column 10</td>
</tr>
<tr>
<td>81 to 88</td>
<td>Full columns 12; partial column 11</td>
</tr>
<tr>
<td>89 to 95</td>
<td>Partial column 12</td>
</tr>
</tbody>
</table>

NOTE: The order of adding PCR reactions (master mix and sample eluate) to the Abbott 96-Well Optical Reaction Plate starts with column 1 (from top to bottom) and moves to each consecutive column from left to right. Be sure to read the column numbers on the top of the Abbott 96-Well Optical Reaction Plate to ensure that correct well(s) are filled with water.

43. Prepare the amplification master mix.

NOTE: All reagent preparation must take place in the dedicated Reagent Preparation Area. Refer to the Contamination Precautions section of the package insert before preparing reagents. Change gloves before handling the amplification reagents.

• Each amplification reagent pack supports up to 24 reactions.
• Prior to opening the amplification reagents, ensure that the contents of the amplification reagent pack are at the bottom by tapping the amplification reagent pack in an upright position on the bench to bring the liquid to the bottom of the vials.
• Identify the amplification reagents as follows:
  • Activation Reagent (Reagent 1): clear bottle, teal cap
  • Oligonucleotide Reagent (Reagent 2): black bottle, white cap
  • Thermostable rTth DNA Polymerase Enzyme (Reagent 3): clear bottle, white cap
• Remove and discard caps.
• Prepare the master mix by using a PRECISION 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) to the Thermostable rTth DNA Polymerase Enzyme bottle (Reagent 3). Mix the enzyme vial containing the reaction mixture (master mix) by gentlypipetting up and down 5 to 7 times. Avoid creating foam.
• If performing 25 to 48 reactions, prepare the amplification master mix from 2 amplification reagent packs.
• If performing 49 to 72 reactions, prepare the amplification master mix from 3 amplification reagent packs.
• If performing 73 to 96 reactions, prepare the amplification master mix from 4 amplification reagent packs.

NOTE: The Abbott m2000rt protocol (step 50) must be started within 50 minutes of the addition of the activation reagent into the first enzyme bottle (step 43).

44. Pipette the contents of the master mix from the enzyme bottle(s) into a single-use RNase/DNase-free tube or container. Mix by gently pipetting up and down 5 to 7 times. Avoid creating foam.

45. Using a PRECISION PIPETTE DEDICATED FOR REAGENT USE ONLY, dispense 50 μL aliquots of the amplification master mix into each well of the Abbott 96-Well Optical Reaction Plate that will be used to run the samples and controls. A calibrated repeat pipettor may be used.

• Add the master mix in an order starting with column 1 (from top to bottom), and moving to each consecutive column from left to right.
• Visually verify that 50 μL has been dispensed into each well.
• Transfer the Abbott 96-Well Optical Reaction Plate on the StrataCooler 96 Benchtop Cooler or Eppendorf PCR Cooler to the Sample Preparation Area.

Sample Preparation Area

46. Using a precision pipettor and a fresh, sterile 200 μL aerosol barrier pipette tip for each sample, transfer 50 μL of each eluted sample to the Abbott 96-Well Optical Reaction Plate on the StrataCooler 96 Benchtop Cooler or Eppendorf PCR Cooler. During the transfer of each sample, mix the reaction by pipetting up and down 3 to 5 times. Visually verify that a total of 100 μL has been dispensed into each well.

47. Seal the Abbott 96-Well Optical Reaction Plate according to the instructions in the Abbott m2000rt Operations Manual, Operating Instructions section.
48. Remove the Abbott 96-Well Optical Reaction Plate from the StrataCooler 96 Benchtop Cooler or Eppendorf 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 5000g for 5 minutes.

49. Transfer the Abbott 96-Well Optical Reaction Plate in the Abbott Splash-Free Support Base to the Amplification Area.

NOTE: Do not transfer the StrataCooler 96 Benchtop Cooler or Eppendorf PCR Cooler to the Amplification Area.

Amplification Area

50. Place the Abbott 96-Well Optical Reaction Plate in the Abbott m2000rt instrument, select the test order created (step 41), and initiate the Abbott RealTime HIV-1 Qualitative assay protocol, as described in the Abbott m2000rt Operations Manual, Operating Instructions section. At the completion of the run, assay results are reported on the Abbott m2000rt. Refer to the RESULTS section of the package insert for further details.

POST PROCESSING PROCEDURES

1. At the end of each run, clear and clean all work areas:
   - For Abbott Manual Sample Preparation Method users (Protocols III and IV), clean sample racks, temperature blocks, and magnetic racks by soaking in a 0.5% sodium hypochlorite solution for approximately 10 minutes and then rinsing completely with water and thoroughly air drying. Decontaminate and wipe work area according to laboratory guidelines. Clean the StrataCooler 96 Benchtop Cooler or Eppendorf PCR Cooler as described in the StrataCooler 96 Benchtop Cooler instruction manual or Eppendorf PCR Cooler Instructions for Use and return to the Reagent Preparation Area.


3. Decontaminate and dispose of all specimens, controls, reagents, and other potentially contaminated materials in accordance with local, state, and federal regulations.

4. Abbott mSample Preparation SystemDNA reagents are single use only. The remaining reagents and liquid waste must be discarded in accordance with local, state, and federal regulations.

WARNING: Do not mix any oxidizing agents, such as sodium hypochlorite, with the mLysisDNA, mMicroparticlesDNA, and mWash1DNA in the mSample Preparation SystemDNA reagents. Do not mix any oxidizing agents, such as sodium hypochlorite, with the liquid waste. Toxic gases may be generated from these mixtures. Pressure may build up in the closed container of the mixtures. It is the responsibility of each facility to characterize its waste and ensure that it is handled and disposed of in accordance with local, state, and federal regulations.

5. Remove and discard all disposables and solid waste in accordance with local, state, and federal regulations.

6. 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.

QUALITY CONTROL PROCEDURES

Abbott m2000rt Optical Calibration

Refer to the Calibration Procedures section in the Abbott m2000rt Operations Manual for a detailed description of how to perform an Abbott m2000rt Optical Calibration. Optical calibration of the Abbott m2000rt instrument is required for the accurate measurement and discrimination of dye fluorescence during the Abbott RealTime HIV-1 Qualitative assay. The following Abbott m2000rt Optical Calibration Plates are used to calibrate the Abbott m2000rt instrument for the Abbott RealTime HIV-1 Qualitative assay:

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

Detection of Inhibition

A defined, consistent quantity of IC is introduced into each specimen and control at the beginning of sample preparation and detected on the Abbott m2000rt instrument to demonstrate proper specimen processing and assay validity. The IC is comprised of an RNA sequence unrelated to the HIV-1 target sequence. A flag or an error code is displayed when IC cycle number (CN) value of a specimen or control exceeds the validity range. Refer to the Abbott m2000rt Operations Manual for an explanation of the corrective actions for errors.

Negative and Positive Controls

A negative control and a positive control are required for every run to verify that the sample processing, the amplification, and the detection steps are performed correctly. The Abbott RealTime HIV-1 Qualitative Controls need to be processed together with the specimens prior to running the amplification portion of the assay. A flag is displayed when a control result is out of range. If negative control or positive control is out of range, all of the specimens and controls from that run must be reprocessed, beginning with sample preparation. HIV-1 must not be detected in the negative control. HIV-1 detected in the negative control is indicative of contamination from other specimens or amplified product introduced during sample preparation or during preparation of the Abbott 96-Well Optical Reaction Plate.

To remove contamination, clean the Abbott m2000sp and m2000rt and all work areas according to the Abbott m2000sp and m2000rt Operations Manuals and the instructions in the POST PROCESSING PROCEDURES section of this package insert. Following cleaning, repeat sample processing for controls and specimens. If negative controls are persistently reactive, contact your Abbott representative.

IC results for the negative control and positive control that are outside the validity limit indicate the occurrence of inhibition during sample preparation or during the amplification reaction steps of the assay. Repeat the processing for controls and specimens.

Monitoring the Laboratory for the Presence of Contamination

It is recommended that the following procedure be done at least once a month to monitor laboratory surfaces and equipment for contamination. It is very important to test all areas that may have been exposed to processed specimens and controls and/or amplification product. These include routinely handled objects such as pipettes, the Abbott m2000sp and m2000rt function keys, bench surfaces and other equipment that may be present in the work areas.

1. Add 1.5 mL RNase/DNase-free water to a new master mix tube.
2. Saturate the cotton tip of an applicator (Puritan or equivalent) in the RNase/DNase-free water from the master mix tube.
3. Using the saturated cotton tip of the applicator, wipe the area to be monitored using a sweeping motion. Place the applicator into the master mix tube.
4. Swirl the cotton tip in RNase/DNase-free water 10 times, then press the applicator along the inside of the tube so that the liquid drains back into the solution at the bottom of the master mix tube. Discard the applicator.
5. Cap the master mix tube and vortex.
6. Remove the caps from the master mix tubes and test the samples according to the appropriate assay protocol instructions in this package insert.
7. Contamination is indicated by the detection of HIV-1 in the swab samples.
   - If contamination is present, the instrument will report “HIV-1 Detected.”
   - If there is no contamination, the instrument will report “Not Detected.”
8. If contamination is detected on the equipment, follow the cleaning and decontaminating guidelines given in that equipment’s operations manual and in the instructions in the POST PROCESSING PROCEDURES section of this package insert. If HIV-1 is detected on surfaces, clean the contaminated areas with 1.0% (v/v) sodium hypochlorite solution, followed by 70% ethanol or water.

NOTE: Chlorine solutions may pit equipment and metal. Use sufficient amounts or repeated applications of 70% ethanol until chlorine residue is no longer visible.

9. Repeat testing of the contaminated area by following steps 1 through 6.
10. If the presence of contamination is detected again, repeat steps 8 and 9 until no HIV-1 amplification is detected.

RESULTS

A specimen tested by the Abbott RealTime HIV-1 Qualitative assay will have a result of “HIV-1 Detected” or “Not Detected.” When using the dried blood spot application, “HIV-1 Detected” specimen results will also display the Cycle Number (CN) value. The Cycle Number value is the PCR cycle at which the fluorescence has risen above the predefined fluorescence threshold established for determining HIV-1 detection. Specimens with the Abbott RealTime HIV-1 Qualitative assay No Cycle Number will be reported for HIV-1 “Not Detected” dried blood spot specimens. The Cycle Number will not be reported for any plasma specimens.
and Abbott RealTime HIV-1 Qualitative assay were performed with 3 lots of amplification reagents on 3 Abbott RealTime HIV-1 Qualitative assay was 110 copies/mL in plasma using the plasma procedure.

Probit analysis of the data determined that the concentration of HIV-1 RNA in plasma detected with 95% probability was 80 copies/mL (95% Confidence Interval (CI) 65 - 118 copies/mL). The LOD of the Abbott RealTime HIV-1 Qualitative assay is 110 copies/mL in plasma using the plasma procedure.

Probit analysis of the data determined that the concentration of HIV-1 RNA in whole blood detected with 95% probability was 2,469 copies/mL (95% Cl 1,939 - 4,040 copies/mL). The LOD of the Abbott RealTime HIV-1 Qualitative assay is 2,500 copies/mL in whole blood using the DBS procedure.

Detection of WHO 2nd International Standard (NIBSC 97/650)
Assay performance was also evaluated by testing dilutions of WHO 2nd International Standard for HIV-1 (NIBSC 97/650). The results are summarized in Tables 3 and 4 for the plasma and DBS procedures, respectively.

Probit analysis of the data determined that the concentration of HIV-1 RNA in plasma detected with 95% probability was 149 IU/mL (95% CI 120 - 217 IU/mL) using the plasma procedure.

Probit analysis of the data determined that the concentration of HIV-1 RNA in plasma detected with 95% probability was 3,085 IU/mL (95% CI 120 - 217 IU/mL) using the DBS procedure.

Table 1. Plasma Procedure, VQA Viral Standard

<table>
<thead>
<tr>
<th>Concentration (copies/mL)</th>
<th>Number Tested</th>
<th>Number Detected</th>
<th>Percent Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>45</td>
<td>32</td>
<td>71</td>
</tr>
<tr>
<td>50</td>
<td>45</td>
<td>36</td>
<td>80</td>
</tr>
<tr>
<td>75</td>
<td>45</td>
<td>42</td>
<td>93</td>
</tr>
<tr>
<td>100</td>
<td>45</td>
<td>44</td>
<td>98</td>
</tr>
<tr>
<td>150</td>
<td>45</td>
<td>45</td>
<td>100</td>
</tr>
<tr>
<td>200</td>
<td>45</td>
<td>45</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2. DBS Procedure, VQA Viral Standard

<table>
<thead>
<tr>
<th>Concentration (copies/mL)</th>
<th>Number Tested</th>
<th>Number Detected</th>
<th>Percent Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>800</td>
<td>45</td>
<td>31</td>
<td>69</td>
</tr>
<tr>
<td>1000</td>
<td>45</td>
<td>32</td>
<td>71</td>
</tr>
<tr>
<td>1500</td>
<td>45</td>
<td>41</td>
<td>91</td>
</tr>
<tr>
<td>2000</td>
<td>45</td>
<td>41</td>
<td>91</td>
</tr>
<tr>
<td>2500</td>
<td>45</td>
<td>42</td>
<td>93</td>
</tr>
<tr>
<td>3000</td>
<td>45</td>
<td>44</td>
<td>98</td>
</tr>
</tbody>
</table>

Probit analysis of the data determined that the concentration of HIV-1 RNA in plasma detected with 95% probability was 149 IU/mL (95% CI 120 - 217 IU/mL) using the plasma procedure.

Probit analysis of the data determined that the concentration of HIV-1 RNA in whole blood detected with 95% probability was 2,469 copies/mL (95% Cl 1,939 - 4,040 copies/mL). The LOD of the Abbott RealTime HIV-1 Qualitative assay is 2,500 copies/mL in whole blood using the DBS procedure.

Detection of WHO 2nd International Standard (NIBSC 97/650)
Assay performance was also evaluated by testing dilutions of WHO 2nd International Standard for HIV-1 (NIBSC 97/650). The results are summarized in Tables 3 and 4 for the plasma and DBS procedures, respectively.

Table 3. Plasma Procedure, WHO 2nd International Standard for HIV-1 (NIBSC 97/650)

<table>
<thead>
<tr>
<th>Concentration (IU/mL)</th>
<th>Number Tested</th>
<th>Number Detected</th>
<th>Percent Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>45</td>
<td>27</td>
<td>60</td>
</tr>
<tr>
<td>80</td>
<td>45</td>
<td>39</td>
<td>87</td>
</tr>
<tr>
<td>120</td>
<td>45</td>
<td>39</td>
<td>87</td>
</tr>
<tr>
<td>160</td>
<td>45</td>
<td>44</td>
<td>98</td>
</tr>
<tr>
<td>240</td>
<td>45</td>
<td>44</td>
<td>98</td>
</tr>
<tr>
<td>320</td>
<td>45</td>
<td>45</td>
<td>100</td>
</tr>
</tbody>
</table>

Probit analysis of the data determined that the concentration of HIV-1 RNA in plasma detected with 95% probability was 149 IU/mL (95% CI 120 - 217 IU/mL) using the plasma procedure.

Probit analysis of the data determined that the concentration of HIV-1 RNA in whole blood detected with 95% probability was 2,469 copies/mL (95% Cl 1,939 - 4,040 copies/mL). The LOD of the Abbott RealTime HIV-1 Qualitative assay is 2,500 copies/mL in whole blood using the DBS procedure.

Detection of WHO 2nd International Standard (NIBSC 97/650)
Assay performance was also evaluated by testing dilutions of WHO 2nd International Standard for HIV-1 (NIBSC 97/650). The results are summarized in Tables 3 and 4 for the plasma and DBS procedures, respectively.

Table 4. DBS Procedure, WHO 2nd International Standard for HIV-1 (NIBSC 97/650)

<table>
<thead>
<tr>
<th>Concentration (IU/mL)</th>
<th>Number Tested</th>
<th>Number Detected</th>
<th>Percent Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1600</td>
<td>45</td>
<td>28</td>
<td>62</td>
</tr>
<tr>
<td>2400</td>
<td>45</td>
<td>39</td>
<td>87</td>
</tr>
<tr>
<td>3000</td>
<td>45</td>
<td>42</td>
<td>93</td>
</tr>
<tr>
<td>5000</td>
<td>45</td>
<td>45</td>
<td>100</td>
</tr>
<tr>
<td>7000</td>
<td>45</td>
<td>45</td>
<td>100</td>
</tr>
<tr>
<td>9000</td>
<td>45</td>
<td>45</td>
<td>100</td>
</tr>
</tbody>
</table>

Probit analysis of the data determined that the concentration of HIV-1 RNA in plasma detected with 95% probability was 149 IU/mL (95% CI 120 - 217 IU/mL) using the plasma procedure.

Probit analysis of the data determined that the concentration of HIV-1 RNA in whole blood detected with 95% probability was 2,469 copies/mL (95% Cl 1,939 - 4,040 copies/mL). The LOD of the Abbott RealTime HIV-1 Qualitative assay is 2,500 copies/mL in whole blood using the DBS procedure.

Seroconversion Sensitivity
The diagnostic sensitivity of the Abbott RealTime HIV-1 Qualitative assay was evaluated by testing sequential plasma specimens from 13 HIV seroconversion panels. These panels are commercially available and pre-characterized for HIV infection. The Abbott RealTime HIV-1 Qualitative assay detected HIV-1 in 57 out of 82 total number of bleeds compared with 16 out of 82 that were detected by an HIV-1 antibody test (Abbott HIVAB HIV-1/2 (rDNA) EIA List No. 3A77). The first reactive bleed (i.e., panel member) for Abbott RealTime HIV-1 Qualitative assay occurred earlier in all 13 panels (median 10.0 days) compared to the Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA. The seroconversion sensitivity is presented in Table 5.
The overall agreement between the Abbott RealTime HIV-1 Qualitative assay and the Roche Amplicor HIV-1 DNA Test version 1.5 was 97.8% (275/288) for the DBS and plasma assay procedures, respectively (95% CI: 96.5% - 98.9%).

**Performance for Detection of HIV-1 Infection in Infants**

The performance of the Abbott RealTime HIV-1 Qualitative assay in detecting HIV-1 infection was evaluated by testing specimens randomly collected from infants approximately 6 weeks to 18 months old who were born to HIV-1 positive mothers. The plasma samples from 367 subjects and DBS samples from 288 subjects were tested with the Abbott RealTime HIV-1 Qualitative assay. The whole blood sample from each subject was tested with Roche Amplicor HIV-1 DNA Test version 1.5 following instructions for use.

The overall agreement between the Abbott RealTime HIV-1 Qualitative results and the Roche Amplicor HIV-1 DNA Test version 1.5 results was 95.5% and 97.8% for the DBS and plasma assay procedures, respectively (Table 6 and Table 7).

**Table 5. Serocconversion Sensitivity**

<table>
<thead>
<tr>
<th>Panel ID</th>
<th>Number of Panel Members Tested</th>
<th>Roche Amplicor HIV-1 Qualitative</th>
<th>Abbott RealTime HIV-1 Qualitative</th>
<th>Days to First Reactive Result</th>
<th>Difference in Days to First Reactive Result (Based on Bleed Date)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRB943</td>
<td>7</td>
<td>6</td>
<td>3</td>
<td>5</td>
<td>14 9</td>
</tr>
<tr>
<td>PRB950</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>18</td>
<td>28 10</td>
</tr>
<tr>
<td>PRB951</td>
<td>6</td>
<td>4</td>
<td>1</td>
<td>8</td>
<td>19 11</td>
</tr>
<tr>
<td>PRB952</td>
<td>6</td>
<td>5</td>
<td>2</td>
<td>7</td>
<td>17 10</td>
</tr>
<tr>
<td>PRB954</td>
<td>7</td>
<td>4</td>
<td>0</td>
<td>10</td>
<td>21 11</td>
</tr>
<tr>
<td>PRB955</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>12 12</td>
</tr>
<tr>
<td>PRB956</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>40</td>
<td>50 12</td>
</tr>
<tr>
<td>PRB957</td>
<td>7</td>
<td>4</td>
<td>2</td>
<td>14</td>
<td>23 9</td>
</tr>
<tr>
<td>PRB962</td>
<td>6</td>
<td>4</td>
<td>0</td>
<td>7</td>
<td>17 10</td>
</tr>
<tr>
<td>PRB963</td>
<td>7</td>
<td>4</td>
<td>0</td>
<td>9</td>
<td>21 12</td>
</tr>
<tr>
<td>PRB964</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>15</td>
<td>22 7</td>
</tr>
<tr>
<td>PRB965</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>12 12</td>
</tr>
<tr>
<td>PRB966</td>
<td>10</td>
<td>5</td>
<td>2</td>
<td>35</td>
<td>48 13</td>
</tr>
</tbody>
</table>

**Table 6. Agreement Between Abbott RealTime HIV-1 Qualitative DBS Procedure and Roche Amplicor HIV-1 DNA Test Version 1.5**

<table>
<thead>
<tr>
<th>Abbott RealTime HIV-1 Qualitative DBS Procedure</th>
<th>Detected</th>
<th>Not Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roche Amplicor HIV-1 DNA Test Version 1.5</td>
<td>Detected</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Not Detected</td>
<td>12</td>
</tr>
</tbody>
</table>

Agreement = 95.5% (275/288) 95% CI: (92.40 - 97.57)

**Table 7. Agreement Between Abbott RealTime HIV-1 Qualitative Plasma Procedure and Roche Amplicor HIV-1 DNA Test Version 1.5**

<table>
<thead>
<tr>
<th>Abbott RealTime HIV-1 Qualitative Plasma Procedure</th>
<th>Detected</th>
<th>Not Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roche Amplicor HIV-1 DNA Test Version 1.5</td>
<td>Detected</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>Not Detected</td>
<td>0</td>
</tr>
</tbody>
</table>

Agreement = 97.8% (359/367) 95% CI: (95.75 - 99.05)

**NOTE:** Neither pregnant nor postpartum women were included as subjects in this study. The Abbott RealTime HIV-1 Qualitative assay has not been evaluated in pregnant women.

**Detection of HIV-1 Subtypes and Groups**

The performance of the Abbott RealTime HIV-1 Qualitative assay with HIV-1 subtypes/groups was evaluated by testing 10 clinical specimens of Group M subtypes A, B, C, D, CRF01-AE, F, CRF02-AG, and G, 10 clinical specimens of Group O, and purified RNA transcripts of Group M subtype H and Group N (10 replicates of each transcript). The results are summarized in Table 8.

**Table 8: Subtype Detection**

<table>
<thead>
<tr>
<th>Group/Subtypes</th>
<th>n(^a)</th>
<th>Detected by Abbott RealTime HIV-1 Qualitative assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group M/Subtype A</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Group M/Subtype B</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Group M/Subtype C</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Group M/Subtype D</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Group M/Subtype AE</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Group M/Subtype F</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Group M/Subtype AG</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Group M/Subtype G</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Group M/Subtype H</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Group O</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Group N</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

\(^a\) “n” represents the number of clinical specimens tested. For Group M/Subtype H and Group N, “n” represents the number of replicates of transcript samples tested.

The Abbott RealTime HIV-1 Qualitative assay detected all groups and subtypes tested.

**Specificity**

The specificity of the Abbott RealTime HIV-1 Qualitative assay was evaluated for both the plasma and DBS procedures by testing 550 HIV-1 seronegative plasma specimens and 550 HIV-1 seronegative whole blood specimens. Both plasma and whole blood specimens were collected from each of the 550 subjects. For each assay procedure, the specimens were tested on 2 Abbott m2000 Systems with 4 lots of amplification reagents. HIV-1 was not detected for 550 out of 550 samples in both specimen types, resulting in 100.0% specificity (95% CI 99.33% - 100.00%) for both the plasma and DBS assay procedures in this representative study.

The specificity of the Abbott RealTime HIV-1 Qualitative assay was further evaluated by testing plasma 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 (n=10), anti-nuclear antibodies (n=10), rheumatoid factor (n=10), multiple sclerosis (n=7), multiple myeloma (n=10), HBsAg (n=10), anti-HTLV-I (n=10), anti-HCV (n=10). Also tested were flu vaccinees (n=10) and HBV vaccinees (n=10). HIV-1 was not detected in any of the specimens tested. In addition, HIV-1 was not detected in all the specimens that had been spiked with HIV-1 RNA. 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 Qualitative assay.

**Reproducibility**

The reproducibility of the Abbott RealTime HIV-1 Qualitative assay was evaluated using the Abbott m2000sp. A four-member HIV-1 RNA panel was prepared consisting of 1 negative member and 3 positive members at 1,000,000, 100,000 and 10,000 copies/mL. The panel was tested by 3 operators. Each operator, using a unique combination of reagent
lot and instrument pair, tested 10 replicates of each panel member once per day for 5 days, for a total of 50 replicates per operator (150 total replicates per panel member). The Abbott RealTime HIV-1 Qualitative assay also provides a manual sample preparation option. The reproducibility between the manual and the automated sample preparation methods was determined by testing the same HIV-1 RNA panel as evaluated in the Reproducibility study. The panel was tested by a single operator using both sample preparation methods and using 1 reagent lot and 1 Abbott m2000rt instrument. For each method, the operator tested 10 replicates of each panel member once per day for 5 days, for a total of 50 replicates (200 total replicates for all panel members). The overall agreement between the manual and Abbott m2000sp sample preparation methods was 99.0% (95% CI 96.43 – 99.88%).

Cross-Reactivity

The following viruses and microorganisms were evaluated for potential cross-reactivity in the Abbott RealTime HIV-1 Qualitative assay. Each potential cross-reactant (purified nucleic acids, viral lysate, clinical specimen, or cloned plasmid DNA) was added to HIV-1 negative samples and samples that contained 1,000 copies/mL HIV-1 RNA.

<table>
<thead>
<tr>
<th>Human Immunodeficiency virus 2</th>
<th>Human herpesvirus 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human T-lymphotropic virus 1</td>
<td>Varicella-zoster virus</td>
</tr>
<tr>
<td>Hepatitis B virus</td>
<td>Vaccinia virus</td>
</tr>
<tr>
<td>Hepatitis C virus</td>
<td>BK human polyomavirus</td>
</tr>
<tr>
<td>Epstein-Barr virus</td>
<td>Neisseria gonorrhoeae</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>Chlamydia trachomatis</td>
</tr>
<tr>
<td>Human papillomavirus 16</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>Human papillomavirus 18</td>
<td>Staphylococcus epidermidis</td>
</tr>
<tr>
<td>Herpes simplex virus 1</td>
<td>Mycobacterium gordonae</td>
</tr>
<tr>
<td>Herpes simplex virus 2</td>
<td>Mycobacterium smegmatis</td>
</tr>
<tr>
<td>Human herpesvirus B</td>
<td>Candida albicans</td>
</tr>
</tbody>
</table>

No interference in the Abbott RealTime HIV-1 Qualitative assay results (HIV-1 Detected or Not Detected) was observed in the presence of the potential cross-reactants for all positive and negative samples tested.

Potentially Interfering Substances

The susceptibility of the Abbott RealTime HIV-1 Qualitative assay to interference by elevated levels of endogenous substances was evaluated for the DBS procedure. HIV-1 negative samples (human whole blood) and samples spiked with 10,000 copies/mL of HIV-1 RNA were made into DBS and further tested.

No interference in the Abbott RealTime HIV-1 Qualitative assay results (HIV-1 Detected or Not Detected) was observed in the presence of the following substances for all positive and negative samples tested:

- Hemoglobin 2 g/L
- Triglycerides 37 mM
- Bilirubin 342 µM
- Protein 120 g/L

Carryover

Potential sample carryover in the automated Abbott m2000 systems when used with the Abbott RealTime HIV-1 Qualitative assay was evaluated by testing high titer HIV-1 positive samples (with a target concentration of 1,000,000 copies/mL) interspersed with negative samples. The Abbott RealTime HIV-1 Qualitative assay did not exhibit detectable carryover from high positive samples to negative samples.

BIBLIOGRAPHY


TECHNICAL ASSISTANCE

For technical assistance, call Abbott Molecular Technical Services at 1-800-553-7042 (within the US) or +1-6122-580 (outside the US), or visit the Abbott Molecular website at http://www.abbottmolecular.com.

THE PURCHASE OF THIS PRODUCT ALLOWS THE PURCHASER TO
USE IT FOR AMPLIFICATION OF NUCLEIC ACID SEQUENCES AND FOR DETECTION OF NUCLEIC ACID SEQUENCES FOR HUMAN IN VITRO DIAGNOSTICS. NO GENERAL PATENT OR OTHER LICENSE OF ANY KIND OTHER THAN THIS SPECIFIC RIGHT OF USE FROM PURCHASE IS GRANTED HEREBY. THIS PROVISION DOES NOT PROHIBIT THE RESALE OF THIS PRODUCT.

Armored RNA® is a patented technology jointly developed by Ambion, Inc. and Cenetron Diagnostics, LLC. U.S. patents #5,677,124, #5,919,625, #5,939,262 and patents pending.

Armored RNA is a registered trademark of Ambion, Inc. ProClin is a registered trademark of Rohm and Haas. StrataCooler is a registered trademark of Stratagene. Eppendorf is a registered trademark of Eppendorf AG. FAM and ROX are trademarks of Life Technologies Corporation. VIC is a registered trademark of Life Technologies Corporation. Abbott RealTime, m, m2000, m2000rt, and m2000sp are trademarks of Abbott Laboratories.

Abbott Molecular Inc. is the legal manufacturer of the:
Abbott RealTime HIV-1 Qualitative Amplification Reagent Kit (List No. 4N66-90) and
Abbott RealTime HIV-1 Qualitative Control Kit (List No. 4N66-80)

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

NOTE: Changes Highlighted

Key to Symbols used
- List Number
- Lot Number
- Expiration Date
- Negative Control
- High Positive Control
- Store at ≤ -10°C
- Consult instructions for use
- Caution: Consult Instructions For Use (Infection Risk)
- 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 Qualitative Controls are used to establish run validity of the Abbott RealTime HIV-1 Qualitative assay when used for the qualitative detection of Human Immunodeficiency Virus Type 1 (HIV-1) nucleic acids from human plasma and dried blood spots (DBS).

Intended User
The intended users for the Abbott RealTime HIV-1 Qualitative Controls are laboratory and healthcare professionals.

Contents
1. **CONTROL** Abbott RealTime HIV-1 Negative Control (2G31Z) (12 vials, 1.8 mL per vial). Negative human plasma tested and found to be nonreactive for HBsAg, anti-HIV-1/HIV-2, anti-HCV, HIV RNA, HCV RNA, and HBV DNA. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.
2. **CONTROL** Abbott RealTime HIV-1 High Positive Control (2G31X) (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, anti-HIV-1/HIV-2, anti-HCV, HIV RNA, HCV RNA, and HBV DNA. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

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

- The Abbott RealTime HIV-1 Qualitative Control Kit must only be used with the Abbott RealTime HIV-1 Qualitative assay (List No. 4N66-80).

**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 bactericidal 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 Qualitative Control Kit (List No. 4N66-80) contain the following components:
- 2-Methyl-2H-isothiazol-3-one
- Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one
- Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one

The following warnings apply:

**Warning**
- May cause an allergic skin reaction.
- Avoid breathing mist / vapours / spray.
- Wear protective gloves / protective clothing / eye protection.
- Contaminated work clothing should not be allowed out of the workplace.
- IF ON SKIN: Wash with plenty of water.
- IF skin irritation or rash occurs: Get medical advice / attention.
- Take off contaminated clothing and wash it before reuse.
- 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.

www.abbottmolecular.com
Abbott Molecular Inc.
Des Plaines, IL 60018 USA

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