WHO Prequalification of Diagnostics Programme
PUBLIC REPORT

Product: Abbott RealTi*m*e HIV-1 (m2000sp)
Number: PQDx 0145-027-00

Abbott RealTi*m*e HIV-1 (*m*2000sp) assay with product code 2G31, which includes 2G31-80 and 2G31-70 and 2G31-90 (plasma specimens), and product code 02G31-010 [1] (DBS specimens), 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 listed on 17 October 2011.

Summary of prequalification status for Abbott RealTi*me* HIV-1 (*m*2000sp)

	Date	Outcome
Status on PQ list	17 October 2011	Listed
Dossier assessment	28 September 2011	MR
Site inspection(s) of the quality management system	12 November 2019	MR
Product performance evaluation	plasma (FT) 30 June 2015 (DBS)	MR

MR: Meets Requirements, FT: Fast-tracked

Report amendments and product changes

This public report has since been amended. Amendments may have arisen because of changes to the prequalified product for which the 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.

Version	Summary of amendment	Date of report amendment
2.0-4.0	Inclusion of product code 02G31-010, allowing the use of dried blood spot (DBS) specimens in addition to plasma specimens. Series of editorial changes on the versions.	23 June 2016, 30 June 2016
6.0	Change of manufacturing process from manual to automated at the supplier for incoming materials (oligonucleotides).	24 August 2016

[1] product code 02G31-010 was added to allow for the use of dried blood spot (DBS) specimens in addition to plasma specimens.

7.0	Changes in the DBS protocol.	7 October 2016.
8.0	Modified specimen processing protocol resulted in updated labelling and Instructions for Use.	23 April 2018
9.0	1. The Notified Body number on the Abbott RealTime HIV-1 Quantitative and Qualitative kit labels and package inserts has been updated to reflect the new notified body Polskie Centrum Badan I Certyfikacji S.A. (PCBC) Notified Body number of 1434. 2. The word "Abbott" has been aligned to the centre of the Abbott logo (where applicable). Labelling (labels and IFU) has been revised, and version numbers have been updated.	12 December 2019
10.0	Updated Abbott's European Authorized Representative (EC Rep) legal entity name from Abbott GmbH & Co. KG to Abbott GmbH. Labelling changes to comply with the labelling requirements for products registered under IVDR.	21 October 2021
11.0	Correction of the product code for the Abbott RealTime HIV-1 Amplification Reagent Kit from 02G31-10 to 02G31-010.	12 January 2023
12.0	1. Updated the IFU to adjust the number of new and partial Internal Control (IC) vials in the Sample Preparation Reagent and IC Requirements table in the DBS Sample Processing (Assay Protocol III) section. 2. Clarified in the footnote to specify using the same lot of IC and referencing Appendix 1 for partial IC storage requirements.	13 July 2023

Intended use:

According to the claim of Abbott Molecular Inc, " *The Abbott RealTime HIV-1 assay is an in vitro reverse transcription polymerase chain reaction (RT-PCR) assay for the quantitation of Human Immunodeficiency Virus type 1 (HIV-1) in whole blood spotted on cards as dried blood spots (DBS) (i.e. obtained via venipuncture or capillary blood) or human plasma from HIV-1 infected individuals. The Abbott RealTime HIV-1 is intended for use in conjunction with clinical presentation and other laboratory markers as an indicator of disease prognosis and for use as an aid in assessing viral response to antiretroviral treatment as measured by changes in DBS or plasma HIV-1 RNA levels. This assay is not intended to be used as a screening test for HIV-1 or as a diagnostic test to confirm the presence of HIV-1 infection.*

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

Assay principle:

According to the claim of Abbott Molecular Inc,

"*The Abbott RealTime HIV-1 assay uses RT-PCR to generate amplified product from the RNA genome of HIV-1 in clinical specimens. An RNA sequence that is unrelated to the HIV-1 target sequence is introduced into each specimen at the beginning of sample preparation. This unrelated RNA sequence is simultaneously amplified by RT-PCR, and serves as an internal control (IC) to demonstrate that the process has proceeded correctly for each sample. The amount of HIV-1 target sequence that is present at each amplification cycle is measured through the use of fluorescent-labeled oligonucleotide probes on the Abbott m2000rt instrument. The probes do not generate signal unless they are specifically bound to the amplified product. The amplification cycle at which fluorescent signal is detected by the Abbott m2000rt is proportional to the log of the HIV-1 RNA concentration present in the original sample".*

Test kit contents:

Component	Details
Abbott RealTime HIV-1 Controls (2G31-80)	Negative Control (2G31Z) 8 vials x 1.8 mL Low Positive Control (2G31W) 8 vials x1.8 mL High Positive Control (2G31X) 8 vials, 1.8 mL
Abbott RealTime HIV-1 Calibrator Kit (2G31-70)	Abbott RealTime HIV-1 Calibrator A 12 vials x1.8 mL. Abbott RealTime HIV-1 Calibrator B 12 vials, 1.8 mL.
Abbott RealTime HIV-1 Amplification Reagent Kit (02G31-010)-for plasma and DBS	Abbott RealTime HIV-1 Internal Control 4 vials, 1.2 mL per vial. Abbott RealTime HIV-1 Amplification Reagent Pack (4 packs x 24 tests/pack)
Abbott RealTime HIV-1 Amplification Reagent Kit (2G31-90)-for plasma only	Abbott RealTime HIV-1 Internal Control (2G31Y) 4 vials x 1.2 mL. Abbott RealTime HIV-1 Amplification Reagent Pack (2G31) (4 packs x 24 tests/pack)

Materials required but not provided:

Component	Details
Instrumentation	Abbott *m*2000sp Instrument (9K14-02)
	Abbott *m*2000rt Instrument (9K15-01)
Reagents	Abbott *m* Sample Preparation System RNA (4 X 24 Preps) (04J70-24) for plasma and DBS processing.
	Abbott *m*Sample Preparation System DBS Buffer Kit (List No. 09N02-001) for DBS processing only.
	Abbott *m*2000rt Optical Calibration kit (4J71-93)
Software	**For plasma only product (product code 2G31-90):** Abbott RealTi*m*e HIV-1 m2000 ROW System Combined Application CD-ROM 1L68 **For plasma and DBS product (product code 2G31-10):** Abbott RealTi*m*e HIV-1 m2000 ROW System Combined Application CD-ROM 01L68-14 or higher
Optional	Abbott RealTi*m*e HIV-1 UNG Protocol (2G31-66)
Consumables	Disposable Tips (DiTis), 1000 µL (4J7110) Disposable Tips (DiTis), 200 µL (4J7117) Biohazard Bags (4J7145) 5 mL Reaction Vessels (4J7120) 200 ml Reagent Vessels (4J7160) 96 Deep Well Plates (4J7130) 96-Well Optical Reaction Plates (4J7170) Optical Adhesive Covers (4J7175) Master Mix Tube (4J7180) Adhesive Cover Applicator (9K3201) Splash-Free Support Base (9K3101) 13 mm Sample Racks (4J7282) **Additional materials required if using DBS Sample Type:** • 15.8 mm well diameter heat block (to fit 15 mm diameter Master Mix Tubes) • m2000 System 13mm DBS PoST Set (List No. 09N03-001) Recommended: perforated Munktell paper card, Whatman 903 or Ahlstrom 226

Storage:

Component	Storage temperature
Abbott RealTi*m*e HIV-1 Calibrator A and Calibrator B	-10°C or colder
Abbott RealTi*m*e HIV-1 Negative, Low Positive, and High Positive Controls	-10°C or colder
Abbott RealTi*m*e HIV-1 Amplification Reagent Pack (2G31-90) **OR** Abbott RealTi*m*e HIV-1 Amplification Reagent Kit (02G31-010)	-10°C or colder when not in use -15 to -25 ˚C
Abbott *m*Sample Preparation System RNA (4X24 Preps)	15-30°C
Abbott *m*Sample Preparation System DBS Buffer Kit	15-30°C

Maximum shelf-life upon manufacture:

Component	Shelf life
Abbott RealTi*m*e HIV-1 Amplification Reagent Kit (02G31-010)	18 months
Abbott RealTi*m*e HIV-1 Amplification Reagent Kit (2G31-90 and 02G31-010)	
Abbott RealTi*m*e HIV-1 Internal Control 2G31Y	18 months
Thermostable rTth Polymerase Enzyme 56685	Per control date on vendor certificate of analysis
HIV-1 Oligonucleotide Reagent 2G31L	18 months
Activation Reagent 93591	18 months
Abbott RealTi*m*e HIV-1 Control Kit (2G31-80)	18 months
Abbott RealTi*m*e HIV-1 Calibrator Kit (2G31-70)	18 months
Abbott *m*Sample Preparation System RNA Kit 04J70-24	18 months
Abbott *m*Sample Preparation System DBS Buffer Kit	18 months

Limitations/warnings:

- This assay is not intended to be used as a screening test for HIV-1 or as a diagnostic test to confirm the presence of HIV-1 infection.

- Specimen preparation and storage are critical to ensuring accurate results and should be performed strictly in accordance to Manufacturer's instructions. Of note:
  - After plasma preparation, plasma 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 must be kept at –70°C or colder. Multiple freeze-thaw cycles should be avoided. If frozen, thaw plasma specimens at 15 to 30°C or 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.
  - Plasma specimens should not be frozen in non-gel blood collection tubes.

Notes:

- The currently prequalified DBS protocol requires a single 70 µL dried blood spot.
- Freshly drawn whole blood (ACD-A and EDTA only) may be held at 15 to 30°C for up to 24 hours or at 2 to 8°C for up to 48 hours prior to processing into plasma or DBS specimen.

Prioritization for Prequalification

Based on the established eligibility criteria, Abbott RealTi*m*e HIV-1 (m2000sp) was given priority for the WHO prequalification assessment.

Product dossier assessment

Abbott Molecular Inc. submitted a product dossier for Abbott RealTi*m*e HIV-1 (m2000sp) as per the 'Instructions for compilation of a product dossier' (PQDx_018 v1). The information (data and documentation) submitted in the product dossier was reviewed in accordance with the 'Internal report on the screening and assessment of a product dossier' (PQDx_009 v2) by WHO staff and external experts (assessors) appointed by WHO.

Based on the product dossier screening and assessment findings, the product dossier for Abbott RealTi*m*e HIV-1 (m2000sp) assay meets WHO prequalification requirements.

Manufacturing site inspection

A desk assessment of ***Abbott Molecular Inc***., located at *1300 East Touhy Avenue, 60018 Des Plaines, IL, USA,* was conducted on 12 November 2019. At the time of considering the product application for Prequalification, the Manufacturer of the product had a well-established quality management system and manufacturing practices in place that would support the manufacture of a product of consistent quality.

Routine inspections of the Manufacturing site will be conducted with copies of the WHO Public Inspection Report (WHOPIR) published on the WHO Prequalification web page as per Resolution WHA57.14 of the World Health Assembly. Note that a WHOPIR reflects the information on the most current inspection performed at a manufacturing site for in vitro diagnostic products and gives a summary of the inspection findings.

Information on the most current inspection can be found at:
https://extranet.who.int/pqweb/inspection-services/prequalification-reports/whopirs-vitro-diagnostics

All published WHOPIRs are with the agreement of the Manufacturer.

Based on the desk assessment, the quality management system for Abbott RealTi*m*e HIV-1 (m2000sp) meets WHO prequalification requirements.

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 on plasma, Abbott RealTi*m*e HIV-1 (m2000sp) assay has been found eligible to undergo the WHO fast track[2] procedure. Subsequently, the product was not required to undergo a laboratory evaluation for its use with human plasma.

Performance evaluation using dried blood spot (DBS) specimens

The performance of Abbott RealTi*m*e HIV-1 (m2000sp) with dried blood spot specimens was evaluated by WHO at the Institute of Tropical Medicine, Antwerp, Belgium, in Q3-Q4 2015. The evaluation was conducted with an early development open-mode version protocol provided by the manufacturer at the time of the evaluation. The results below do not reflect the current performance of the CE-marked WHO prequalified assay version using DBS specimens.

In this limited performance evaluation on a panel of 323 specimens, we found an initial bias (95% CI) of -0.42 log copies/mL ([-0.52] - [-0.32]) compared to the reference results for samples >1,000 copies/mL. The upward and downward misclassification rates around the threshold of 1,000 copies/mL were 10.3% and 24.0%, respectively. The upward and downward misclassification rates around the threshold of 5,000 copies/mL were 2.1% and 22.0%, respectively. The sensitivity (95% CI) was 76.0% (68.1%-82.5%), and the specificity (95% CI) was 89.7% (82.7%-94.2%) compared to the reference results at a threshold of 1,000 copies/mL. At 5,000 copies/mL, sensitivity and specificity were 78.0% (67.3%-86.1%) and 97.9% (94.3%-99.3%), respectively. In this study, the invalid rate was 1.1 %.

<u>Limitations of the evaluation:</u>

1. The reference method used to compare results obtained from DBS specimens was plasma, for which a viral load result was obtained using the Roche Cobas AmpliPrep/Cobas TaqMan HIV-1 Version 2.0, the standard assay in the evaluating site. This may have contributed to increased bias and misclassification rates (Sollis 2014) (Amendola 2014). Discrepant results were not retested on plasma using the

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

Abbott platform, given that the protocol used in the evaluation has now been made obsolete.

2. The evaluation was conducted using the early development open-mode version protocol provided by the manufacturer at the time. The manufacturer has since developed a new protocol and added DBS as an additional specimen type.

The performance of this product on DBS using the recommended protocol was reviewed as part of an assessment of the submission of a change request for the addition of DBS as a specimen type was accepted in October 2016.

The new instructions for use, including DBS testing and processing protocol, can be found in the labelling section.

Labelling

1. **Labels**
2. **Instructions for use**

1. Labels

1.1 Abbott RealTi*m*e Amplification Reagent Pack (2G31-90)

Abbott RealTi*me* HIV-1

Amplification Reagent Kit

Σ 96

REF 02G31-010

In Vitro Test

IVD

CE 1434

INTERNAL CONTROL 4 x 1.2mL

AMPLIFICATION REAGENT PACK 4

www.abbottmolecular.com/ifu

51-603152/R4

H317, P261, P280, P272, P302+P352, P333+P313, P362+P364, P501

GTIN

LOT

REF

Abbott RealTi*me* HIV-1

Amplification Reagent Kit

-15°C

-25°C

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

www.abbottmolecular.com

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

Abbott

1.2 HIV-1 Amplification Reagent Kit (List No. 02G31-010)

(de) In-vitro-Diagnostikum. Der Abbott RealTi*me* HIV-1 Assay ist ein In-vitro-Reverse-Transkription-Polymerasekettenreaktions-Assay (RT-PCR) zur quantitativen Bestimmung des Human Immunodeficiency Virus Typ 1 (HIV-1) in von HIV-1-infizierten Personen stammendem Humanplasma. Der Abbott RealTi*me* HIV-1 Assay dient zur Verwendung in Verbindung mit dem klinischen Bild und anderen Labormarkern als Indikator für die Krankheitsprognose und als Hilfsmittel bei der Beurteilung des viralen Ansprechens auf antiretrovirale Behandlung, wobei die Veränderung der HIV-1 RNA-Konzentrationen im Plasma gemessen wird. Dieser Assay ist nicht zur Verwendung als Screening-Test für HIV-1 oder als diagnostischer Test zur Bestätigung einer HIV-1-Infektion vorgesehen.

Inhalt:

1. **INTERNAL CONTROL** Abbott RealTi*me* HIV-1 Interne Kontrolle (4 Fläschchen, 1,2 ml pro Fläschchen). <0,01 % nicht infektiöse Armored RNA® mit internen Kontrollsequenzen in negativem Humanplasma. Negatives Humanplasma wurde getestet und war nicht reaktiv für HBsAg, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, HBV DNA und anti-HCV.
   Konservierungsmittel: 0,1 % ProClin® 300 und 0,15 % ProClin 950.
2. **AMPLIFICATION REAGENT PACK** Abbott RealTi*me* HIV-1 Amplifikations-Reagenzpackung (4 Packungen, 24 Tests/Packung).
   Jede Reagenzpackung enthält:
   - 1 Fläschchen (0,141 ml) Thermostabiles rTth-Polymerase-Enzym (2,9 bis 3,5 Einheiten/µl) in gepufferter Lösung.
   - 1 Fläschchen (1,10 ml) HIV-1 Oligonukleotidreagenz. <0,1 % synthetische Oligonukleotide (4 Primer, 2 Sonden und 1 Quencher-Oligonukleotid) und <0,3 % dNTPs in einer gepufferten Lösung mit einem Referenzfarbstoff. Konservierungsmittel: 0,1 % ProClin 300 und 0,15 % ProClin 950.
   - 1 Fläschchen (0,40 ml) Aktivierungsreagenz. 30 mmol/l Manganchloridlösung. Konservierungsmittel: 0,1 % ProClin 300 und 0,15 % ProClin 950.

ProClin ist ein eingetragenes Warenzeichen von Rohm and Haas.
Armored RNA ist ein eingetragenes Warenzeichen von Ambion.
Abbott RealTi*me* ist ein Warenzeichen von Abbott Laboratories.

(fr) Pour diagnostic *in vitro*. RealTi*me* HIV-1 est un dosage *in vitro* par RT-PCR (reverse transcription-polymerase chain reaction) pour la mesure quantitative du virus de l'immunodéficience humaine de type 1 (VIH-1) dans le plasma d'individus infectés par le VIH-1. Le dosage Abbott RealTi*me* HIV-1 doit être utilisé en association avec les avis cliniques et d'autres marqueurs de laboratoire comme indicateur du pronostic de la maladie et comme assistance dans l'évaluation de la réponse virale au traitement antirétroviral, mesurée en fonction des variations des niveaux d'ARN du VIH-1. Ce dosage n'est pas destiné au dépistage du VIH-1 ou à la confirmation du diagnostic d'une infection par le VIH-1.

Contenu :

1. **INTERNAL CONTROL** Contrôle interne Abbott RealTi*me* HIV-1 (4 flacons de 1,2 ml chacun). < 0,01 % d'Armored RNA® non infectieux comprenant des séquences de contrôle interne dans du plasma humain négatif. Le plasma humain négatif a été analysé et trouvé non réactif pour l'AgHBs, l'ARN VIH, l'ARN VHC, l'ADN VHB et pour les anticorps anti-VIH-1/VIH-2 et anti-VHC.
   Conservateurs : ProClin® 300 à 0,1 % et ProClin 950 à 0,15 %.
2. **AMPLIFICATION REAGENT PACK** Coffret-réactifs pour amplification Abbott RealTi*me* HIV-1 (4 boîtes de 24 tests chacune).
   Chaque coffret-réactifs contient :
   - 1 flacon (0,141 ml) d'enzyme rTth polymérase thermostable (2,9 à 3,5 unités/µl) dans une solution tampon.
   - 1 flacon (1,10 ml) de réactif d'oligonucléotides de VIH-1. < 0,1 % d'oligonucléotides synthétiques (4 amorces, 2 sondes et 1 oligonucléotide désactiveur) et < 0,3 % de dNTPs dans une solution tampon contenant un fluorochrome de référence. Conservateurs : ProClin 300 à 0,1 % et ProClin 950 à 0,15 %.
   - 1 flacon (0,40 ml) de réactif d'activation. 30 mM de solution de chlorure de manganèse. Conservateurs : ProClin 300 à 0,1 % et ProClin 950 à 0,15 %.

ProClin est une marque déposée de Rohm and Haas.
Armored RNA est une marque déposée d'Ambion.
Abbott RealTi*me* est une marque commerciale d'Abbott Laboratories.

(es) Para uso en diagnóstico *in vitro*. Abbott RealTi*me* HIV-1 es un ensayo de reacción en cadena de la polimerasa previa transcripción inversa (RT-PCR) para la determinación cuantitativa del virus de la inmunodeficiencia humana del tipo 1 (VIH-1) en plasma humano de pacientes infectados por el VIH-1. El ensayo Abbott RealTi*me* HIV-1 se usa junto con pruebas clínicas y otros marcadores de laboratorio como indicador del pronóstico de la enfermedad y como ayuda en la evaluación de la respuesta del virus al tratamiento antirretroviral, según los cambios de concentración del RNA del VIH-1 medidos en el plasma. Este ensayo no está diseñado como análisis de cribado del VIH-1 ni como análisis de diagnóstico para confirmar la presencia de una infección por VIH-1.

Contenido:

1. **INTERNAL CONTROL** Control interno Abbott RealTi*me* HIV-1 (4 tubos de 1,2 ml cada uno). <0,01% Armored RNA® no infeccioso con secuencias de control interno en plasma humano negativo. El plasma humano negativo ha sido analizado y no se ha encontrado reactividad para HBsAg, ni para el RNA del VIH, ni para el RNA del VHC, ni reactividad de anticuerpos anti-VIH-1/VIH-2, ni anti-VHC ni para el DNA del VHB. Conservantes: ProClin® 300 al 0,1% y ProClin 950 al 0,15%.
2. **AMPLIFICATION REAGENT PACK** Envase de reactivos de amplificación Abbott RealTi*me* HIV-1 (4 envases de 24 análisis cada uno). Cada envase de reactivo contiene:
   - 1 frasco (0,141 ml) de Thermostable rTth Polymerase Enzyme (enzima polimerasa rTth termoestable) (2,9 a 3,5 unidades/µl) en solución tamponada.
   - 1 frasco (1,10 ml) de HIV-1 Oligonucleotide Reagent (reactivo de oligonucleótidos VIH-1). <0,1% oligonucleótidos sintéticos (4 cebadores, 2 sondas y 1 oligonucleótido extintor) y <0,3% dNTPs en solución tamponada con fluoróforos de referencia. Conservantes: ProClin 300 al 0,1% y ProClin 950 al 0,15%.
   - 1 frasco (0,40 ml) de Activation Reagent (reactivo de activación). Solución de cloruro de manganeso 30 mM. Conservantes: ProClin 300 al 0,1% y ProClin 950 al 0,15%.

ProClin es una marca comercial registrada de Rohm and Haas.
Armored RNA es una marca comercial registrada de Ambion.
Abbott RealTi*me* es una marca comercial de Abbott Laboratories.

(it) Per uso diagnostico *in vitro.* Il dosaggio Abbott RealTi*me* HIV-1 è un dosaggio basato sul principio della RT-PCR (trascrizione inversa-reazione a catena della polimerasi) *in vitro* per la determinazione quantitativa del virus dell'immunodeficienza umana di tipo 1 (HIV-1) in campioni di plasma umano prelevati da soggetti con infezione da HIV-1. Il dosaggio Abbott RealTi*me* HIV-1 deve essere utilizzato congiuntamente alla valutazione del quadro clinico e di altri marker di laboratorio quale indicatore prognostico della malattia, nonché come ausilio nel controllo della risposta al trattamento antiretrovirale, misurata in base ai cambiamenti dei livelli di HIV-1 RNA nel plasma. Il presente dosaggio non deve essere utilizzato come test di screening per l'HIV-1 o come test diagnostico per confermare la presenza dell'infezione da HIV-1.

Contenuto:

1. **INTERNAL CONTROL** Controllo interno Abbott RealTi*me* HIV-1 (4 provette, 1,2 ml per provetta). Armored RNA® non infettivo <0,01% con sequenze di controllo interno in plasma umano negativo. Plasma umano negativo analizzato e risultato non reattivo per l'HBsAg, l'HIV RNA, l'HCV RNA, per gli anticorpi anti-HIV-1/
   HIV-2, per l'HBV DNA e per gli anticorpi anti-HCV.
   Conservanti: ProClin® 300 allo 0,1% e ProClin 950 allo 0,15%.
2. **AMPLIFICATION REAGENT PACK** Confezione dei reagenti per l'amplificazione Abbott RealTi*me* HIV-1 (4 confezioni, 24 test/confezione). Ciascuna confezione dei reagenti contiene:
   - 1 flacone (0,141 ml) di enzima termostabile polimerasi rTth (da 2,9 a 3,5 unità/µl) in una soluzione tamponata.
   - 1 flacone (1,10 ml) di reagente di oligonucleotidi dell'HIV-1. Oligonucleotidi sintetici <0,1% (4 primer, 2 sonde e 1 oligonucleotide "quencher") e dNTP <0,3% in una soluzione tamponata con un colorante di riferimento. Conservanti: ProClin 300 allo 0,1% e ProClin 950 allo 0,15%.
   - 1 flacone (0,40 ml) di reagente di attivazione. 30 mM di soluzione di cloruro di manganese. Conservanti: ProClin 300 allo 0,1% e ProClin 950 allo 0,15%.

ProClin è un marchio commerciale registrato di Rohm and Haas.
Armored RNA è un marchio commerciale registrato di Ambion.
Abbott RealTi*me* è un marchio commerciale di Abbott Laboratories.

Abbott RealTi*me* HIV-1

Amplification Reagent Kit

(4x24 Tests)

(en) For *In Vitro* Diagnostic Use. The Abbott RealTi*me* HIV-1 assay is an *in vitro* reverse transcription-polymerase chain reaction (RT-PCR) assay for the quantitation of Human Immunodeficiency Virus type 1 (HIV-1) in human plasma from HIV-1 infected individuals. The Abbott RealTi*me* HIV-1 assay is intended for use in conjunction with clinical presentation and other laboratory markers as an indicator of disease prognosis and for use as an aid in assessing viral response to antiretroviral treatment as measured by changes in plasma HIV-1 RNA levels. This assay is not intended to be used as a screening test for HIV-1 or as a diagnostic test to confirm the presence of HIV-1 infection.

Contents:

1. **INTERNAL CONTROL** Abbott RealTi*me* HIV-1 Internal Control (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, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, HBV DNA, and anti-HCV. Preservatives: 0.1% ProClin® 300 and 0.15% ProClin 950.
2. **AMPLIFICATION REAGENT PACK** Abbott RealTi*me* HIV-1 Amplification Reagent Pack (4 packs, 24 tests/pack). Each Reagent Pack contains:
   - 1 Bottle (0.141 mL) Thermostable rTth Polymerase Enzyme (2.9 to 3.5 Units/µL) in buffered solution.
   - 1 Bottle (1.10 mL) HIV-1 Oligonucleotide Reagent. <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.

CE 1434

REF 2G31-90

IVD

-10°C

ProClin is a registered trademark of Rohm and Haas.
Armored RNA is a registered trademark of Ambion.
Abbott RealTi*me* is a trademark of Abbott Laboratories.

51-602111/R5

GTIN

LOT

REF

CAUTION: Handle human sourced materials as potentially infectious. Consult instructions for use. / ACHTUNG: Humanmaterial gilt als potentiell infektiös und muss mit der entsprechenden Vorsicht gehandhabt werden. Siehe Gebrauchsanweisung. / ATTENTION : Manipuler les produits d'origine humaine comme s'ils étaient potentiellement infectieux. Consulter les instructions d'utilisation. / ATENCIÓN: maneje los productos de origen humano como potencialmente infecciosos. Consulte las instrucciones de uso. / ATTENZIONE: Trattare i materiali di origine umana come potenzialmente infettivi. Consultare le istruzioni per l'uso. / ATENÇÃO: manusear os materiais de origem humana como potencialmente infecciosos. Consultar as instruções de utilização.

Abbott RealTi*me* HIV-1

Amplification Reagent Kit

(4x24 Tests)

(pt) Para utilização *in vitro*. O ensaio Abbott RealTi*me* HIV-1 é um ensaio, para utilização *in vitro*, de transcriptase reversa associada à reacção em cadeia da polimerase (RT-PCR) para a quantificação do vírus da imunodeficiência humana tipo 1 (HIV-1) em plasma humano de indivíduos infectados pelo HIV-1. O ensaio Abbott RealTi*me* HIV-1 destina-se a ser utilizado em conjunto com dados clínicos e outros marcadores laboratoriais como indicador de prognóstico de doença e como meio auxiliar na avaliação da resposta viral à terapêutica anti-retroviral, medida através das alterações nos níveis plasmáticos de ARN do HIV-1. Este ensaio não se destina a ser utilizado como teste de rastreio do HIV-1 nem como teste de diagnóstico confirmatório da presença de infecção pelo HIV-1.

Conteúdo:

1. **INTERNAL CONTROL** Abbott RealTi*me* HIV-1 Internal Control (4 frascos, 1,2 ml por frasco). <0,01% de Armored RNA® (ARN protegido) não-infeccioso com sequências de controlo interno em plasma humano negativo. Plasma humano negativo testado e considerado não-reactivo para HBsAg, ARN do HIV, ARN do HCV, ADN do HBV, anticorpos anti-HIV-1/HIV-2 e anti-HCV.
   Conservantes: 0,1% de ProClin® 300 e 0,15% de ProClin 950.
2. **AMPLIFICATION REAGENT PACK** Abbott RealTi*me* HIV-1 Amplification Reagent Pack (4 embalagens, 24 testes/embalagem). Cada embalagem de reagentes contém:
   - 1 Frasco (0,141 ml) de enzima rTth da polimerase termoestável (2,9 a 3,5 Unidades/µl) numa solução tamponada.
   - 1 Frasco (1,10 ml) de reagente de oligonucleótidos do HIV-1. <0,1% de oligonucleótidos sintéticos (4 *primers*, 2 sondas e 1 oligonucleótido desactivador) e <0,3% de dNTPs numa solução tamponada com um corante de referência. Conservantes: 0,1% de ProClin 300 e 0,15% de ProClin 950.
   - 1 Frasco (0,40 ml) de reagente de activação. Solução de cloreto de manganésio 30 mM. Conservantes: 0,1% de ProClin 300 e 0,15% de ProClin 950.

ProClin é uma marca comercial registada de Rohm and Haas.
Armored RNA é uma marca comercial registada de Ambion.
Abbott RealTi*me* é uma marca comercial de Abbott Laboratories.

H317
P261, P280, P272, P302+P352, P333+P313, P362+P364, P501

Product of USA / Produkt aus USA / Produit aux Etats-Unis / Producto de EE.UU. / Prodotto degli USA / Fabricado nos EUA

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

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65205 Wiesbaden, Germany

Abbott

1.3 Abbott RealTi*m*e HIV-1 Calibrator Kit (List No. 2G31-70)

Abbott RealTime HIV-1

Calibrator Kit

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

Contents:

1. CAL A Abbott Real*Time* HIV-1 Calibrator A (12 vials, 1.8 mL per vial). Noninfectious Armored RNA® with HIV-1 sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, HBV DNA, and anti-HCV. Preservatives: 0.1% ProClin® 300 and 0.15% ProClin 950.
2. CAL B Abbott Real*Time* HIV-1 Calibrator B (12 vials, 1.8 mL per vial). Noninfectious Armored RNA with HIV-1 sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, HBV DNA, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

H317
P261, P280, P272, P302+P352,
P333+P313, P362+P364, P501

REF 2G31-70

IVD

51-602102/R5

ProClin is a registered trademark of Rohm and Haas.

Armored RNA is a registered trademark of Ambion.

Abbott Real*Time* is a trademark of Abbott Laboratories.

CAUTION: Handle human sourced materials as potentially infectious. Consult instructions for use. / ACHTUNG: Humanmaterial gilt als potentiell infektiös und muss mit der entsprechenden Vorsicht gehandhabt werden. Siehe Gebrauchsanweisung. / ATTENTION : Manipuler les produits d'origine humaine comme s'ils étaient potentiellement infectieux. Consulter les instructions d'utilisation. / ATENCIÓN: maneje los productos de origen humano como potencialmente infecciosos. Consulte las instrucciones de uso. / ATTENZIONE: Trattare i materiali di origine umana come potenzialmente infettivi. Consultare le istruzioni per l'uso. / ATENÇÃO: manusear os materiais de origem humana como potencialmente infecciosos. Consultar as instruções de utilização.

GTIN

LOT

REF

(de) In-vitro-Diagnostikum. Die Abbott RealTime HIV-1 Kalibratoren dienen zur Kalibrierung des Abbott RealTime HIV-1 Assays bei der quantitativen Bestimmung von Human Immundeficiency Virus Typ 1 (HIV-1) RNA in von HIV-1-infizierten Personen stammendem Humanplasma.

Inhalt:

1. CAL A Abbott RealTime HIV-1 Kalibrator A (12 Fläschchen, 1,8 ml pro Fläschchen). Nicht infektiöse Armored RNA® mit HIV-1-Sequenzen in negativem Humanplasma. Negatives Humanplasma wurde getestet und war nicht reaktiv für HBsAg, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, HBV DNA und anti-HCV. Konservierungsmittel: 0,1 % ProClin® 300 und 0,15 % ProClin 950.
2. CAL B Abbott RealTime HIV-1 Kalibrator B (12 Fläschchen, 1,8 ml pro Fläschchen). Nicht infektiöse Armored RNA mit HIV-1-Sequenzen in negativem Humanplasma. Negatives Humanplasma wurde getestet und war nicht reaktiv für HBsAg, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, HBV DNA und anti-HCV, Konservierungsmittel: 0,1 % ProClin 300 und 0,15 % ProClin 950.

ProClin ist ein eingetragenes Warenzeichen von Rohm and Haas.

Armored RNA ist ein eingetragenes Warenzeichen von Ambion.

Abbott RealTime ist ein Warenzeichen von Abbott Laboratories.

(fr) Pour diagnostic *in vitro*. Les calibrateurs Abbott RealTime HIV-1 sont utilisés pour la calibration du dosage Abbott RealTime HIV-1 lors de la mesure quantitative de l'ARN du virus de l'immunodéficience humaine de type 1 (HIV-1) dans le plasma humain d'individus infectés par le VIH-1.

Contenu :

1. CAL A Calibrateur A Abbott RealTime HIV-1 (12 flacons de 1,8 ml chacun). Armored RNA® non infectieux comprenant des séquences de VIH-1 dans du plasma humain négatif. Le plasma humain négatif a été testé et trouvé non réactif pour l'AgHBs, l'ARN VIH, l'ARN VHC, l'ADN VHB et pour les anticorps anti-VIH-1/VIH-2 et anti-VHC. Conservateurs : ProClin® 300 à 0,1 % et ProClin 950 à 0,15 %.
2. CAL B Calibrateur B Abbott RealTime HIV-1 (12 flacons de 1,8 ml chacun). Armored RNA non infectieux comprenant des séquences de VIH-1 dans du plasma humain négatif. Le plasma humain négatif a été testé et trouvé non réactif pour l'AgHBs, l'ARN VIH, l'ARN VHC, l'ADN VHB et pour les anticorps anti-VIH-1/VIH-2 et anti-VHC. Conservateurs : ProClin® 300 à 0,1 % et ProClin 950 à 0,15 %.

ProClin est une marque déposée de Rohm and Haas.

Armored RNA est une marque déposée d'Ambion.

Abbott RealTime est une marque commerciale d'Abbott Laboratories.

(es) Para uso en diagnóstico *in vitro*. Los calibradores Abbott RealTime HIV-1 se utilizan para la calibración del ensayo Abbott RealTime HIV-1 en la determinación cuantitativa del RNA del virus de la inmunodeficiencia humana del tipo 1 (VIH-1) en plasma humano de pacientes infectados por el VIH-1.

Contenido:

1. CAL A Calibrador A Abbott RealTime HIV-1 (12 tubos de 1,8 ml cada uno). Armored RNA® no infeccioso con secuencias de VIH-1 en plasma humano negativo. El plasma humano negativo se ha analizado y no se ha encontrado reactividad para HBsAg, ni para el RNA del VIH, ni para el RNA del VHC, ni reactividad de anticuerpos anti-VIH-1/VIH-2, ni anti-VHC ni para el DNA del VHB. Conservantes: ProClin® 300 al 0,1% y ProClin 950 al 0,15%.
2. CAL B Calibrador B Abbott RealTime HIV-1 (12 tubos de 1,8 ml cada uno). Armored RNA no infeccioso con secuencias de VIH-1 en plasma humano negativo. El plasma humano negativo se ha analizado y no se ha encontrado reactividad para HBsAg, ni para el RNA del VIH, ni para el RNA del VHC, ni reactividad de anticuerpos anti-VIH-1/VIH-2, ni anti-VHC ni para el DNA del VHB. Conservantes: ProClin 300 al 0,1% y ProClin 950 al 0,15%.

ProClin es una marca comercial registrada de Rohm and Haas.

Armored RNA es una marca comercial registrada de Ambion.

Abbott RealTime es una marca comercial de Abbott Laboratories.

(it) Per uso diagnostico *in vitro*. I calibratori Abbott RealTime HIV-1 vengono utilizzati per la calibrazione del dosaggio Abbott RealTime HIV-1 per la determinazione quantitativa dell'RNA del virus dell'immunodeficienza umana di tipo 1 (HIV-1) in campioni di plasma umano prelevati da soggetti con infezione da HIV-1.

Contenuto:

1. CAL A Calibratore A Abbott RealTime HIV-1 (12 provette, 1,8 ml per provetta). Armored RNA® non infettivo con sequenze di HIV-1 in plasma umano negativo. Plasma umano negativo analizzato e risultato non reattivo per l'HBsAg, l'HIV RNA, l'HCV RNA, per gli anticorpi anti-HIV-1/HIV-2, per l'HBV DNA e per gli anticorpi anti-HCV. Conservanti: ProClin® 300 allo 0,1% e ProClin 950 allo 0,15%.
2. CAL B Calibratore B Abbott RealTime HIV-1 (12 provette, 1,8 ml per provetta). Armored RNA non infettivo con sequenze di HIV-1 in plasma umano negativo. Plasma umano negativo analizzato e risultato non reattivo per l'HBsAg, l'HIV RNA, l'HCV RNA, per gli anticorpi anti-HIV-1/HIV-2, per l'HBV DNA e per gli anticorpi anti-HCV. Conservanti: ProClin 300 allo 0,1% e ProClin 950 allo 0,15%.

ProClin è un marchio commerciale registrato di Rohm and Haas.

Armored RNA è un marchio commerciale registrato di Ambion.

Abbott RealTime è un marchio commerciale di Abbott Laboratories.

Abbott RealTime HIV-1

Calibrator Kit

(pt) Para utilização *in vitro*. Os Abbott Real*Time* HIV-1 Calibrators destinam-se à calibração do ensaio Abbott Real*Time* HIV-1, quando utilizado para a determinação quantitativa do ARN do vírus da imunodeficiência humana tipo 1 (HIV-1) em plasma humano de indivíduos infectados pelo HIV-1.

Conteúdo:

1. CAL A Abbott Real*Time* HIV-1 Calibrator A (12 frascos, 1,8 ml por frasco). Armored RNA® (ARN protegido) não-infeccioso com sequências de HIV-1 em plasma humano negativo. Plasma humano negativo testado e considerado não-reactivo para HBsAg, ARN do HIV, ARN do HCV, ADN do HBV, anticorpos anti-HIV-1/HIV-2 e anti-HCV. Conservantes: 0,1% de ProClin® 300 e 0,15% de ProClin 950.
2. CAL B Abbott Real*Time* HIV-1 Calibrator B (12 frascos, 1,8 ml por frasco). ARN protegido não-infeccioso com sequências de HIV-1 em plasma humano negativo. Plasma humano negativo testado e considerado não-reactivo para HBsAg, ARN do HIV, ARN do HCV, ADN do HBV, anticorpos anti-HIV-1/HIV-2 e anti-HCV. Conservantes: 0,1% de ProClin 300 e 0,15% de ProClin 950.

ProClin é uma marca comercial registada de Rohm and Haas.

Armored RNA é uma marca comercial registada de Ambion.

Abbott Real*Time* é uma marca comercial de Abbott Laboratories.

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Abbott

1.4 Label for Abbott RealTi*m*e HIV-1 Calibrator A (List No. 2G31A)

Top Edge

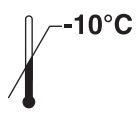
A

REF 2G31A

1.8mL

Abbott RealTi*me* HIV-1

CAL A



Store at ≤ - 10°C

Infection Risk

Exp.

LOT

51-602112/R6

Abbott

Colors: PMS 299 C
PMS 185 C
BLACK

1.5 Label for the Abbott RealTi*me* HIV-1 Calibrator B (List No. 2G31B)

Top Edge

B

REF 2G31B

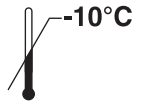
1.8mL

HIV-1 CAL B

Abbott RealTi*me*

HIV-1

CAL B



Store at ≤ - 10°C

Infection Risk

Exp.

LOT

51-602101/R6

Abbott

Colors: PMS 299 C
PMS 185 C
BLACK

Labeling: Duan

51-602101R6.indd 1 8/25/2014 4:01:52 PM

1.6 Abbott RealTi*m*e HIV-1 Control Kit (List No. 2G31-80)

Abbott RealTime HIV-1

Control Kit

(en) For *In Vitro* Diagnostic Use. The Abbott Real*Time* HIV-1 Controls are used to establish run validity of the Abbott Real*Time* HIV-1 assay when used for the quantitative determination of human immunodeficiency virus type 1 (HIV-1) RNA in human plasma from HIV-1 infected individuals.

Contents:

1. **CONTROL –** Abbott Real*Time* HIV-1 Negative Control (8 vials, 1.8 mL per vial). Negative human plasma tested and found to be nonreactive for HBsAg, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, HBV DNA, and anti-HCV. Preservatives: 0.1% ProClin® 300 and 0.15% ProClin 950.
2. **CONTROL L** Abbott Real*Time* HIV-1 Low Positive Control (8 vials, 1.8 mL per vial). Noninfectious Armored RNA® with HIV-1 sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, HBV DNA, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.
3. **CONTROL H** Abbott Real*Time* HIV-1 High Positive Control (8 vials, 1.8 mL per vial). Noninfectious Armored RNA with HIV-1 sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, HBV DNA, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

ProClin is a registered trademark of Rohm and Haas.

Armored RNA is a registered trademark of Ambion.

Abbott Real*Time* is a trademark of Abbott Laboratories.

CAUTION: Handle human sourced materials as potentially infectious. Consult instructions for use. / ACHTUNG: Humanmaterial gilt als potentiell infektiös und muss mit der entsprechenden Vorsicht gehandhabt werden. Siehe Gebrauchsanweisung. / ATTENTION : Manipuler les produits d'origine humaine comme s'ils étaient potentiellement infectieux. Consulter les instructions d'utilisation. / ATENCIÓN: maneje los productos de origen humano como potencialmente infecciosos. Consulte las instrucciones de uso. / ATTENZIONE: Trattare i materiali di origine umana come potenzialmente infettivi. Consultare le istruzioni per l'uso. / ATENÇÃO: manusear os materiais de origem humana como potencialmente infecciosos. Consultar as instruções de utilização.

CE 1434

REF 2G31-80

IVD

-10°C

51-602107/R5

GTIN

LOT

REF

(de) In-vitro-Diagnostikum. Die Abbott RealTime HIV-1 Kontrollen dienen zur Herstellung der Testgültigkeit des Abbott RealTime HIV-1 Assays bei der quantitativen Bestimmung von Human Immunodeficiency Virus Typ 1 (HIV-1) RNA in von HIV-1-infizierten Personen stammendem Humanplasma.

Inhalt:

1. **CONTROL –** Abbott RealTime HIV-1 Negative Kontrolle (8 Fläschchen, 1,8 ml pro Fläschchen). Negatives Humanplasma wurde getestet und war nicht reaktiv für HBsAg, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, HBV DNA und anti-HCV. Konservierungsmittel: 0,1 % ProClin® 300 und 0,15 % ProClin 950.
2. **CONTROL L** Abbott RealTime HIV-1 Niedrig Positive Kontrolle (8 Fläschchen, 1,8 ml pro Fläschchen). Nicht infektiöses Armored RNA® mit HIV-1-Sequenzen in negativem Humanplasma. Negatives Humanplasma wurde getestet und war nicht reaktiv für HBsAg, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, HBV DNA und anti-HCV. Konservierungsmittel: 0,1 % ProClin 300 und 0,15 % ProClin 950.
3. **CONTROL H** Abbott RealTime HIV-1 Hoch Positive Kontrolle (8 Fläschchen, 1,8 ml pro Fläschchen). Nicht infektiöses Armored RNA mit HIV-1-Sequenzen in negativem Humanplasma. Negatives Humanplasma wurde getestet und war nicht reaktiv für HBsAg, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, HBV DNA und anti-HCV. Konservierungsmittel: 0,1 % ProClin 300 und 0,15 % ProClin 950.

ProClin ist ein eingetragenes Warenzeichen von Rohm and Haas.

Armored RNA ist ein eingetragenes Warenzeichen von Ambion.

Abbott RealTime ist ein Warenzeichen von Abbott Laboratories.

(fr) Pour diagnostic *in vitro*. Les contrôles Abbott RealTime HIV-1 sont utilisés pour établir la validité des analyses effectuées avec le dosage Abbott RealTime HIV-1 lors de la mesure quantitative de l'ARN du virus de l'immunodéficience humaine de type 1 (VIH-1) dans le plasma humain d'individus infectés par le VIH-1.

Contenu :

1. **CONTROL –** Contrôle négatif Abbott RealTime HIV-1 (8 flacons de 1,8 ml chacun). Le plasma humain négatif a été testé et trouvé non réactif pour l'AgHBs, l'ARN VIH, l'ARN VHC, l'ADN VHB et pour les anticorps anti-VIH-1/VIH-2 et anti-VHC. Conservateurs : ProClin® 300 à 0,1 % et ProClin 950 à 0,15 %.
2. **CONTROL L** Contrôle positif bas Abbott RealTime HIV-1 (8 flacons de 1,8 ml chacun). Armored RNA® non infectieux contenant des séquences de VIH-1 dans du plasma humain négatif. Le plasma humain négatif a été testé et trouvé non réactif pour l'AgHBs, l'ARN VIH, l'ARN VHC, l'ADN VHB et pour les anticorps anti-VIH-1/VIH-2 et anti-VHC. Conservateurs : ProClin 300 à 0,1 % et ProClin 950 à 0,15 %.
3. **CONTROL H** Contrôle positif haut Abbott RealTime HIV-1 (8 flacons de 1,8 ml chacun). Armored RNA non infectieux contenant des séquences de VIH-1 dans du plasma humain négatif. Le plasma humain négatif a été testé et trouvé non réactif pour l'AgHBs, l'ARN VIH, l'ARN VHC, l'ADN VHB et pour les anticorps anti-VIH-1/VIH-2 et anti-VHC. Conservateurs : ProClin 300 à 0,1 % et ProClin 950 à 0,15 %.

ProClin est une marque déposée de Rohm and Haas.

Armored RNA est une marque déposée d'Ambion.

Abbott RealTime est une marque commerciale d'Abbott Laboratories.

(es) Para uso en diagnóstico *in vitro*. Los controles Abbott RealTime HIV-1 se usan para establecer la validez del ensayo Abbott RealTime HIV-1 en la determinación cuantitativa del ARN del virus de la inmunodeficiencia humana del tipo 1 (VIH-1) en plasma humano de pacientes infectados por el VIH-1.

Contenido:

1. **CONTROL –** Control negativo Abbott RealTime HIV-1 (8 tubos de 1,8 ml cada uno). El plasma humano negativo ha sido analizado y no se ha reconocido reactividad para el HBsAg, ni para el RNA del VIH, RNA del VHC, ni reactividad de anticuerpos anti-VIH-1/VIH-2, ni anti-VHC ni para el DNA del VHB. Conservante: ProClin® 300 al 0,1 % y ProClin 950 al 0,15%.
2. **CONTROL L** Control positivo bajo Abbott RealTime HIV-1 (8 tubos de 1,8 ml cada uno). Armored RNA® no infeccioso con secuencias de VIH-1 en plasma humano negativo. El plasma humano negativo se ha analizado y no se ha encontrado reactividad para HBsAg, ni para el RNA del VIH, ni para el RNA del VHC, ni reactividad de anticuerpos anti-VIH-1/VIH-2, ni anti-VHC ni para el DNA del VHB. Conservante: ProClin 300 al 0,1 % y ProClin 950 al 0,15%.
3. **CONTROL H** Control positivo alto Abbott RealTime HIV-1 (8 tubos de 1,8 ml cada uno). Armored RNA no infeccioso con secuencias de VIH-1 en plasma humano negativo. El plasma humano negativo se ha analizado y no se ha encontrado reactividad para HBsAg, ni para el RNA del VIH, ni para el RNA del VHC, ni reactividad de anticuerpos anti-VIH-1/VIH-2, ni anti-VHC ni para el DNA del VHB. Conservante: ProClin 300 al 0,1% y ProClin 950 al 0,15%.

ProClin es una marca comercial registrada de Rohm and Haas.

Armored RNA es una marca comercial registrada de Ambion.

Abbott RealTime es una marca comercial de Abbott Laboratories.

(it) Per uso diagnostico *in vitro* i controlli Abbott RealTime HIV-1 vengono utilizzati per stabilire la validità dell'analisi con il dosaggio Abbott RealTime HIV-1 per la determinazione quantitativa dell'RNA del virus dell'immunodeficienza umana di tipo 1 (HIV-1) in campioni di plasma umano prelevati da soggetti con infezione da HIV-1.

Contenuto:

1. **CONTROL –** Controllo negativo Abbott RealTime HIV-1 (8 provette, 1,8 ml per provetta). Plasma umano negativo analizzato e risultato non reattivo per l'HBsAg, l'HIV RNA, l'HCV RNA, per gli anticorpi anti-HIV-1/HIV-2, per l'HBV DNA e per gli anticorpi anti-HCV. Conservanti: ProClin® 300 allo 0,1% e ProClin 950 allo 0,15%.
2. **CONTROL L** Controllo positivo basso Abbott RealTime HIV-1 (8 provette, 1,8 ml per provetta). Armored RNA® non infettivo con sequenze di HIV-1 in plasma umano negativo. Plasma umano analizzato e risultato non reattivo per l'HBsAg, l'HIV RNA, l'HCV RNA, per gli anticorpi anti-HIV-1/HIV-2, per l'HBV DNA e per gli anticorpi anti-HCV Conservanti: ProClin 300 allo 0,1% e ProClin 950 allo 0,15%.
3. **CONTROL H** Controllo positivo alto Abbott RealTime HIV-1 (8 provette, 1,8 ml per provetta). Armored RNA non infettivo con sequenze di HIV-1 in plasma umano negativo. Plasma umano analizzato e risultato non reattivo per l'HBsAg, l'HIV RNA, l'HCV RNA, per gli anticorpi anti-HIV-1/HIV-2, per l'HBV DNA e per gli anticorpi anti-HCV. Conservanti: ProClin 300 allo 0,1% e ProClin 950 allo 0,15%.

ProClin è una marca comercial registrada de Rohm and Haas.

Armored RNA è una marca comercial registrada de Ambion.

Abbott RealTime è un marchio commerciale di Abbott Laboratories.

Abbott RealTime HIV-1

Control Kit

(pt) Para utilização *in vitro*. Os Abbott Real*Time* HIV-1 Controls são utilizados para estabelecer a validade do ensaio Abbott Real*Time* HIV-1 quando utilizado para a determinação quantitativa de ARN do vírus da imunodeficiência humana tipo 1 (HIV-1) em plasma humano de indivíduos infectados pelo HIV-1.

Conteúdo:

1. **CONTROL –** Abbott Real*Time* HIV-1 Negative Control (8 frascos, 1,8 ml por frasco). Plasma humano negativo testado e considerado não-reactivo para HBsAg, ARN do HCV, ADN do HBV, anticorpos anti-HIV-1/HIV-2 e anti-HCV. Conservantes: 0,1% de ProClin® 300 e 0,15% de ProClin 950.
2. **CONTROL L** Abbott Real*Time* HIV-1 Low Positive Control (8 frascos, 1,8 ml por frasco). Armored RNA® (ARN protegido) não-infeccioso com sequências de HIV-1 em plasma humano negativo. Plasma humano negativo testado e considerado não-reactivo para HBsAg, ARN do HIV, ARN do HCV, ADN do HBV, anticorpos anti-HIV-1/HIV-2 e anti-HCV. Conservantes: 0,1% de ProClin 300 e 0,15% de ProClin 950.
3. **CONTROL H** Abbott Real*Time* HIV-1 High Positive Control (8 frascos, 1,8 ml por frasco). ARN protegido não-infeccioso com sequências de HIV-1 em plasma humano negativo. Plasma humano negativo testado e considerado não-reactivo para HBsAg, ARN do HIV, ARN do HCV, ADN do HBV, anticorpos anti-HIV-1/HIV-2 e anti-HCV. Conservantes: 0,1% de ProClin 300 e 0,15% de ProClin 950.

ProClin é uma marca comercial registada de Rohm and Haas.

Armored RNA é uma marca comercial registada de Ambion.

Abbott Real*Time* é uma marca comercial de Abbott Laboratories.

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65205 Wiesbaden, Germany

Abbott

1.7 Abbott RealTi*m*e HIV-1 High Positive Control (List No. 2G31X)

Top Edge

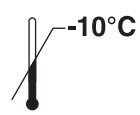
++

REF 2G31X

1.8mL

Abbott RealTi*me*

HIV-1 CONTROL H



Store at ≤ - 10°C

Infection Risk

Exp.

LOT

51-602105/R6

Abbott

Colors: PMS 299 C
PMS 185 C
BLACK

Labeling: Duan

51-602105R6.indd 1 9/23/2014 1:02:34 PM

1.8 Abbott RealTi*m*e HIV-1 Low Positive Control (List No. 2G31W)

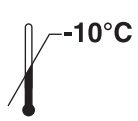
Top Edge

REF 2G31W

1.8mL

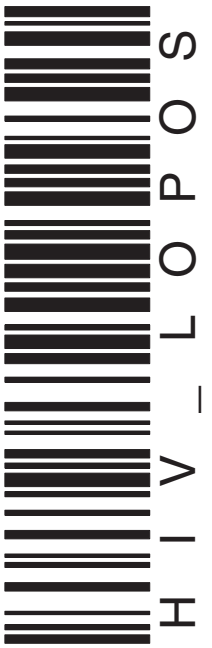
Abbott RealTi*me*

HIV-1 CONTROL L



Store at ≤ - 10°C

Infection Risk



Exp.

LOT

51-602104/R7

Abbott

Colors: PMS 299 C
PMS 185 C
BLACK

1.9 Abbott RealTi*m*e HIV-1 Negative Control (List No. 2G31Z)

Top Edge

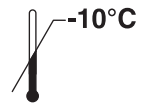
REF 2G31Z

1.8mL

HIV-1 NEG

Abbott RealTi*me*

HIV-1 CONTROL –



Store at ≤ - 10°C

Infection Risk

Exp.

LOT

51-602106/R6

Abbott

Colors: PMS 299 C
PMS 185 C
BLACK

Labeling: Duan

1.10 Abbott RealTi*m*e HIV-1 Internal Control (List No. 2G31Y)

Top Edge

IC

Abbott RealTi*me* HIV-1

INTERNAL CONTROL

-10°C

Store at ≤ - 10°C

Infection Risk

Abbott Molecular Inc.
Des Plaines, Il 60018 USA

Exp.

LOT

REF 2G31Y

1.2 mL

51-602110/R6

Colors: PMS 299 C
PMS 185 C
BLACK

2. Instructions for Use[3]

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

Abbott RealTi*me* HIV-1

en

REF 02G31-010

51-608282/R10

REF 02G31-010

51-608282/R10

NOTE: CHANGES HIGHLIGHTED

Key to Symbols Used

REF	Reference Number
LOT	Lot Number
IVD	In Vitro Diagnostic Medical Device
In Vitro Test	In Vitro Test
	Use By
	Contains sufficient for <n> tests
CONTROL –	Negative Control
CONTROL L	Low Positive Control
CONTROL H	High Positive Control
CAL A	Calibrator A
CAL B	Calibrator B
INTERNAL CONTROL	Internal Control
AMPLIFICATION REAGENT PACK	Amplification Reagent Pack
	Maximum Time Allowed
	Upper Limit of Temperature
	Consult instructions for use
	Caution
	Warning
EC REP	Authorized Representative in the European Community
	Manufacturer

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

NOTICE TO USER

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

CUSTOMER SERVICE INTERNATIONAL: CALL YOUR ABBOTT REPRESENTATIVE

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

NAME

Abbott RealTi*me* HIV-1

INTENDED USE

The Abbott RealTi*me* HIV-1 assay is an in vitro reverse transcription polymerase chain reaction (RT-PCR) assay for the quantitation of Human Immunodeficiency Virus type 1 (HIV-1) in whole blood spotted on cards as dried blood spots (DBS) (i.e. obtained via venipuncture or capillary blood) or human plasma from HIV-1 infected individuals. The Abbott RealTi*me* HIV-1 is intended for use in conjunction with clinical presentation and other laboratory markers as an indicator of disease prognosis and for use as an aid in assessing viral response to antiretroviral treatment as measured by changes in DBS or plasma HIV-1 RNA levels. This assay is not intended to be used as a screening test for HIV-1 or as a diagnostic test to confirm the presence of HIV-1 infection.

INTENDED USER

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

SUMMARY AND EXPLANATION OF THE TEST

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

Quantitative measurement of HIV levels in peripheral blood has greatly contributed to the understanding of the pathogenesis of HIV infection[10,11] and has been shown to be an essential parameter in prognosis and management of HIV infected individuals.[12-17] Decisions regarding initiation or changes in antiretroviral therapy are guided by monitoring plasma or DBS HIV RNA levels (viral load), CD4+ T cell count, and the patient's clinical condition.[17-19] The goal of antiretroviral therapy is to reduce the HIV virus in plasma and DBS to below detectable levels of available viral load tests.[17,20]

HIV RNA levels in plasma or DBS can be quantitated by nucleic acid amplification or signal amplification technologies.[19-23] The Abbott RealTi*me* HIV-1 assay uses Polymerase Chain Reaction (PCR) technology with homogenous real-time fluorescent detection. Partially double-stranded fluorescent probe design allows detection of diverse group M subtypes and group O isolates. The assay is standardized against a viral standard from the Virology Quality Assurance (VQA) Laboratory of the AIDS Clinical Trial Group,[24] and against World Health Organization (WHO) 1st International Standard for HIV-1 RNA (97/656).[25,26] The assay results can be reported in copies/mL or International Units/mL (IU/mL) or their log base 10 equivalents.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The Abbott RealTi*me* HIV-1 assay consists of 3 reagent kits:

- Abbott RealTi*me* HIV-1 Amplification Reagent Kit
- Abbott RealTi*me* HIV-1 Control Kit
- Abbott RealTi*me* HIV-1 Calibrator Kit

The Abbott RealTi*me* HIV-1 assay uses RT-PCR[27] to generate amplified product from the RNA genome of HIV-1 in clinical specimens. An RNA sequence that is unrelated to the HIV-1 target sequence is introduced

into each specimen at the beginning of sample preparation. This unrelated RNA sequence is simultaneously amplified by RT-PCR, and serves as an internal control (IC) to demonstrate that the process has proceeded correctly for each sample. The amount of HIV-1 target sequence that is present at each amplification cycle is measured through the use of fluorescent-labeled oligonucleotide probes on the Abbott *m*2000*rt* instrument. The probes do not generate signal unless they are specifically bound to the amplified product. The amplification cycle at which fluorescent signal is detected by the Abbott *m*2000*rt* is proportional to the log of the HIV-1 RNA concentration present in the original sample.

Sample Preparation

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

The Abbott *m*Sample Preparation System (4 × 24 Preps) uses magnetic particle technology to capture nucleic acids and washes the particles to remove unbound sample components. The bound nucleic acids are eluted and transferred to output tubes or a 96 deep-well plate. The nucleic acids are then ready for amplification. The IC is taken through the entire sample preparation procedure along with the calibrators, controls, and specimens.

Two automated instrument systems, the Abbott *m*2000*sp* or the Abbott *m*1000 System can be used to prepare samples for the Abbott RealTi*m*e HIV-1 assay. The Abbott *m*2000*sp* provides automated sample eluate transfer and reaction assembly in the Abbott 96-Well Optical Reaction Plate, while the Abbott *m*1000 System requires manual sample eluate transfer and reaction assembly.

Alternatively, samples can be prepared manually using the Abbott *m*Sample Preparation System, followed by manual reaction assembly.

Reagent Preparation and Reaction Plate Assembly

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

Abbott *m*1000 System users and manual sample preparation method users manually combine the Abbott RealTi*m*e HIV-1 amplification reagent components to create the amplification master mix and transfer aliquots of the master mix and sample eluates to the reaction plate. The plate is ready, after manual application of the optical seal and centrifugation, for transfer to the Abbott *m*2000*rt*.

Amplification

During the amplification reaction on the Abbott *m*2000*rt*, the target RNA is converted to cDNA by the reverse transcriptase activity of the thermostable rTth DNA polymerase. First, the HIV-1 and IC reverse primers anneal to their respective targets and are extended during a prolonged incubation period. After a denaturation step, in which the temperature of the reaction is raised above the melting point of the double-stranded cDNA:RNA product, a second primer anneals to the cDNA strand and is extended by the DNA polymerase activity of the rTth enzyme to create a double-stranded DNA product.

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

The target sequence for the Abbott RealTi*m*e HIV-1 assay is in the *pol* integrase region of the HIV-1 genome. This region is highly conserved.[28] The primers are designed to hybridize to the *pol* integrase region with the fewest possible mismatches among various subtypes.

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

Detection

During the read cycles of amplification on the Abbott *m*2000*rt*, the temperature is lowered further to allow fluorescent detection of amplification products as the HIV-1 and IC probes anneal to their targets (real-time fluorescence detection). The HIV-1 probe has a fluorescent moiety that is covalently linked to the 5′ end. A short oligonucleotide (quencher oligonucleotide) is complementary to the 5′ end of the HIV-1 probe and has a quencher molecule at its 3′ end. In the absence of HIV-1 target, the HIV-1 probe fluorescence is quenched through hybridization to the quencher oligonucleotide. In the presence of the HIV-1 target sequence, the HIV-1 probe preferentially hybridizes to the target sequence, dissociating from the quencher oligonucleotide, allowing fluorescent detection.

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

The HIV-1 and IC specific probes are each labeled with a different fluorophore, thus allowing for simultaneous detection of both amplified products at each cycle. The amplification cycle at which fluorescent signal is detected by the Abbott *m*2000*rt* is proportional to the log of the HIV-1 RNA concentration present in the original sample.

Optional Amplification Reagent Extended Use Feature

An overview of this feature is provided in Appendix 1 of this package insert.

The optional amplification reagent extended use feature allows amplification reagent packs containing prepared master mix to be stored at −25 to −15°C, capped and protected from light, for up to 7 days before a second use. The internal control (IC) may be used again within 14 days if the vial remains capped at −25 to −15°C until the second use. The amplification reagent extended use feature applies only to samples prepared using the *m*2000*sp* system. Amplification reagent packs and IC can be used a total of 2 times. Throughout this manual, amplification reagent packs and IC that have not yet been used will be referred to as **new** amplification reagent packs and IC (ie, initial use). Amplification reagent packs that have been used once and contain prepared master mix will be referred to as **partial** amplification reagent packs. IC vials that have been used once will be referred to as **partial** vials of IC.

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 RealTi*m*e HIV-1 assay. Refer to the **SPECIAL PRECAUTIONS** section of this package insert.

REAGENTS

Abbott RealTi*m*e HIV-1 Amplification Reagent Kit (List No. 02G31-010)

1. **INTERNAL CONTROL** Abbott RealTi*m*e HIV-1 Internal Control (List No. 2G31Y0002) (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, HIV RNA, HCV RNA, HBV DNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin® 300 and 0.15% ProClin 950.
2. **AMPLIFICATION REAGENT PACK** Abbott RealTi*m*e HIV-1 Amplification Reagent Pack (List No. 2G31) (4 packs, 24 tests/pack)
   - 1 bottle (0.141 mL) Thermostable rTth Polymerase Enzyme (2.9 to 3.5 Units/µL) in buffered solution.
   - 1 bottle (1.10 mL) HIV-1 Oligonucleotide Reagent. < 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.

NOTE: To use the amplification reagent extended use feature, reagent packs with a 6-digit serial number above the barcodes must be used.

Abbott RealTi*m*e HIV-1 Control Kit (List No. 2G31-80)

1. CONTROL – Abbott RealTi*m*e HIV-1 Negative Control (List No. 2G31Z) (8 vials, 1.8 mL per vial) Negative human plasma tested and found to be nonreactive for HBsAg, HIV RNA, HCV RNA, HBV DNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.
2. CONTROL L Abbott RealTi*m*e HIV-1 Low Positive Control (List No. 2G31W) (8 vials, 1.8 mL per vial) Noninfectious Armored RNA with HIV-1 sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HIV RNA, HCV RNA, HBV DNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.
3. CONTROL H Abbott RealTi*m*e HIV-1 High Positive Control (List No. 2G31X) (8 vials, 1.8 mL per vial). Noninfectious Armored RNA with HIV-1 sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HIV RNA, HCV RNA, HBV DNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

Abbott RealTi*m*e HIV-1 Calibrator Kit (List No. 2G31-70)

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

WARNINGS AND PRECAUTIONS

IVD

For In Vitro Diagnostic Use

This assay is not intended to be used as a screening test for HIV-1 or as a diagnostic test to confirm the presence of HIV-1 infection.

Safety Precautions

Refer to the Abbott *m*1000 Operating Manual, Safety Section, the Manual Sample Preparation for Abbott RealTi*m*e RNA Assays Procedure, Handling Precaution Section, or Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals, Hazard Section, for instructions on safety precautions.

CAUTION: This preparation contains human sourced and/or potentially infectious components. Components sourced from human blood have been tested and found to be nonreactive by FDA-licensed tests for antibody to 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 laboratory safety procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories,[29] OSHA Standards on Bloodborne Pathogens,[30] CLSI Document M29-A3,[31] and other appropriate biosafety practices.[32] Therefore all human sourced materials should be considered infectious.

These precautions include, but are not limited to, the following:

- Wear gloves when handling specimens or reagents.
- Do not pipette by mouth.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where these materials are handled.
- Clean and disinfect spills of specimens by including the use of a tuberculocidal disinfectant such as 1.0% sodium hypochlorite or other suitable disinfectant.[29]
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and federal regulations.[32]

Components of the Abbott RealTi*m*e HIV-1 Calibrator Kit (2G31-70) and Abbott RealTi*m*e HIV-1 Control Kit (2G31-80), and the HIV-1 Oligonucleotide Reagent, HIV-1 Internal Control and Activation Reagent contain the following components:

- 2-Methyl-2H-isothiazol-3-one (EC no. 220-239-6)
- 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)
- 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. 220-239-6)(3:1)

The following warnings apply:

Warning

H317	May cause an allergic skin reaction.
P261	Avoid breathing mist/vapours/spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves/protective clothing/ eye protection.
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 before reuse.
P501	Dispose of contents/container in accordance with local regulations.

SPECIAL PRECAUTIONS

Handling Precautions for Plasma Specimens

The Abbott RealTi*m*e HIV-1 assay is only for use with plasma specimens that have been handled and stored in capped tubes as described in the **SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE** section.

Handling Precautions for DBS Specimens

The Abbott RealTi*m*e HIV-1 assay is only for use with whole blood or DBS specimens that have been handled and stored as described in the **SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE** section.

During preparation of samples, compliance with good laboratory practices is essential to minimize the risk of cross-contamination between samples and the inadvertent introduction of ribonucleases (RNases) into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with RNA.

Amplification technologies such as PCR are sensitive to accidental introduction of product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the reagents used become contaminated by accidental introduction of even a few molecules of amplification product. Measures to reduce the risk of contamination in the laboratory include physically separating the activities involved in performing PCR in compliance with good laboratory practices.

Work Areas

Use 3 dedicated areas within the laboratory for performing the Abbott RealTi*m*e HIV-1 assay with the Abbott *m*1000 System or manual sample preparation using the Abbott *m*Sample Preparation System and Abbott *m*2000*rt*:

- The **Reagent Preparation Area** is dedicated to combining the Abbott RealTi*m*e HIV-1 amplification reagent components to create the amplification master mix and transferring aliquots of the master mix to the reaction plate. Laboratory coats, pipettes, pipette tips, and vortexers 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.**
- The **Sample Preparation Area** is dedicated to processing samples (specimens, Abbott RealTi*m*e HIV-1 Controls, and Calibrators), and to adding processed samples, controls, and calibrators to the Abbott 96-Well Optical Reaction Plate. **All reagents used in the Sample Preparation Area should remain in this dedicated area at all times. Laboratory coats, pipettes, pipette tips, and vortexers used in the Sample Preparation Area must remain in this area and not be moved to either the Reagent Preparation Area or the Amplification Area. Do not bring amplification product into the Sample Preparation Area.**
- The **Amplification Area** is dedicated to the amplification and detection of amplified product. Laboratory coats and equipment used in the **Amplification Area** must remain in this area and not be moved to either the **Reagent Preparation Area** or the **Sample Preparation Area**.

Only 2 dedicated areas, Sample Preparation Area and Amplification Area, are recommended when the Abbott *m*2000*sp* and Abbott *m*2000*rt* are used.

Components contained within a kit are intended to be used together. Do not mix components from different kit lots. For example, do not use the negative control from control kit lot X with the positive controls from control kit lot Y.

Do not use kits or reagents after the expiration dates shown on kit labels.

Work area and instrument platforms must be considered potential sources of contamination. Change gloves after contact with potential contaminants (specimens, eluates, and/or amplified product) before handling unopened reagents, negative control, positive controls, calibrators, or specimens. Refer to the Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals for instrument cleaning procedures.

If the Abbott *m*1000 System or Abbott *m*2000*sp* instrument run is aborted, dispose of all commodities and reagents according to the Abbott *m*1000 Operating Manual or the Abbott *m*2000*sp* Operations Manual.

NOTE: New amplification reagents may be saved, stored, and used a second time, as described in this manual.

If the Abbott *m*2000*sp* master mix addition protocol is aborted, seal the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott *m*2000*sp* Operations Manual, Hazards section, along with the gloves used to handle the plate.

If the Abbott *m*2000*rt* instrument run is interrupted or aborted, seal the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott *m*2000*rt* Operations Manual along with the gloves used to handle the plate.

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

NOTE: Autoclaving the sealed Reaction Plate will not degrade the amplified product and may contribute to the release of the amplified product by opening the sealed plate. The laboratory area can become contaminated with amplified product if the waste materials are not carefully handled and contained.

Aerosol Containment

To reduce the risk of nucleic acid contamination due to aerosols formed during manual pipetting, aerosol barrier pipette tips must be used for all manual pipetting. The pipette tips must be used only 1 time. Clean and disinfect spills of specimens and reagents as stated in the Abbott *m*1000 Operating Manual or the Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals.

Contamination and Inhibition

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

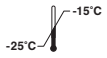
- Wear appropriate personal protective equipment at all times.
- Use powder-free gloves.
- Change gloves after having contact with potential contaminants (such as specimens, eluates, and/or amplified product).
- To reduce the risk of nucleic acid contamination due to aerosols formed during pipetting, pipettes with aerosol barrier tips must be used for all pipetting. The length of the tip should be sufficient to prevent contamination of the pipette barrel. While pipetting, care should be taken to avoid touching the pipette barrel to the inside of the sample tube or container. The use of extended aerosol barrier pipette tips is recommended.
- Change aerosol barrier pipette tips between ALL manual liquid transfers.
- The Abbott *m*Sample Preparation System (4 × 24 Preps) reagents are single use only. Use new reagent troughs or vessels, reaction vessels, and newly opened reagents for every new Abbott RealTi*m*e HIV-1 assay run. At the end of each run, discard all remaining reagents from the worktable as stated in the Abbott *m*1000 Operating Manual or the Abbott *m*2000*sp* Operations Manual and the Abbott *m*Sample Preparation System (4 × 24 Preps) product information sheet.
- Follow instructions in this manual to recap and store amplification reagents that are to be used a second time.

Contamination From External dU-Containing Amplified Product

Laboratories that use or have used HIV-1 amplification assays that include post-PCR processing of the amplified product may be contaminated by dU-containing amplified product. Such contamination may cause inaccurate results in the Abbott RealTi*m*e HIV-1 assay. Refer to the **Monitoring the Laboratory for the Presence of Contamination** section of the package insert. 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 the uracil-N-glycosylase (UNG) (List No. 06L87-02) contamination control procedure if decontamination of the laboratory is unsuccessful. Refer to Appendix 2 for the optional UNG Procedure.

STORAGE INSTRUCTIONS

Abbott RealTi*m*e HIV-1 Amplification Reagent Kit (List No. 02G31-010)



- **New** and **Partial** Abbott RealTi*m*e HIV-1 Amplification Reagent Packs and Internal Control (IC) vials must be stored at –25 to –15°C when not in use. Care must be taken to separate the Abbott RealTi*m*e HIV-1 Amplification Reagent Pack that is in use from direct contact with samples, calibrators, and controls.
- **Partial** amplification reagent packs and IC must be stored at –25 to –15°C, capped, upright, and protected from light, following initial use. If stored this way, **partial** amplification reagent packs with prepared master mix may be used a second time within 7 days of initial use. IC may also be used a second time within 14 days of being thawed, if stored capped at –25 to –15°C.
- After 2 uses, discard partial amplification reagent packs and IC.

Abbott RealTi*m*e HIV-1 Control Kit (List No. 2G31-80)

- The Abbott RealTi*m*e HIV-1 Negative and Positive Controls must be stored at –10°C or colder.

Abbott RealTi*m*e HIV-1 Calibrator Kit (List No. 2G31-70)

- The Abbott RealTi*m*e HIV-1 Calibrator A and Calibrator B must be stored at –10°C or colder.

SHIPPING CONDITIONS

- Abbott RealTi*m*e HIV-1 Amplification Reagent Kit: Ship on dry ice.
- Abbott RealTi*m*e HIV-1 Control Kit: Ship on dry ice.
- Abbott RealTi*m*e HIV-1 Calibrator Kit: Ship on dry ice.

INDICATION OF INSTABILITY OR DETERIORATION OF REAGENTS

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

INSTRUMENT PROCEDURE

The nucleic acid testing (NAT) software must be installed on the Abbott *m*1000 System prior to performing the assay. For detailed information on NAT software installation, refer to the Abbott *m*1000 Operating Manual, Putting into Operation section.

The Abbott RealTi*m*e HIV-1 application files with the extended use feature enabled must be installed on the Abbott *m*2000*sp* and Abbott *m*2000*rt* systems from the Abbott RealTi*m*e HIV-1 *m*2000 ROW System Combined Application CD-ROM (List No. 1L68-014 or higher) prior to performing the assay. For detailed information on application file installation, refer to the Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals, Operating Instructions section.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE

Specimen Collection and Storage

Freshly drawn whole blood (ACD-A and EDTA) may be held at 15 to 30˚C for up to 24 hours or at 2 to 8˚C for up to 48 hours prior to processing.

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

To prepare EDTA and ACD-A plasma specimens, follow the manufacturer's instructions for processing plasma collection tubes. After plasma preparation, plasma may be stored at 15 to 30˚C for up to 24 hours or at 2 to 8˚C for up to 5 days. Plasma specimens may be stored at -20 +/- 10˚C for up to 60 days. If longer storage is required, plasma specimens must be kept at −70˚C or colder.[33,34] Multiple freeze-thaw cycles should be avoided. If frozen, thaw plasma specimens at 15 to 30˚C or at 2 to 8˚C. Once thawed, if plasma specimens are not being processed immediately, they can be stored at 2 to 8˚C for up to 6 hours.

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

- To prepare DBS, use finger prick or EDTA whole blood (not ACD whole blood). If EDTA whole blood needs to be shipped or stored before spotting, the whole blood sample should be maintained under controlled temperature conditions (Refrigerated 2-8˚C storage and shipment for no more than 48 hours. If 15-30˚C temperature is used, it should not exceed 30˚C and 24 hours). Before spotting, mix the blood using a pipette. Spot the blood onto the one-half-inch (12 millimeter) circles on perforated Munktell paper card (or equivalent such as Whatman 903 and Ahlstrom 226), ensuring that the entire circle is covered. It is recommended that at least 70 µL of blood (approximately 3 to 5 blood drops) be used for each circle to ensure full coverage.
- Air dry the card at ambient temperature.
- Package each card in a sealable plastic bag with 2 to 3 desiccant packs. The cards can be stored under ambient conditions for up to 8 weeks. Under conditions of high humidity (85%), the cards can be stored under ambient temperature for up to 2 weeks. Alternatively, cards can be stored at 2 to 8˚C or −10˚C or colder for up to 12 weeks.

Specimen Transport

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

NOTE: If EDTA whole blood needs to be shipped or stored before spotting, the whole blood sample should be maintained under controlled temperature conditions (Refrigerated 2-8˚C storage and shipment for no more than 48 hours. If 15-30˚C temperature is used, it should not exceed 30˚C and 24 hours).

ABBOTT REALTI*M*E HIV-1 ASSAY PROCEDURE

This Abbott RealTi*m*e HIV-1 package insert contains 3 assay protocols:

- Plasma samples prepared for amplification using the Abbott *m*1000 System or the manual sample preparation method follow **ASSAY PROTOCOL I.**
- Plasma Samples prepared for amplification using the Abbott *m*2000*sp* instrument follow **ASSAY PROTOCOL II**.

The Abbott RealTi*m*e HIV-1 assay provides 4 sample volume options (0.2 mL, 0.5 mL, 0.6 mL, and 1.0 mL). (See assay Protocol II step 6 and **RESULTS FOR PLASMA SPECIMENS** section).

- DBS Samples prepared for amplification using the Abbott *m*2000*sp* instrument follow **ASSAY PROTOCOL III**.

Materials Provided

- Abbott RealTi*m*e HIV-1 Amplification Reagent Kit (List No. 02G31-010)

Materials Required But Not Provided

- Abbott RealTi*m*e HIV-1 Calibrator Kit (List No. 2G31-70)
- Abbott RealTi*m*e HIV-1 Control Kit (List No. 2G31-80)
- Abbott *m*Sample Preparation System DBS Buffer Kit (List No. 09N02-001) (if using DBS Sample Type)

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

For Abbott *m*1000 System

Sample Preparation Area

- Abbott *m*1000 System
- Abbott *m*Sample Preparation System (4 × 24 Preps) (List No. 04J70-24)
- Reaction Vessels
- 20 µL to 1000 µL aerosol barrier pipette tips for precision pipettes
- 11.6 to 16 mm Sample Tubes
- Calibrated precision pipettes capable of delivering 20 to 1000 µL
- 200 µL and 1000 µL disposable tips
- Abbott 96 Deep-Well Plate (List No. 04J71-30)
- Vortex Mixer
- Abbott Optical Adhesive Covers (List No. 04J71-75)
- Abbott Adhesive Cover Applicators
- Abbott Splash-Free Support Base (List No. 09K31-01)
- Reagent Troughs
- 1.5 mL Output Tubes
- Centrifuge capable of 5000*g*
- Uracil-N-glycosylase (UNG) (List No. 06L87-02) (Optional)

For Abbott *m*2000*sp* Instrument

Sample Preparation Area

- Abbott *m*2000*sp* with software version 6.0 or higher
- Abbott *m*Sample Preparation System (4 × 24 Preps) (List No. 04J70-24)
- 5 mL Reaction Vessels
- Calibrated precision pipettes capable of delivering 20 to 1000 µL (Calibrated precision pipettes capable of delivering < 20 µL may be required if using UNG.)
- Aerosol barrier pipette tips for 20 to 1000 µL pipettes (Aerosol barrier pipette tips capable of delivering < 20 µL may be required if using UNG.)
- 11.5 to 16 mm Sample Tubes
- 200 µL and 1000 µL disposable tips
- Vortex Mixer
- Abbott Optical Adhesive Covers (List No. 04J71-75)
- Abbott Adhesive Cover Applicators
- Abbott Splash-Free Support Base (List No. 09K31-01)
- Master Mix Tube (List No. 04J71-80)
- 200 mL Reagent Vessels
- Abbott 96-Deep-Well Plate (List No. 04J71-30)
- Uracil-N-glycosylase (UNG) (List No. 06L87-02) (Optional)
- Abbott 96-Well Optical Reaction Plate (List No. 04J71-70)
- 1.4 mL Micro Vial 15 mm Caps (List No. 3N20-01) optional
- Centrifuge capable of 2000*g*
- Abbott RealTi*m*e HIV-1 *m*2000 ROW System Combined Application CD-ROM (List No. 1L68-014 or higher)

Additional materials required if using DBS Sample Type:

- 15.8 mm well diameter heat block (to fit 15 mm diameter Master Mix Tubes)
- Abbott *m*Sample Preparation System DBS Buffer Kit (List No. 09N02-001)
- *m*2000 System 13mm DBS PoST Set (List No. 09N03-001)

For Abbott *m*1000 System

Reagent Preparation Area

- StrataCooler® 96 Benchtop Cooler or Eppendorf PCR Cooler
- Abbott 96-Well Optical Reaction Plate (List No. 04J71-70)
- Calibrated precision pipettes capable of delivering 20 to 1000 µL
- 20 µL to 1000 µL aerosol barrier pipette tips for precision pipettes
- Single-use RNase/DNase-free tube or container
- Vortex Mixer

For Abbott *m*2000*rt* Instrument

Amplification Area

- Abbott *m*2000*rt* instrument
- Abbott RealTi*m*e HIV-1 *m*2000 ROW System Combined Application CD-ROM (List No. 1L68-014 or higher)
- Abbott *m*2000*rt* Optical Calibration Kit (List No. 04J71-93)

Other Materials

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

†**Note: These 3 items are used in the procedure for Monitoring the Laboratory for the Presence of Contamination**. Refer to the **QUALITY CONTROL PROCEDURES** section of this package insert.

Procedural Precautions

- Read the instructions in this package insert carefully before processing samples.
- Amplification reagents and internal control (IC) may be used up to 2 times, as described in this package insert. The Abbott RealTi*m*e HIV-1 Calibrators, Negative Control, Low Positive Control, and High Positive Control vials are intended for single-use only and should be discarded after use.
- Use aerosol barrier pipette tips or disposable pipettes only one time when pipetting specimens, IC, or amplification reagents. To prevent contamination to the pipette barrel while pipetting, care should be taken to avoid touching the pipette barrel to the inside of the sample tube or container. The use of extended aerosol barrier pipette tips is recommended.
- Monitoring procedures for the presence of amplification product can be found in the **QUALITY CONTROL PROCEDURES** section in this package insert.
- To reduce the risk of nucleic acid contamination, clean and disinfect spills of specimens by including the use of a tuberculocidal disinfectant such as 1.0% sodium hypochlorite or other suitable disinfectant.
- The Abbott RealTi*m*e HIV-1 Calibrators and Controls must be prepared in conjunction with the specimens to be tested. The use of the Abbott RealTi*m*e HIV-1 Controls and Calibrators is integral to the performance of the Abbott RealTi*m*e HIV-1 assay. Refer to the **QUALITY CONTROL PROCEDURES** section of this package insert for details.
- **IMPORTANT: Amplification reagents that will be used a second time must be stored at −25 to −15°C within 50 minutes of the initiation of the master mix addition protocol.**

ASSAY PROTOCOL I: ABBOTT *m*1000 SYSTEM OR THE MANUAL SAMPLE PREPARATION METHOD AND ABBOTT *m*2000*rt* INSTRUMENT

For a detailed description of how to perform an Abbott *m*1000 System and Abbott *m*2000*rt* instrument protocol, refer to the Abbott *m*1000 Operating Manual, Operation section and the Abbott *m*2000*rt* Operations Manual, Operating Instructions section.

Laboratory personnel must be trained to operate the Abbott *m*1000 System and the Abbott *m*2000*rt* instrument. The operator must have a thorough knowledge of the software applications and must follow good laboratory practices.

For plasma samples prepared for amplification using the Abbott *m*1000 System or the manual sample preparation method and using the optional UNG procedure, refer to Appendix 2**.**

1. Thaw assay controls and IC at 15 to 30°C or at 2 to 8°C. Thaw calibrators at 15 to 30°C or at 2 to 8°C only if performing a calibration run; see **QUALITY CONTROL PROCEDURES** section of this package insert.
   - Once thawed, assay controls, IC, and calibrators can be stored at 2 to 8°C for up to 24 hours before use.
   - Vortex each assay calibrator and each control 3 times for 2 to 3 seconds before use. Ensure that the contents of each vial are at the bottom after vortexing by tapping the vials on the bench to bring liquid to the bottom of the vial.
2. Thaw amplification reagents at 15 to 30°C or at 2 to 8°C and store at 2 to 8°C until required for the amplification master mix procedure.
   - Once thawed, the amplification reagents can be stored at 2 to 8°C for up to 24 hours if not used immediately.

NOTE: Use 1 set of sample preparation reagent bottles, 1 vial of IC, and 1 Abbott RealTi*m*e HIV-1 Amplification Reagent Pack to support up to 24 reactions. Use a second set of reagents to support 25 to 48 reactions. A maximum of 48 reactions can be performed per run using an Abbott *m*1000 instrument.

Sample Preparation Area

For sample preparation using the Abbott *m*1000 System, follow steps 3 through 10. For the manual sample preparation method refer to the Extraction Protocol Section of the Manual Sample Preparation for Abbott RealTi*m*e RNA Assays Procedure (List No. 06L73).

3. Gently invert the Abbott *m*Sample Preparation bottles to ensure a homogeneous solution. If crystals are observed in any of the reagent bottles upon opening, allow the reagent to equilibrate at room temperature until the crystals disappear. Do not use the reagents until the crystals have dissolved.
4. Vortex each IC 3 times for 2 to 3 seconds before use.
5. Use a calibrated precision **PIPETTE DEDICATED FOR INTERNAL CONTROL USE ONLY** to add 500 µL of IC to each bottle of *m*Lysis Buffer. Mix by gently inverting the container 5 to 10 times to minimize foaming.
6. **A total of 48 samples can be processed in each run**. A negative control, a low positive control, and a high positive control are included in each run, therefore allowing a maximum of 45 specimens to be processed per run.
   - The Abbott RealTi*m*e HIV-1 assay minimum sample volume and associated rack requirements on the Abbott *m*1000 System are:

		Abbott RealTi*m*e HIV-1 Minimum Sample Volume Assay Application		
Rack	**Tube Diameter[a]**	**0.2 mL**	**0.5 mL**	**1.0 mL**
13 mm	11.6 mm - 14.0 mm	0.7 mL	1.0 mL	1.5 mL
16 mm	15.0 mm - 16.0 mm	1.0 mL	1.3 mL	1.8 mL

[a] Refers to sample tube outer diameter

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

     NOTE: For every stored specimen, the following actions must be done in the order described: vortex the specimen first and follow with centrifugation. If these actions are not performed in this order, then invalid results may occur.
   - Vortex each specimen 3 times for 2 to 3 seconds.
   - Centrifuge specimens at 2000g for 5 minutes before loading onto the Abbott *m*1000 worktable. Aliquot each specimen into clean tubes or vials if necessary. Refer to the Abbott *m*1000 Operating Manual for tube sizes. Avoid touching the inside of the cap when opening tubes.
7. Place the calibrators (if applicable), low and high positive controls, the negative control, and the patient specimens into the Abbott *m*1000 sample rack. Follow directions for performing a user-defined protocol, as described in the Abbott *m*1000 Operating Manual, Operation section.
8. Place the Reaction Vessels into the Abbott *m*1000 1 mL subsystem carrier.
9. Load the Abbott *m*Sample Preparation System reagents and the 1.5 mL Output Tubes on the Abbott *m*1000 System worktable as described in the Abbott *m*1000 Operating Manual, Operation section.
10. Initiate the Abbott *m*1000 protocol as described in the Abbott *m*1000 Operating Manual, Operation section. From the Protocol screen, select the appropriate application file corresponding to the sample volume being tested.
    - **The assembly of the amplification master mix and sample eluates into the Abbott 96-Well Optical Reaction Plate (step 17) must be initiated within 1 hour after completion of Sample Preparation.**

Amplification Area

11. Switch on and initialize the Abbott *m*2000*rt* instrument.

    NOTE: The Abbott *m*2000*rt* instrument requires 15 minutes to warm up.

12. Create the Abbott *m*2000*rt* test order. Refer to the Operating Instructions section of the Abbott *m*2000*rt* Operations Manual. From the Protocol screen, select the appropriate application file corresponding to the sample volume being tested.
    - Enter calibrator (needed if a calibration curve has not been stored on the Abbott *m*2000*rt*) and control lot specific values in the test order for accurate calibration and control evaluation. Lot-specific values are specified in each Abbott RealTi*m*e HIV-1 Calibrator and Control Kit Card.

Reagent Preparation Area

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

13. Prepare the amplification master mix.
    - Each Amplification Reagent Pack supports up to 24 reactions.
    - Prior to opening the amplification reagents, ensure that the contents of the vials are at the bottom by tapping the vials in an upright position on the bench 5 to 10 times to bring the liquid to the bottom of the vials.
    - Prepare the master mix by using a **PIPETTE DEDICATED FOR REAGENT USE ONLY** to add 271 µL of the HIV-1 Activation Reagent (Reagent 1) and 949 µL of the HIV-1 Oligonucleotide Reagent (Reagent 2) together in the Thermostable rTth DNA Polymerase Enzyme bottle (Reagent 3).
    - If performing 25 to 48 reactions, prepare a second amplification master mix with a second Amplification Reagent Pack.
    - **The Abbott *m*2000*rt* protocol (step 20) must be initiated within 40 minutes of the addition of Activation Reagent into the first rTth Enzyme Reagent bottle (step 13).**
14. Pipette the contents of the master mix from the enzyme bottle(s) into a single-use RNase/DNase-free tube and vortex to mix.
15. Place an Abbott 96-Well Optical Reaction Plate in a StrataCooler 96 or Eppendorf PCR Cooler stored as indicated in the instruction manual. Using a **DEDICATED PIPETTE**, dispense 50 µL aliquots of the amplification master mix into the Abbott 96-Well Optical Reaction Plate. A calibrated repeat pipettor may be used. Visually verify that 50 µL has been dispensed into each well.
16. Transfer the Abbott 96-Well Optical Reaction Plate on the StrataCooler 96 or Eppendorf PCR Cooler to the Sample Preparation Area.

Sample Preparation Area

17. In the Sample Preparation Area, transfer 50 µL of sample eluate to the Abbott 96-Well Optical Reaction Plate on the StrataCooler 96 or Eppendorf PCR Cooler. **Use a separate pipette tip for each sample eluate transfer**. During the transfer of each sample, mix the reaction by pipetting up and down 3 to 5 times. Visually verify that 100 µL has been dispensed into each well.
18. Seal the Abbott 96-Well Optical Reaction Plate according to the instructions in the Abbott *m*2000*rt* Operations Manual.
19. Remove the Abbott 96-Well Optical Reaction Plate from the StrataCooler 96 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 5,000*g* for 5 minutes. Transfer to the Amplification Area.
    NOTE: Do not transfer the StrataCooler 96 or Eppendorf PCR Cooler to the Amplification Area.

Amplification Area

20. Place the Abbott 96-Well Optical Reaction Plate in the Abbott *m*2000*rt* instrument. From the Protocol screen, select the appropriate application file corresponding to the sample volume being tested. Initiate the Abbott RealTi*m*e HIV-1 protocol, as described in the Abbott *m*2000*rt* Operations Manual, Operating Instructions section.

POST PROCESSING PROCEDURES FOR PROTOCOL I

1. Clean the StrataCooler 96 or Eppendorf PCR Cooler as described in the instruction manual and return to the Reagent Preparation Area.
2. Remove the 1.5 mL Output Tubes from the worktable and dispose of according to the Abbott *m*1000 Operating Manual.
3. Place the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose of according to the Abbott *m*2000*rt* Operations Manual along with the gloves used to handle the plate.
4. Clean the Splash-Free Support Base before next use, according to the Abbott *m*2000*rt* Operations Manual.
5. For manual sample preparation method users, refer to the Clean Up Section of the Manual Sample Preparation for Abbott RealTi*m*e RNA Assays Procedure (List No. 06L73).

ASSAY PROTOCOL II: PLASMA SAMPLES USING THE ABBOTT *m*2000*sp* AND THE ABBOTT *m*2000*rt* INSTRUMENTS

For a detailed description of how to perform an Abbott *m*2000*sp* instrument and Abbott *m*2000*rt* instrument protocol, refer to the Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals, Operating Instructions sections. The *m*2000*sp* protocol run requires Abbott *m*2000*sp* Software Version 6.0 or higher. Please follow Abbott *m*2000*sp* Operations Manual (List 9K20) version 6 or higher.

Laboratory personnel must be trained to operate the Abbott *m*2000*sp* and Abbott *m*2000*rt* instruments. The operator must have a thorough knowledge of the applications run on the instruments and must follow good laboratory practices.

For plasma Samples prepared for amplification using the Abbott *m*2000*sp* instrument and using the optional UNG procedure, refer to Appendix 2.

1. Thaw assay controls and IC at 15 to 30°C or at 2 to 8°C. Thaw calibrators at 15 to 30°C or at 2 to 8°C only if performing a calibration run; see **QUALITY CONTROL PROCEDURES** section of this package insert.
    - Once thawed, assay controls, IC, and calibrators can be stored at 2 to 8°C for up to 24 hours before use.
    - Vortex each assay calibrator and each control 3 times for 2 to 3 seconds before use. Ensure that the contents of each vial are at the bottom after vortexing by tapping the vials on the bench to bring liquid to the bottom of the vial.
2. Select **new** and/or **partial** amplification reagent packs to be used in the run. Refer to the Abbott *m*2000*sp* Operations Manual (List No. 9K20 version 6 or higher), Operating Instructions section, for instructions pertaining to amplification reagent pack inventory management. Amplification reagent packs must have the same lot number.
    Thaw **new** amplification reagents at 15 to 30°C or at 2 to 8°C and store at 2 to 8°C until required for the amplification master mix procedure. Once thawed, the **new** amplification reagents can be stored at 2 to 8°C for up to 24 hours if not used immediately.
    NOTE: Partial amplification reagent packs being used a second time should NOT be stored at 2 to 8°C before use. They should be kept at −25 to −15°C until needed for master mix addition. Once removed from the freezer, cumulative room temperature exposure should not exceed 25 minutes, including instances where packs are removed from storage, but not used. If 25 minutes is exceeded, discard the partial amplification reagent packs.

The following table shows the number of sample preparation reagents and internal control vials needed based on the number of reactions.

Sample Preparation Reagents and Internal Control Requirements

Reagent	1 to 24 Reactions	25 to 48 Reactions	49 to 72 Reactions	73 to 96 Reactions
*m*Microparticles	1 bottle	2 bottles	2 bottles	2 bottles
*m*Lysis	1 bottle	2 bottles	3 bottles	4 bottles
*m*Wash 1	1 bottle	2 bottles	3 bottles	4 bottles
*m*Wash 2	1 bottle	2 bottles	3 bottles	4 bottles
*m*Elution Buffer	1 bottle	2 bottles	3 bottles	4 bottles
Internal Control[a]	1 **new** vial or 1 **partial** vial	1 **new** vial or 2 **partial** vials	2 **new** vials or 3 **partial** vials	2 **new** vials or 4 **partial** vials

[a] A combination of **new** and **partial** vials of Internal Control may be used.

3. Gently invert the Abbott *m*Sample Preparation bottles to ensure a homogeneous solution. If crystals are observed in any of the reagent bottles upon opening, allow the reagent to equilibrate at room temperature until the crystals disappear. Do not use the reagents until the crystals have dissolved.

4. Vortex each IC 3 times for 2 to 3 seconds before use.
5. Use a calibrated precision **PIPETTE DEDICATED FOR INTERNAL CONTROL USE ONLY** to add 500 µL of IC to each bottle of *m*Lysis Buffer. Mix by gently inverting the container 5 to 10 times to minimize foaming. **Partial** vials of IC can be recapped and stored at −25 to −15°C for a second use.
6. **A total of 96 samples can be processed in each run**, **with the exception of the 1.0 mL Assay Application**. A negative control, a low positive control, and a high positive control are included in each run, therefore allowing a maximum of 93 specimens to be processed per run. For the 1.0 mL Assay Application, a total of 48 samples can be processed in each run, allowing a maximum of 45 specimens to be processed per run.
   - The Abbott RealTi*m*e HIV-1 assay minimum sample volume and associated rack requirements on the Abbott *m*2000*sp* are:

		Abbott RealTi*m*e HIV-1 Minimum Sample Volume Assay Application			
Rack	**Tube Diameter**[a]	**0.2 mL**	**0.5 mL**	**0.6 mL**	**1.0 mL**
13 mm	11.5 - 14.0 mm	0.4 - 0.8 mL	0.7 - 1.2 mL	0.8 - 1.3 mL	1.2 - 1.7 mL
16 mm	14.5 - 16.0 mm	0.4 - 1.0 mL	0.8 - 1.4 mL	0.9 - 1.5 mL	1.3 - 1.9 mL

[a] Refers to sample tube outer diameter. Minimum sample volume varies with tube geometry and size. Refer to the Abbott *m*2000*sp* Operations Manual and **QUICK REFERENCE GUIDE FOR SAMPLE TUBE SIZES AND VOLUMES** for recommended sample input volume.

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

     NOTE: For every stored specimen, the following actions must be done in the order described: vortex the specimen first and follow with centrifugation. If these actions are not performed in this order, then invalid results may occur.
   - Vortex each specimen 3 times for 2 to 3 seconds.
   - Centrifuge specimens at 2000*g* for 5 minutes before loading onto the Abbott *m*2000*sp* worktable. Aliquot each specimen into clean tubes or vials if necessary. Refer to the Abbott *m*2000*sp* Operations Manual for tube sizes. Avoid touching the inside of the cap when opening tubes.
7. Place the low and high positive controls, the negative control, the calibrators, if applicable, and the patient specimens into the Abbott *m*2000*sp* sample rack. If used, bar codes on tube labels must face right for scanning.
8. Place the 5 mL Reaction Vessels into the Abbott *m*2000*sp* 1 mL subsystem carrier.
9. Load the Abbott *m*Sample Preparation System reagents and the Abbott 96 Deep-Well Plate on the Abbott *m*2000*sp* worktable as described in the Abbott *m*2000*sp* Operations Manual, Operating Instructions section.
10. From the Protocol screen, select the appropriate application file corresponding to the sample volume being tested. Initiate the sample extraction protocol as described in the Abbott *m*2000s*p* Operations Manual, Operating Instruction section.
    - Enter calibrator (needed if a calibration curve has not been stored on the Abbott *m*2000*rt*) and control lot specific values in the **Sample Extraction: Worktable Setup, Calibrator and Control** fields. Lot-specific values are specified in each Abbott RealTi*m*e HIV-1 Calibrator and Control Kit Card.
    - **The Abbott *m*2000*sp* 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.
11. Load the amplification reagents and the master mix tube (if needed) on the Abbott *m*2000*sp* worktable after sample preparation is completed. The following table shows the number of amplification reagent packs needed based on the number of reactions. If only 1 amplification reagent pack is being used, no master mix tube is required.

Amplification Reagent Pack Requirements[a]			
1 to 24 Reactions	**25 to 48 Reactions**	**49 to 72 Reactions**	**73 to 96 Reactions**
1 if **new**; up to 4 with **partial** packs	2 if **new**; up to 4 with **partial** packs	3 if **new**; up to 4 with **partial** packs	4 **new** or **partial** packs

[a] Refer to the Abbott *m*2000*sp* Operations Manual (List No. 9K20 version 6 or higher) for instructions on inventory management to determine the maximum number of reactions that can be tested with the **partial** packs selected.

- **Partial amplification reagent packs can only be used on the same Abbott *m*2000*sp* instrument used for the reagent pack's initial preparation. Using an amplification reagent pack for a second time on a different instrument will result in an error, which may delay the run.**
- **Partial and new amplification reagent packs may be used together.**

IMPORTANT: Partial amplification reagent packs should be stored at −25 to −15°C until immediately before the second use. Confirm that master mix is thawed before placing partial pack(s) on the Abbott *m*2000*sp* worktable. Once removed from −25 to −15°C storage, partial amplification reagent packs being used a second time must be used within 25 minutes or discarded. This applies to cumulative room temperature exposure, including instances where packs are removed from storage, but not used.

- Ensure that the contents of **new** amplification reagent packs are at the bottom of the vials prior to opening the amplification reagents by tapping the vials in an upright position on the bench 5 to 10 times.
- Do not tap partial amplification reagent packs being used a second time. Tapping may result in loss of master mix volume in the cap.
- Remove caps. If a **new** amplification reagent pack will be stored for a second use, the vials will need to be recapped for storage. If planning to reuse the original caps to recap the reagent vials, save the original caps. If planning to use fresh caps to recap the reagent vials, original caps may be discarded.
- **Partial amplification reagent packs are loaded to the left of new amplification reagent packs on the Abbott *m*2000*sp* worktable.**
- **Ensure that amplification reagent packs are firmly seated on the instrument.**

12. Select the appropriate deep-well plate that matches the corresponding sample preparation extraction. Initiate the Abbott *m*2000*sp* Master Mix Addition protocol. Follow the instructions as described in the Abbott *m*2000*sp* Operations Manual, Operating Instructions section.

    NOTE: The operator should not manually fill any empty/unfilled wells in the Abbott 96-Well Optical Reaction Plate.
    - After sample extraction is complete, the Abbott *m*2000*sp* automatically fills any empty wells in the Abbott 96-Well Optical Reaction Plate when there are greater than 48 samples processed within a run. Plate fill is not performed for runs containing 48 samples or fewer.
    - If prompted by the instrument, Reagent Carrier 2 should remain in place, minimally containing the reagent vessel for *m*Elution Buffer (Reagent Carrier 2, location 6). If this reagent vessel has been unloaded, place a new reagent vessel with the *m*Elution Buffer label into Reagent Carrier 2, location 6. System fluid will be added to the reagent vessel and used to fill empty wells. Once this process is complete, the system will continue with the master mix addition.

      NOTE: System instructions for use of the automated plate-filling feature are found in the Abbott *m*2000*sp* Operations Manual (List No. 9K20 version 6 or higher), section 5, Operating Instructions, Sample Extraction—Closed Mode.
    - **The Abbott *m*2000*rt* protocol (step 16) must be started within 50 minutes of the initiation of the Master Mix Addition protocol (step 12).**

      NOTE: If the run is aborted for any reason subsequent to step 12, a new 96-well PCR plate must be used if the Abbott *m*2000*sp* Master Mix Addition Protocol (step 12) will be repeated.

13. Switch on and initialize the Abbott *m*2000*rt* instrument in the Amplification Area.

    NOTE: The Abbott *m*2000*rt* 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 *m*2000*sp* instrument has completed addition of samples and master mix according to the Abbott *m*2000*sp* Operations Manual, Operating Instructions section.

15. Place the sealed optical reaction plate into the Abbott Splash-Free Support Base for transfer to the Abbott *m*2000*rt* instrument.

16. Place the Abbott 96-Well Optical Reaction Plate in the Abbott *m*2000*rt* instrument. From the Protocol screen, select the appropriate application file corresponding to the sample volume being tested. Initiate the Abbott RealTi*m*e HIV-1 protocol, as described in the Abbott *m*2000*rt* Operations Manual, Operating Instructions section.

    NOTE: Test order transfer through the use of CD-ROM or network connection with export and import features of the *m*2000*sp* and *m*2000*rt* software is recommended. If creating the Abbott *m*2000*rt* test order manually, enter sample IDs in the corresponding PCR tray locations according to the "Wells for Selected Plate" grid, found on the detail screen of the "PCR Plate Results" on the Abbott *m*2000*sp*. See Section 5 of the Abbott *m*2000*sp* Operations Manual.

17. If a prepared **partial** amplification reagent pack is to be used a second time, cap the 3 reagent vials with the saved caps or new caps (List No. 3N20-01) and promptly store the reagents at −25 to −15°C, protected from light, and in an upright position. Discard any amplification reagent packs that are exhausted or have been used twice.

IMPORTANT: Amplification reagents that will be used a second time must be stored at −25 to −15°C within 50 minutes of the initiation of the master mix addition protocol.

ASSAY PROTOCOL III: DBS SAMPLES USING THE ABBOTT *m*2000*sp* AND THE ABBOTT *m*2000*rt* INSTRUMENTS

For a detailed description of how to perform an Abbott *m*2000*sp* instrument and Abbott *m*2000*rt* instrument protocol, refer to the Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals, Operating Instructions sections. The DBS protocol requires Abbott *m*2000*sp* Software Version 6.0 or higher. Please follow Abbott *m*2000*sp* Operations Manual (List 9K20 version 6 or higher).

A total of 96 samples can be processed in each run. A negative control, a low positive control, and a high positive control are included in each run, therefore allowing a maximum of 93 DBS specimens to be processed per run when calibrators are not included. Process Calibrators and Controls directly as liquid samples; step 1 through step 5 are for DBS only. Do not process plasma specimens on any run where DBS protocol is used. For each DBS sample, a single Abbott Master Mix Tube should be used for the entire sample processing procedure. Ensure DBS samples are labeled throughout processing.

For DBS Samples prepared for amplification using the Abbott *m*2000*sp* instrument and using the optional UNG procedure follow, refer to Appendix 2.

1. Fill the Abbott Master Mix Tube with 1.3 mL of *m*DBS buffer from the Abbott *m*Sample Preparation system DBS Buffer Kit (List No. 09N02-001).

   NOTE: Do not use *m*Lysis Buffer or any other reagents for this step.

2. Hold perforated DBS paper card above the Abbott Master Mix Tube.

3. Push the DBS circle out of the card using a clean pipette tip, one DBS circle per Master Mix Tube. Each DBS should be approximately one-half-inch (12 millimeters) in diameter. **USE A NEW PIPETTE TIP FOR EACH DBS SAMPLE TO PREVENT CROSS CONTAMINATION.**

4. Ensure that the DBS circle is fully submerged in the *m*DBS Buffer by tapping the tube or using the pipette tip to push the DBS into the buffer.

NOTE: If a pipette tip is used to push the DBS into the buffer, ensure that the pipette tip does not cause DBS buffer volume loss due to liquid containment in the tip and/or absorption of the buffer by the tip filter.

5. Manually shake or swirl the sample tubes and then place them in a heat block set at 55°C. Do not vortex the samples. Incubate for 30 minutes (±2 minutes) at 55°C.

6. Meanwhile, thaw assay controls and internal control (IC) at 15 to 30°C or at 2 to 8°C. Thaw calibrators at 15 to 30°C or at 2 to 8°C only if performing a calibration run.

NOTE: Once thawed, assay controls, IC, and calibrators can be stored at 2 to 8°C for up to 24 hours before use.

7. Vortex each assay calibrator and each control 3 times for 2 to 3 seconds before use. Ensure that the contents of each vial are at the bottom by tapping the vials on the bench to bring liquid to the bottom of the vial. Ensure bubbles or foam is not generated; if present, remove with a sterile pipette tip, using a new tip for each vial.

8. Thaw amplification reagents at 15 to 30°C or at 2 to 8°C and store at 2 to 8°C until required for the amplification master mix procedure.

9. Select **new** and/or **partial** amplification reagent packs to be used in the run. Refer to the Abbott *m*2000*sp* Operations Manual (List No. 9K20 version 6 or higher), Operating Instructions section, for instructions pertaining to amplification reagent pack inventory management. Amplification reagent packs must have the same lot number.
   Thaw **new** amplification reagents at 15 to 30°C or at 2 to 8°C and store at 2 to 8°C until required for the amplification master mix procedure. Once thawed, the **new** amplification reagents can be stored at 2 to 8°C for up to 24 hours if not used immediately.

   NOTE: Partial amplification reagent packs being used a second time should NOT be stored at 2 to 8°C before use. They should be kept at −25 to −15°C until needed for master mix addition. Once removed from the freezer, cumulative room temperature exposure should not exceed 25 minutes, including instances where packs are removed from storage, but not used. If 25 minutes is exceeded, discard the partial amplification reagent packs.

The following table shows the number of sample preparation reagents and internal control vials needed based on the number of reactions.

Sample Preparation Reagents and Internal Control Requirements

Reagent	1 to 24 Reactions	25 to 48 Reactions	49 to 72 Reactions	73 to 96 Reactions
*m*Microparticles	1 bottle	2 bottles	2 bottles	2 bottles
*m*Lysis	1 bottle	2 bottles	3 bottles	4 bottles
*m*Wash 1	1 bottle	2 bottles	3 bottles	4 bottles
*m*Wash 2	1 bottle	2 bottles	3 bottles	4 bottles
*m*Elution Buffer	1 bottle	2 bottles	3 bottles	4 bottles
Internal Control[a]	1 **new** vial	2 **new** vials	2 **new** vials	3 **new** vials

[a] A combination of new and partial vials of the same lot of Internal Control may be used. Refer to Appendix 1 for partial IC storage requirements.

10. Gently invert the Abbott *m*Sample Preparation bottles to ensure a homogeneous solution. If crystals are observed in any of the reagent bottles upon opening, allow the reagent to equilibrate at room temperature until the crystals disappear. Do not use the reagents until the crystals have dissolved.

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

12. Use a calibrated precision pipette **DEDICATED FOR INTERNAL CONTROL USE ONLY** to add 750 µL of IC to each bottle of mLysis Buffer. Mix by gently inverting the container 5 to 10 times to minimize foaming.

13. Place the low and high positive controls, the negative control, and the calibrators, if applicable, into the Abbott *m*2000*sp* sample racks.

14. After the incubation is complete, manually shake or swirl the DBS sample tubes and then place them into the Abbott *m*2000*sp* sample racks.

    NOTE: Ensure that the Abbott *m*2000*sp* sample racks have been calibrated specifically for the Abbott RealTi*m*e HIV-1 DBS procedure.

15. Load the 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.

16. Load filled sample racks onto the Abbott *m*2000*sp* 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.
17. Place the 5 mL Reaction Vessels into the Abbott *m*2000*sp* 1 mL subsystem carrier.
18. Load the Abbott mSample Preparation System reagents and the Abbott 96 Deep-Well Plate on the Abbott *m*2000*sp* worktable as described in the Abbott *m*2000*sp* Operations Manual, Operating Instructions section.
19. From the Protocol screen, select the HIV-1 DBS viral load application file. Initiate the sample extraction protocol as described in the Abbott *m*2000*sp* Operations Manual, Operating Instruction section.
20. Enter calibrator (needed if a calibration curve has not been stored on the Abbott *m*2000*rt*) and control lot specific values in the Sample Extraction: Worktable Setup, Calibrator and Control fields. Lot-specific values are specified in each Abbott RealTi*m*e HIV-1 Calibrator and Control Kit Card.
21. The Abbott *m*2000*sp* Master Mix Addition protocol (step 23) must be initiated within 1 hour after completion of Sample Preparation.

    NOTE: Change gloves before handling the amplification reagents.
22. Load the amplification reagents and the master mix tube (if needed) on the Abbott *m*2000*sp* worktable after sample preparation is completed. The following table shows the number of amplification reagent packs needed based on the number of reactions. If only 1 amplification reagent pack is being used, no master mix tube is required.

Amplification Reagent Pack Requirements[a]			
1 to 24 Reactions	**25 to 48 Reactions**	**49 to 72 Reactions**	**73 to 96 Reactions**
1 if **new**; up to 4 with **partial** packs	2 if **new**; up to 4 with **partial** packs	3 if **new**; up to 4 with **partial** packs	4 **new** or **partial** packs

[a] Refer to the Abbott *m*2000*sp* Operations Manual (List No. 9K20 version 6 or higher) for instructions on inventory management to determine the maximum number of reactions that can be tested with the partial packs selected.

- Partial amplification reagent packs can only be used on the same Abbott *m*2000*sp* instrument used for the reagent pack's initial preparation. Using an amplification reagent pack for a second time on a different instrument will result in an error, which may delay the run.
- Partial and new amplification reagent packs may be used together.

IMPORTANT: Partial amplification reagent packs should be stored at – 25 to – 15°C until immediately before the second use. Confirm that master mix is thawed before placing partial pack(s) on the Abbott *m*2000*sp* worktable. Once removed from – 25 to – 15°C storage, partial amplification reagent packs being used a second time must be used within 25 minutes or discarded. This applies to cumulative room temperature exposure, including instances where packs are removed from storage, but not used.

- Ensure that the contents of new amplification reagent packs are at the bottom of the vials prior to opening the amplification reagents by tapping the vials in an upright position on the bench 5 to 10 times.
- Do not tap partial amplification reagent packs being used a second time. Tapping may result in loss of master mix volume in the cap.
- Remove caps. If a new amplification reagent pack will be stored for a second use, the vials will need to be recapped for storage. If planning to reuse the original caps to recap the reagent vials, save the original caps. If planning to use fresh caps to recap the reagent vials, original caps may be discarded.
- Partial amplification reagent packs are loaded to the left of new amplification reagent packs on the Abbott *m*2000*sp* worktable.
- Ensure that amplification reagent packs are firmly seated on the instrument.

23. Select the appropriate deep-well plate that matches the corresponding sample preparation extraction. Initiate the Abbott *m*2000*sp* Master Mix Addition protocol. Follow the instructions as described in the Abbott *m*2000*sp* Operations Manual, Operating Instructions section.

    NOTE: The operator should not manually fill any empty/unfilled wells in the Abbott 96-Well Optical Reaction Plate.

    - After sample extraction is complete, the Abbott *m*2000*sp* automatically fills any empty wells in the Abbott 96-Well Optical Reaction Plate with *m*Elution buffer when there are greater than 48 samples processed within a run. Plate fill is not performed for runs containing 48 samples or fewer.
    - If prompted by the instrument, Reagent Carrier 2 should remain in place, minimally containing the reagent vessel for mElution Buffer (Reagent Carrier 2, location 6). If this reagent vessel has been unloaded, place a new reagent vessel with the mElution Buffer label into Reagent Carrier 2, location 6. System fluid will be added to the reagent vessel and used to fill empty wells. Once this process is complete, the system will continue with the master mix addition.

    NOTE: System instructions for use of the automated plate-filling feature are found in the Abbott *m*2000*sp* Operations Manual (List No. 9K20 version 6 or higher), section 5, Operating Instructions, Sample Extraction-Closed Mode.

    - The Abbott *m*2000*rt* protocol (step 27) must be started within 50 minutes of the initiation of the Master Mix Addition protocol (step 23).

    NOTE: If the run is aborted for any reason subsequent to step 23, a new Abbott 96-Well Optical Reaction Plate must be used if the Abbott *m*2000*sp* Master Mix Addition Protocol (step 23) will be repeated.
24. Switch on and initialize the Abbott *m*2000*rt* instrument in the Amplification Area.

    NOTE: The Abbott *m*2000*rt* requires 15 minutes to warm-up.

    NOTE: Remove gloves before returning to the sample preparation area.
25. Seal the Abbott 96-Well Optical Reaction Plate after the Abbott *m*2000*sp* instrument has completed addition of samples and master mix according to the Abbott *m*2000*sp* Operations Manual, Operating Instructions section.
26. Place the sealed optical reaction plate into the Abbott Splash-Free Support Base for transfer to the Abbott *m*2000*rt* instrument.
27. Place the Abbott 96-Well Optical Reaction Plate in the Abbott *m*2000*rt* instrument. From the Protocol screen, select the HIV-1 DBS viral load application file. Initiate the protocol as described in the Abbott *m*2000*rt* Operations Manual, Operating Instructions section.

NOTE: Test order transfer through the use of CD-ROM or network connection with export and import features of the *m*2000*sp* and *m*2000*rt* software is recommended. If creating the Abbott *m*2000*rt* test order manually, enter sample IDs in the corresponding PCR tray locations according to the "Wells for Selected Plate" grid, found on the detail screen of the "PCR Plate Results" on the Abbott *m*2000*sp*. See Section 5 of the Abbott *m*2000*sp* Operations Manual.

28. If a prepared partial amplification reagent pack is to be used a second time, cap the 3 reagent vials with the saved caps or new caps (List No. 3N20-01) and promptly store the reagents at – 25 to – 15°C, protected from light, and in an upright position. Discard any amplification reagent packs that are exhausted or have been used twice.

IMPORTANT: Amplification reagents that will be used a second time must be stored at – 25 to – 15°C within 50 minutes of the initiation of the master mix addition protocol.

POST PROCESSING PROCEDURES FOR PROTOCOL II AND III

1. Remove the Abbott 96 Deep-Well Plate from the worktable and dispose of according to the Abbott *m*2000*sp* Operations Manual.
2. Place the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott *m*2000*rt* Operations Manual along with the gloves used to handle the plate.
3. Clean the Abbott Splash-Free Support Base before next use, according to the Abbott *m*2000*rt* Operations Manual.

QUALITY CONTROL PROCEDURES

Abbott *m*2000*rt* Optical Calibration

Refer to the Calibration Procedures section in the Abbott *m*2000*rt* Operations Manual for a detailed description of how to perform an Abbott *m*2000*rt* Optical Calibration.

Optical calibration of the Abbott *m*2000*rt* instrument is required for the accurate measurement and discrimination of dye fluorescence during the Abbott RealTi*m*e HIV-1 assay.

The following Abbott *m*2000*rt* Optical Calibration Plates are used to calibrate the Abbott *m*2000*rt* instrument for the Abbott RealTi*m*e HIV-1 assay:

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

Assay Calibration

For a detailed description of how to perform an assay calibration refer to the Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals, Operating Instructions sections.

A calibration curve is required to quantitate the HIV-1 RNA concentration of specimens and controls. Two assay calibrators are run in replicates of 3 to generate a calibration curve (logarithm of HIV-1 concentration versus the threshold cycle [C_T] at which a reactive level of fluorescent signal is detected). The calibration curve slope and intercept are calculated and stored on the instrument. The concentration of HIV-1 RNA in a sample is calculated from the stored calibration curve. Results are automatically reported on the Abbott *m*2000*rt* workstation.

Follow the procedure for sample extraction, master mix addition, amplification and detection protocols as stated in the Abbott *m*2000*sp* Operations Manual, and the Abbott *m*2000*rt* Operations Manual.

Once an Abbott RealTi*m*e HIV-1 calibration is accepted and stored, it may be used for 6 months. During this time, all subsequent samples may be tested without further calibration unless:

- An Abbott RealTi*m*e HIV-1 Amplification Reagent Kit with a new lot number is used.
- An Abbott *m*Sample Preparation System (4 × 24 Preps) with a new lot number is used.
- An Abbott RealTi*m*e HIV-1 application file for a different sample volume is used.
- A new Abbott RealTi*m*e HIV-1 application specification file is installed.
- Pure Dye optical re-calibration of the Abbott RealTi*m*e HIV-1 assay-specific dyes (FAM, VIC, or ROX) is performed per the Calibration Procedures section of the Abbott *m*2000*rt* Operations Manual.

Detection of Inhibition

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

A defined, consistent quantity of IC is introduced into each specimen, calibrator, and control at the beginning of sample preparation and measured on the Abbott *m*2000*rt* instrument to demonstrate proper specimen processing and assay validity. The IC is comprised of an RNA sequence unrelated to the HIV-1 target sequence.

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

An error control flag is displayed when a specimen or control fails to meet this specification. Refer to the Abbott *m*2000*rt* Operations Manual for an explanation of the corrective actions for the error control flag. Specimens whose IC C_T value exceeds the established range must be retested starting with sample preparation.

Negative and Positive Controls

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

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

An error control flag is displayed when a control result is out of range. Refer to the Abbott *m*2000*rt* Operations Manual for an explanation of the corrective actions for the error control flag. If negative or positive controls are out of range, all of the specimens and controls from that run must be reprocessed, beginning with sample preparation.

The presence of HIV-1 must not be detected in the negative control. HIV-1 detected in the negative control is indicative of contamination by other samples or by amplified product introduced during sample preparation or during preparation of the Abbott 96-Well Optical Reaction Plate. To avoid contamination, clean the Abbott *m*1000 System or Abbott *m*2000*sp* instrument and the Abbott *m*2000*rt* instrument and repeat sample processing for controls and specimens following the **Procedural Precautions**. If negative controls are persistently reactive, contact your Abbott representative.

Monitoring the Laboratory for the Presence of Contamination

It is recommended that this test be done at least once a month to monitor laboratory surfaces and equipment for contamination by amplification product. It is very important to test all areas that may have been exposed to processed specimens, controls, and calibrators, and/or amplification product. This includes routinely handled objects such as pipettes, the Abbott *m*2000*sp* and Abbott *m*2000*rt* function keys, laboratory bench surfaces, microcentrifuges, and centrifuge adaptors.

1. Add 0.8 mL RNase-free water to a 1.7 mL molecular biology grade microcentrifuge tube.
2. Saturate the cotton tip of an applicator (Puritan or equivalent) in the RNase-free water from the microcentrifuge tube.
3. Using the saturated cotton tip of the applicator, wipe the area to be monitored using a sweeping motion. Place the applicator into the microcentrifuge tube.
4. Swirl the cotton tip in RNase-free water 10 times, and then press the applicator along the inside of the tube so that the liquid drains back into the solution at the bottom of the microcentrifuge tube. Discard the applicator.
5. Pipette 0.5 mL of *m*Wash 1 buffer to a clean tube using the pipette dedicated for Internal Control use.
6. Add 20 µL of the *m*Wash 1 buffer to each microcentrifuge tube.
7. Cap the microcentrifuge tube.
8. Test this sample according to the assay procedure section of this package insert.
   - Transfer liquid from the microcentrifuge tube to a 5 mL Reaction Vessel.
   - Bring the volume to 1.5 mL with RNase-free water.
9. The presence of contamination is indicated by the detection of HIV-1 nucleic acid in the swab samples.
10. If HIV-1 nucleic acid is detected on equipment, follow the cleaning and decontaminating guidelines given in that equipment's operations manual. If HIV-1 nucleic acid is detected on surfaces, clean the contaminated areas with 1.0% (v/v) sodium hypochlorite solution, followed by 70% ethanol or water.

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

RESULTS FOR PLASMA SPECIMENS

Calculation

The concentration of viral HIV-1 RNA in a sample or control is calculated from the stored calibration curve. The Abbott *m*2000*rt* instrument automatically reports the results on the Abbott *m*2000*rt* workstation. Assay results can be reported in copies/mL, log [copies/mL], International Units (IU)/mL, or log [IU/mL]; (1 IU = 0.58 copies, 1 copy = 1.74 IU).

Interpretation of Results

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

[a] 40 Copies/mL

[b] 75 Copies/mL

[c] 150 Copies/mL

[d] ULQ = upper limit of quantitation

RESULTS FOR DBS SPECIMENS

The reported sample concentration result from the *m*2000*rt* DBS protocol run represents the HIV-1 viral concentration in the plasma of the whole blood specimen from which the DBS specimen is obtained. The Abbott *m*2000*rt* instrument automatically reports the results on the Abbott *m*2000*rt* workstation. Assay results can be reported in copies/mL, log [copies/mL], International Units (IU)/mL, or log [IU/mL]; (1 IU = 0.58 copies, 1 copy= 1.74 IU).

Interpretation of Results

Result	Interpretation
Not Detected	Target not detected
<2.92 Log [Copies/mL][a]	Detected
2.92 to 7.00 Log [Copies/mL]	
>7.00 Log [Copies/mL]	> ULQ[b]

[a] 839 Copies/mL

[b] ULQ = upper limit of quantitation

The concentration values for the controls and calibrators provided in their kit cards represent the HIV-1 target concentrations in these plasma equivalent samples. When an assay run is performed with the DBS protocol, the concentration values reported for controls and calibrators will reflect DBS equivalents. This scenario has no impact on results for DBS specimens.

LIMITATIONS OF THE PROCEDURE

- **FOR IN VITRO DIAGNOSTIC USE**
- Optimal performance of this test requires appropriate specimen collection, storage, and transport to the test site (refer to the **SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE** section of this package insert).
- Whole blood specimens for human plasma (collected in ACD-A or EDTA tubes) and DBS (collected in EDTA tubes) may be used with the Abbott RealTi*m*e HIV-1 assay. The use of other anticoagulants has not been validated with the Abbott RealTi*m*e HIV-1 assay.
- Use of the Abbott RealTi*m*e HIV-1 assay is limited to personnel who have been trained in the procedures of a molecular diagnostic assay and the Abbott *m*1000 and Abbott *m*2000 systems.
- The instruments and assay procedures reduce the risk of contamination by amplification product. However, nucleic acid contamination from the calibrators, positive controls, or specimens must be controlled by good laboratory practices and careful adherence to the procedures specified in this package insert.
- As with any diagnostic test, results from the Abbott RealTi*m*e HIV-1 assay should be interpreted in conjunction with other clinical and laboratory findings. A specimen with a result of "Not Detected" cannot be presumed to be negative for HIV-1 RNA.

SPECIFIC PERFORMANCE CHARACTERISTICS FOR PLASMA SPECIMENS

The performance characteristics were determined using the Abbott RealTi*m*e HIV-1 assay with Abbott *m*2000*sp* sample preparation and 1.0 mL sample volume, unless otherwise specified.

Limit of Detection (LOD)

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

Limit of Detection, 1.0 mL Sample Volume

The LOD of the Abbott RealTi*m*e HIV-1 assay is 40 copies/mL with the 1.0 mL sample volume procedure.

The LOD was determined by testing dilutions of a viral standard from the Virology Quality Assurance (VQA) Laboratory of the AIDS Clinical Trial Group. Dilutions were made in HIV-1 negative human plasma. Testing was performed with 3 lots of amplification reagents on 3 Abbott *m*2000 Systems. The results, representative of the analytical sensitivity performance of the Abbott RealTi*m*e HIV-1 assay, are summarized in **Table 1**.

Table 1.

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

[a] One replicate generated an invalid replicate error message and was deleted from the data analysis.

Probit analysis of the data determined that the concentration of HIV-1 RNA detected with 95% probability was 25 copies/mL (95% CI 20 to 33).

Limit of Detection, 0.6 mL Sample Volume

The LOD of the Abbott RealTi*m*e HIV-1 assay is 40 copies/mL with the 0.6 mL sample volume procedure.

The LOD for the 0.6 mL sample volume procedure was determined as described for the 1.0 mL sample volume procedure. The results, representative of the analytical sensitivity performance of the Abbott RealTi*m*e HIV-1 assay, are summarized in **Table 2**.

Table 2.

Conc. (Copies/mL)	Number Tested	Number Detected	Percent Detected
100	57	57	100
75	57	56	98
60	57	57	100
50	57	54	95
40	57	54	95
30	57	55	96
20	57	44	77
10	57	27	47
5	57	13	23

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

Limit of Detection, 0.5 mL Sample Volume

The LOD of the Abbott RealTi*m*e HIV-1 assay is 75 copies/mL with the 0.5 mL sample volume procedure.

The LOD for the 0.5 mL sample volume procedure was determined as described for the 1.0 mL sample volume procedure. The results, representative of the analytical sensitivity performance of the Abbott RealTi*m*e HIV-1 assay, are summarized in **Table 3**.

Table 3.

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

[a] One replicate generated an invalid replicate error message and was deleted from the data analysis.

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

Limit of Detection, 0.2 mL Sample Volume

The LOD of the Abbott RealTi*m*e HIV-1 assay is 150 copies/mL with the 0.2 mL sample volume procedure.

The LOD for the 0.2 mL sample volume procedure was determined as described for the 1.0 mL sample volume procedure. The results, representative of the analytical sensitivity performance of the Abbott RealTi*m*e HIV-1 assay, are summarized in **Table 4**.

Table 4.

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

[a] Eight replicates were invalid due to an instrument error and were deleted from the data analysis.

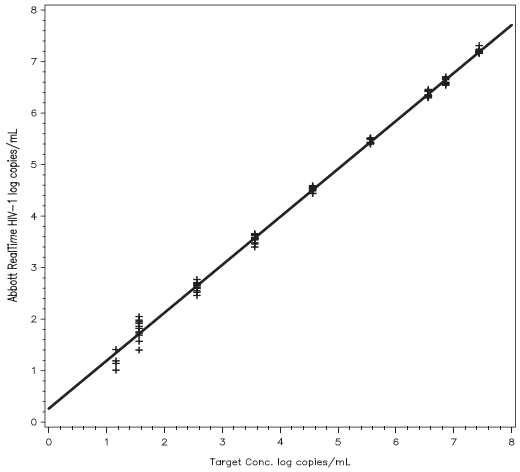
Probit analysis of the data determined that the concentration of HIV-1 RNA detected with 95% probability was 119 copies/mL (95% CI 102 to 150).

Linear Range

The upper limit of quantitation (ULQ) for the Abbott RealTi*m*e HIV-1 assay is 10 million copies/mL, and the lower limit of quantitation is equivalent to the LOD (40 copies/mL for the 1.0 mL and 0.6 mL sample volume procedure, 75 copies/mL for the 0.5 mL sample volume procedure, and 150 copies/mL for the 0.2 mL sample volume procedure).

A 9-member panel prepared by diluting armored HIV-1 RNA from 7.44 log copies/mL to 1.16 log copies/mL in HIV-1 negative human plasma was tested. Linearity analysis was performed following the NCCLS EP6-A guideline.[35] The results, representative of the Abbott RealTi*m*e HIV-1 assay linearity, are shown in **Figure 1**.

Figure 1.



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

Precision

The precision of the Abbott RealTi*m*e HIV-1 assay was evaluated for the 1.0 mL sample volume procedure using the Abbott *m*1000 and Abbott *m*2000*sp* sample preparation systems and the manual sample preparation method. The Abbott RealTi*m*e HIV-1 assay is designed to achieve an inter-assay standard deviation (SD) of less than or equal to 0.25 log copies of HIV-1 RNA per mL for samples containing HIV-1 concentrations from 500 to 5 million copies/mL. A 7-member HIV-1 RNA panel was prepared by diluting an HIV-1 viral stock (panel members 1 through 3) and armored HIV-1 RNA (panel members 4 through 7) in negative human plasma. For the precision studies with the Abbott *m*1000 and the Abbott *m*2000*sp*, the panel members were tested in replicates of 5 in a total of 15 runs on 3 instrument systems, with 3 lots of amplification reagents. For the precision study using the manual sample preparation method, panel members were tested in replicates of 2 for the first run on each instrument and replicates of 3 for each subsequent run for a total of 15 runs on 3 Abbott *m*2000*rt* instruments with 3 lots of amplification reagents. Precision analysis was performed following the NCCLS EP10-A2 guideline.[36] Within-run, between-run, and inter-assay (within-run and between-run) standard deviations were determined. The results, representative of the precision of the Abbott RealTi*m*e HIV-1 assay, are summarized in **Tables 5**, **6**, and **7**.

Table 5.

Precision with the Abbott *m*1000 System

Panel Member	n	Conc. Mean (copies/mL)	Conc. Mean (log copies/mL)	Within-Run SD Component	Between-Run SD Component	Inter-Assay SD[a]
1	75	57	1.75	0.21	0.00	0.21
2	75	573	2.76	0.08	0.00	0.08
3	75	5,000	3.70	0.05	0.02	0.06
4	73[b,c]	35,751	4.55	0.03	0.01	0.04
5	75	315,065	5.50	0.07	0.03	0.07
6	74[b]	2,947,538	6.47	0.05	0.04	0.07
7	75	5,347,285	6.73	0.04	0.05	0.07

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

[b] Two replicates were inhibited and were deleted from the data analysis.

[c] HIV-1 RNA was not detected in 1 replicate.

Table 6.

Precision with the Abbott *m*2000 System

Panel Member	n	Conc. Mean (copies/mL)	Conc. Mean (log copies/mL)	Within-Run SD Component	Between-Run SD Component	Inter-Assay SD[a]
1	74[b]	72	1.86	0.18	0.07	0.19
2	75	652	2.81	0.08	0.00	0.08
3	75	5,417	3.73	0.04	0.02	0.05
4	75	39,458	4.60	0.04	0.03	0.05
5	74[c]	358,587	5.55	0.03	0.03	0.04
6	75	3,102,654	6.49	0.03	0.02	0.04
7	75	5,953,879	6.77	0.04	0.04	0.05

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

[b] HIV-1 RNA was not detected in 1 replicate.

[c] One replicate was inhibited and was deleted from the data analysis.

Table 7.

Precision with Manual Sample Preparation Method

Panel Member	n	Conc. Mean (copies/mL)	Conc. Mean (log copies/mL)	Within-Run SD Component	Between-Run SD Component	Inter-Assay SD[a]
1	40[b]	46	1.66	0.21	0.07	0.22
2	41[c]	471	2.67	0.11	0.09	0.14
3	42	4,474	3.65	0.05	0.10	0.11
4	42	34,503	4.54	0.02	0.06	0.07
5	42	362,283	5.56	0.04	0.08	0.09
6	42	3,597,099	6.56	0.03	0.04	0.05
7	42	6,552,825	6.82	0.05	0.05	0.07

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

[b] HIV-1 RNA was not detected in 2 replicates.

[c] One replicate was inhibited and deleted from the data analysis.

Potentially Interfering Substances

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

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

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

Drugs at concentrations in excess of the peak plasma or serum levels were tested in 5 pools. No interference in the performance of the Abbott RealTi*m*e HIV-1 assay was observed in the presence of the following drug pools for all positive and negative samples tested:

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

Specificity

The target specificity of the Abbott RealTi*m*e HIV-1 assay is greater than or equal to 99.5% after resolution.

The specificity of the Abbott RealTi*m*e HIV-1 assay was evaluated by testing 187 HIV-1 seronegative plasma specimens. The specimens were tested on 3 Abbott *m*2000 instrument systems with 3 lots of amplification reagents. HIV-1 RNA was not detected, resulting in 100% (187/187) specificity (95% CI 98.05 to 100.00) in this representative study.

The specificity of the assay was further evaluated by testing 70 specimens that had been either obtained from individuals diagnosed or screened for an autoimmune disorder or serologically characterized as positive for the following markers: systemic lupus erythematosus (SLE), anti-nuclear antibodies (ANA), rheumatoid factor (RF), HBsAg, anti-HTLV-I/II, anti-HCV, and anti-HIV-2. HIV-1 RNA was not detected in any of the specimens tested. The results demonstrated that the presence of an autoimmune disorder or serologic markers for autoimmune disease or viral pathogens other than HIV-1 did not affect the Abbott RealTi*m*e HIV-1 assay.

Cross-Reactivity

The following viruses and microorganisms were evaluated for potential cross-reactivity in the Abbott RealTi*m*e HIV-1 assay. Purified nucleic acid or viral lysate from each microorganism or virus was added to HIV-1 RNA negative samples and samples that contained 10,000 copies/mL HIV-1 RNA.

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

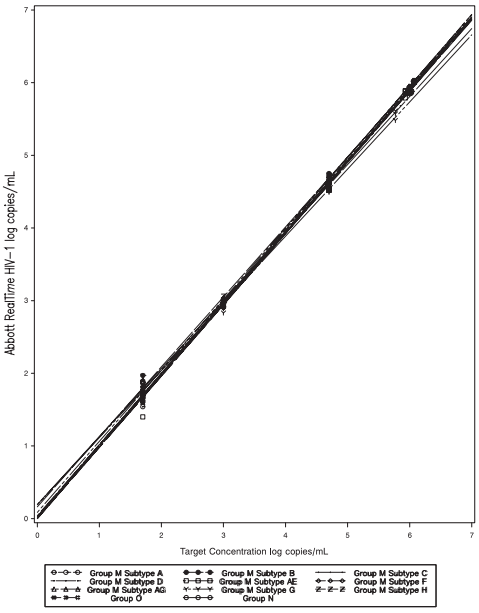
No interference in the performance of the Abbott RealTi*m*e HIV-1 assay was observed in the presence of the potential cross-reactants for all positive and negative samples tested.

Detection of HIV-1 Subtypes and Groups

The performance of the Abbott RealTi*m*e HIV-1 assay with HIV-1 subtypes/groups was evaluated by analysis of purified RNA transcripts from Group M (subtypes A, B, C, D, CRF01-AE, F, CRF02-AG, G, and H), Group O, and Group N, and by testing 10 clinical specimens of each Group M subtype (A, B, C, D, CRF01-AE, F, CRF02-AG, and G), and 10 specimens from Group O.

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

Figure 2.



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

A total of 90 clinical specimens, 10 of each Group M subtype (A, B, C, D, CRF01-AE, F, CRF02-AG, G) and Group O, were tested with the Abbott RealTi*m*e HIV-1 assay and by 2 other HIV-1 quantitative assays referred to as Comparator 1 and Comparator 2. The results are summarized in **Table 8**.

Table 8.

Group/ Subtypes	n	RealTi*m*e Detected	Comparator 1 Detected[a]	Comparator 2 Detected[a]
M/Subtype A	10	10	10 (1)	10 (1)
M/Subtype B	10	10	10 (0)	10 (0)
M/Subtype C	10	10	10 (0)	10 (0)
M/Subtype D	10	10	10 (0)	10 (0)
M/Subtype AE	10	10	10 (0)	10 (0)
M/Subtype F	10	10	10 (0)	10 (0)
M/Subtype AG	10	10	10 (3)	10 (1)
M/Subtype G	10	10	10 (2)	10 (1)
Group O	10	10	0 (NA)	7 (7)

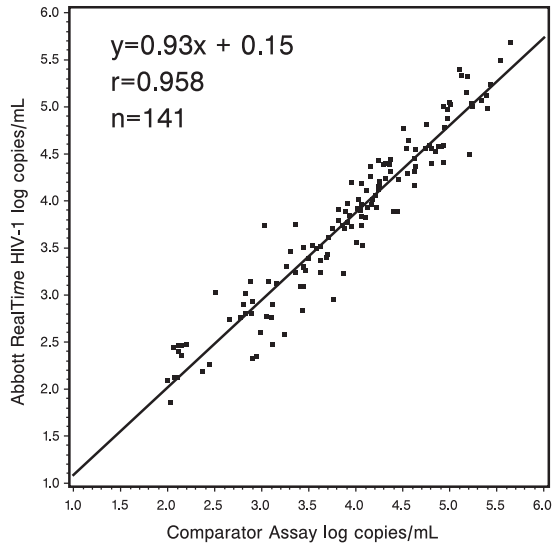
[a] The numbers in parentheses are the number of specimens that had lower quantitation values by more than 1.00 log copies/mL when compared to Abbott RealTi*m*e HIV-1 assay.

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

Correlation

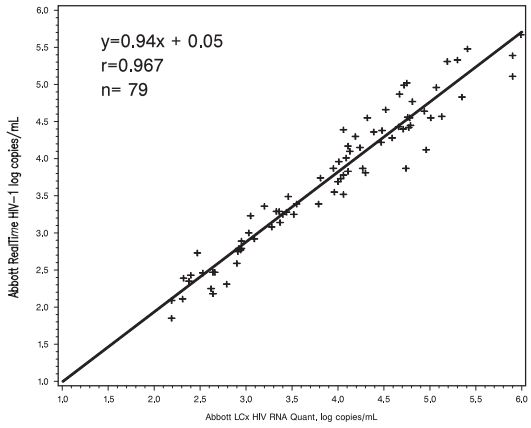
Method comparison analysis was performed following NCCLS EP9-A2.[37] Specimens from 141 HIV-1 infected patients were tested with the Abbott RealTi*m*e HIV-1 assay and a comparator assay. The correlation plot is shown in **Figure 3**.

Figure 3.



Specimens from 79 HIV-1 infected patients (a subset of the 141 tested) were tested with the Abbott LCx HIV RNA Quantitative assay. The correlation plot is shown in **Figure 4**.

Figure 4.



SPECIFIC PERFORMANCE CHARACTERISTICS FOR DBS SPECIMENS

Limit of Detection

The LOD of the Abbott RealTi*m*e HIV-1 assay is 839 copies/mL with the DBS sample type.

The limit of detection was determined by analysis of an HIV-1 viral dilution series from the VQA (Virology Quality Assurance laboratory) standard. Twenty-eight samples at each concentration level were tested across 4 runs using 4 lots of amplification reagents. The detection rate for each dilution panel member was summarized across the four lots of reagents. The results, representative of the analytical sensitivity performance of the Abbott RealTi*m*e HIV-1 assay, are summarized in **Table 9**.

Table 9.

Conc. (Copies/mL)	Number Tested	Number Detected	Percent Detected
3000	27[a]	27	100
1000	28	27	96
500	28	24	86
250	28	10	36
125	28	4	14

[a] One replicate was invalid and was excluded from the analysis.

Probit analysis of the data determined that the concentration of HIV-1 RNA detected with 95% probability was 839 copies/mL (95% CI 624 to 1387 copies/mL).

An additional limit of detection study was performed by testing another HIV-1 viral dilution series. A minimum of 36 samples at each concentration level were tested across 15 runs using 1 lot of amplification reagents. The detection rate for each dilution panel member was summarized across the runs. The results, representative of the analytical sensitivity performance of the Abbott RealTi*m*e HIV-1 assay, are summarized in **Table 10**.

Table 10.

Conc. (Copies/mL)	Number Tested	Number Detected	Percent Detected (%)
5012	36	36	100
2512	36	36	100
1000	59[a,b]	57	97
501	60[a]	49	82
251	36	13	36

[a] 24 additional replicates were run for these panel members.
[b] One replicate was invalid and was excluded from the analysis.

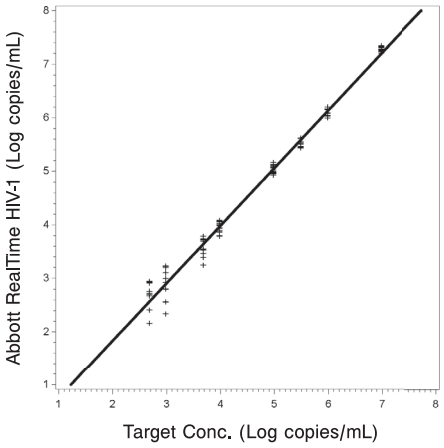
Probit analysis of the data determined that the concentration of HIV-1 RNA detected with 95% probability was 828 copies/mL (95% CI 671 to 1192 copies/mL).

Linear Range

The upper limit of quantitation (ULQ) for the Abbott RealTi*me* HIV-1 assay is 10 million copies/mL and the lower limit of quantitation is equivalent to the LOD (839 copies/mL) for the DBS claim.

A dilution series of HIV-1 Armored RNA covering the range from 500 copies/mL to 10,000,000 copies/mL in HIV-1 sero-negative blood was tested. The results, representative of the Abbott RealTi*me* HIV-1 assay linearity, are shown in **Figure 5**.

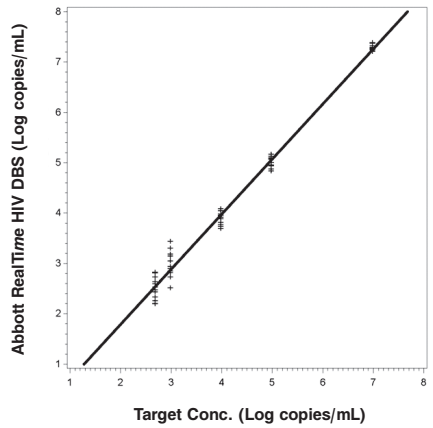
Figure 5.



The Abbott RealTi*me* HIV-1 assay was shown to be linear across the range tested (n = 92, r = 0.995, slope = 1.08, and intercept = -0.32).

An additional dilution series was tested using an HIV-1 viral stock covering the range from 501 copies/mL to 10,000,000 copies/mL in HIV-1 sero-negative blood. The results, representative of the Abbott RealTi*me* HIV-1 assay linearity, are shown in **Figure 6**.

Figure 6.



The Abbott RealTi*me* HIV-1 assay was shown to be linear across the range tested (n = 58, r = 0.994, slope = 1.09, and intercept = -0.40).

Precision

Precision was evaluated by testing HIV-1 panel members targeted to cover the range from 500 Copies/mL to 5,000,000 Copies/mL. Three lots of amplification reagents were run on the three pairs of *m*2000 instrument systems (each lot of reagent assigned to its own instrument pair), once a day for five days. Within-run, between-run, and inter-assay (within-run and between-run) standard deviations (SD) were determined. The results, representative of the precision of the Abbott RealTi*me* HIV-1 assay, are summarized in **Table 11.**

Table 11.

Abbott RealTi*me* HIV-1 Precision for Dried Blood Spot

Panel Member	n	Conc. Mean (copies/mL)	Conc. Mean (log copies/mL)	Within-Run SD Component	Between-Run SD Component	Inter-Assay[a] SD
1	54[b]	417	2.62	0.29	0.00	0.29
2	70[b]	692	2.84	0.26	0.00	0.26
3	74[c]	4531	3.66	0.12	0.09	0.16
4	73[d]	9034	3.96	0.11	0.07	0.13
5	75	108643	5.04	0.05	0.04	0.06
6	75	8130801	6.91	0.05	0.04	0.06

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

[b] Concentration means of Panel Members 1 and 2 are below LOD and the precision estimates reflect only results that are quantitated and, therefore, are for information only.

[c] One replicate was invalid and was excluded from the data analysis.

[d] Two replicates were invalid and were excluded from the data analysis.

Precision was evaluated in an additional study by testing HIV-1 panel members targeted to cover the range from 501 copies/mL to 10,000,000 copies/mL. One lot of amplification reagents was run on three pairs of *m*2000 instrument systems, once a day for five days. Within-run, between-run, and inter-assay (within-run and between-run) standard deviations (SD) were determined. The results, representative of the precision of the Abbott RealTi*me* HIV-1 assay, are summarized in **Table 12**.

Table 12.

Panel Member	n	Conc. Mean (copies/mL)	Conc. Mean (log copies/mL)	Within-Run SD Component	Between-Run SD Component	Inter-Assay[a] SD
1	49[b]	444	2.65	0.23	0.12	0.26
2	57[c]	984	2.99	0.28	0.06	0.29
3	60	10977	4.04	0.11	0.10	0.15
4	60	125458	5.10	0.08	0.05	0.09
5	58[d]	19786971	7.30	0.06	0.06	0.09

[a] Inter-Assay contains within-run and between-run components.

[b] Concentration mean of Panel Member 1 is below LOD and the precision estimates reflect only results that are quantitated and therefore are for information only.

[c] One replicate was invalid and was excluded from the data analysis and two replicates were not detected.

[d] Two replicates were invalid and were excluded from the data analysis.

Specificity

The target specificity of the Abbott RealTi*me* HIV-1 assay is greater than or equal to 99.5% after resolution.

Specificity was determined by testing 120 HIV-1 sero-negative specimens, 60 specimens with each of two lots of amplification reagents. All 120 HIV-1 sero-negative specimens gave results of “Not Detected” for a specificity of 100% (120/120).

Correlation

HIV-1 RNA quantitation was compared between the Abbott RealTi*me* HIV-1 assay using dried blood spots and the CE-marked comparator assay, Abbott RealTi*me* HIV-1 RNA quantitative assay using human plasma. A total of 313 specimens collected from South Africa, Ivory Coast, and Uganda were included in the analysis. These HIV-1 infected patients were tested at Abbott (N=247) and at one external site in South Africa (N=66). For each HIV-1 infected patient dried blood spots prepared from venous blood and capillary blood (finger prick) were tested. The results from specimens that fell within the common assay dynamic range were analyzed by the least squares linear regression method (DBS finger prick versus plasma N=150, DBS venous versus plasma N=150, and DBS finger prick versus DBS venous N=146). The correlation coefficient for HIV-1 viral load in plasma versus DBS finger prick was 0.887, the slope was 0.84 (95% CI 0.77 to 0.91), and the intercept was 0.58 log copies/mL (95% CI 0.26 to 0.90) (**Figure 7**). The correlation coefficient for HIV-1 viral load in plasma versus DBS venous blood was 0.902, the slope was 0.83 (95% CI 0.76 to 0.89), and the intercept was 0.66 log copies/mL (95% CI 0.37 to 0.95) (**Figure 8**). The correlation coefficient for HIV-1 viral load in DBS finger prick versus DBS venous blood was 0.947, the slope was 1.00 (95% CI 0.94 to 1.05), and the intercept was −0.03 log copies/mL (95% CI −0.28 to 0.21) (**Figure 9**). Additionally, Bland-Altman plots for these same comparisons are presented in **Figure 10, Figure 11,** and **Figure 12**.

Figure 7. DBS Finger Prick Versus Plasma

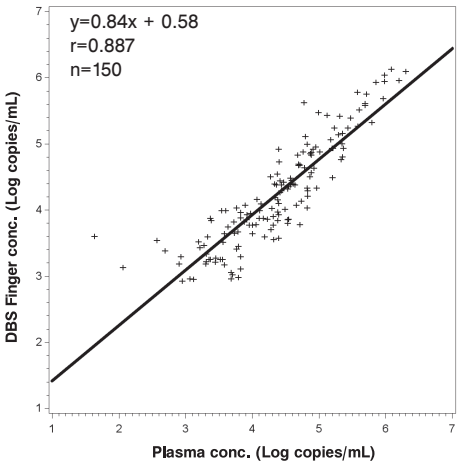


Figure 8. DBS Venous Versus Plasma

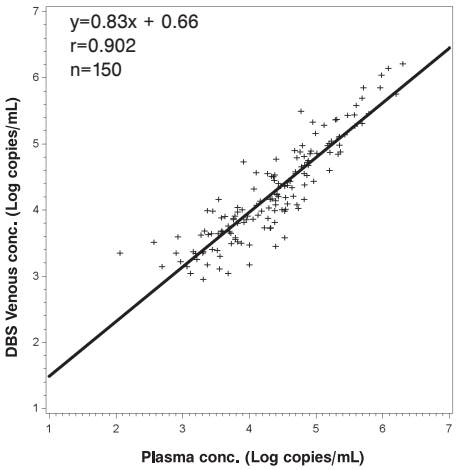


Figure 9. DBS Finger Prick Versus DBS Venous

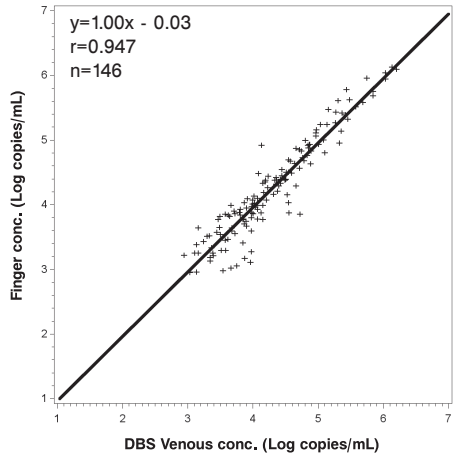


Figure 10. DBS Finger Prick Versus Plasma

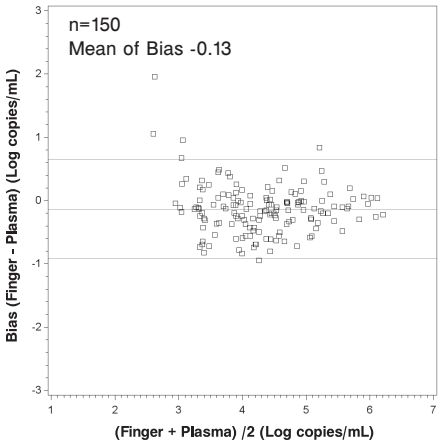


Figure 11. DBS Venous Versus Plasma

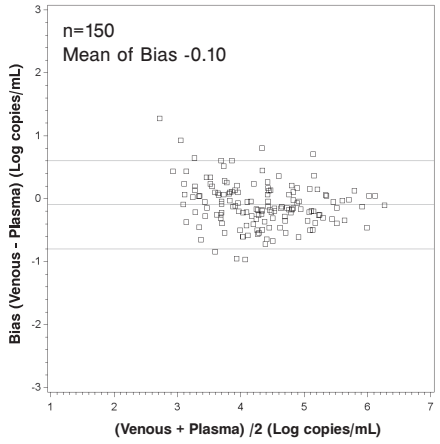
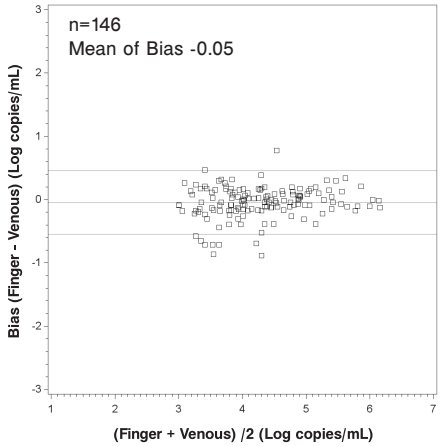


Figure 12. DBS Finger Prick Versus DBS Venous



An additional study was performed to compare HIV-1 RNA quantitation between the Abbott RealTi*m*e HIV-1 assay using dried blood spots and the CE-marked comparator assay, Abbott RealTi*m*e HIV-1 RNA quantitative assay using human plasma. A total of 244 specimens were included in the analysis. These HIV-1 infected patients were tested at Abbott. One DBS test result from each patient was used to compare to the result from matched plasma. Results from specimens that fell within the common assay dynamic range were analyzed by the least squares linear regression method (DBS versus plasma N=119). The correlation coefficient for HIV-1 viral load in plasma versus DBS was 0.929, the slope was 0.76 (95% CI 0.71 to 0.82), and the intercept was 1.20 log copies/mL (95% CI 0.97 to 1.43) (**Figure 13**). Additionally, a Bland-Altman plot is presented in **Figure 14**.

Figure 13. DBS vs. Plasma

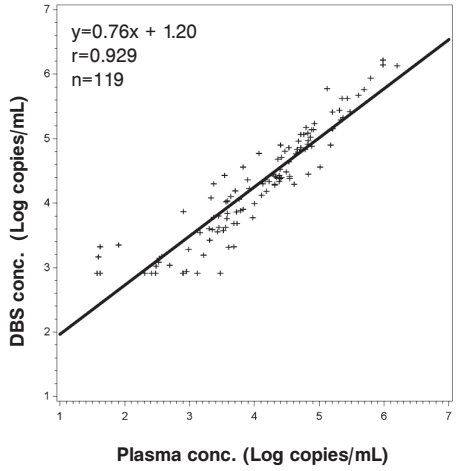
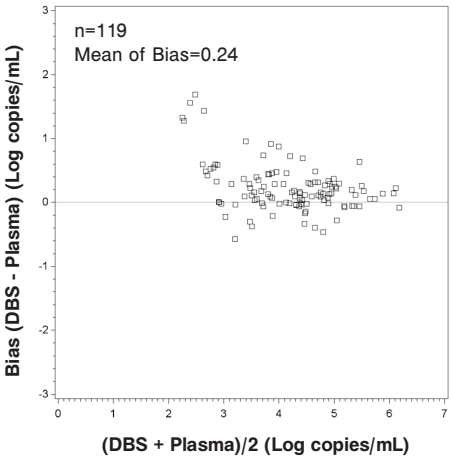


Figure 14. DBS vs. Plasma



BIBLIOGRAPHY

1. Barre-Sinoussi F, Chermann JC, Rey F, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* 1983;220:868-71.
2. Popovic M, Sarngadharan MG, Read E, et al. Detection, isolation and continuous production of cytopathic retroviruses (HTLV-I) from patients with AIDS and pre-AIDS. *Science* 1984;224:497-500.
3. Gallo RC, Salahuddin SZ, Popovic M, et al. Frequent detection and isolation of cytopathic retroviruses (HTLV-I) from patients with AIDS and at risk for AIDS. *Science* 1984;224:500-3.
4. Curran JW, Jaffe HW, Hardy AM, et al. Epidemiology of HIV infection and AIDS in the United States. *Science* 1988;239:610-16.
5. Daar ES, Moudgil T, Meyer RD, Ho DD. Transient high levels of viremia in patients with primary human immunodeficiency virus type 1 infection. *New Engl J Med* 1991;324:961-4.
6. Clark SJ, Saag MS, Decker WD. High titers of cytopathic virus in plasma of patients with symptomatic primary HIV-1 infection. *New Engl J Med* 1991;324:954-60.
7. Albert J, Abrahamsson B, Nagy K, et al. Rapid development of isolate-specific neutralizing antibodies after primary HIV-1 infection and consequent emergence of virus variants which resist neutralization by autologous sera. *AIDS* 1990;4:107-12.
8. Horsburgh CR Jr, Ou CY, Jason J, et al. Duration of human immunodeficiency virus infection before detection of antibody. *Lancet* 1989;334:637-40.
9. Pantaleo G, Graziosi C, Fauci AS. New concepts in the immunopathogenesis of human immunodeficiency virus (HIV) infection. *New Engl J Med* 1993;328:327-35.
10. Ho DD, Neumann AU, Perelson AS, et al. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* 1995;373:123-6.
11. Wei X, Ghosh SK, Taylor ME, et al. Viral dynamics in human immunodeficiency virus type 1 infection. *Nature* 1995;373:117-22.
12. Mellors JW, Rinaldo CR JR, Gupta P, et al. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science* 1996;272:1167-70.
13. Mellors JW, Munoz A, Giorgi JV, et al. Plasma viral load and $CD4^+$ lymphocytes as prognostic markers of HIV-1 infection. *Ann Intern Med* 1997;126(12):946-54.
14. Chene G, Sterne JA, May M, et al. Prognostic importance of initial response in HIV-1 infected patients starting potent antiretroviral therapy: analysis of prospective studies. *Lancet* 2003;362:679-86.
15. Egger M, May M, Chene G, et al. Prognosis of HIV-1 infected drug patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies. *Lancet* 2002;360:119-29.
16. Wood E, Hogg RS, Yip B, et al. Higher baseline levels of plasma human immunodeficiency virus type 1 RNA are associated with increased mortality after initiation of triple-drug antiretroviral therapy. *J Infect Dis* 2003;188:1421-5.
17. US Department of Health and Human Services. 2004 guidelines for the use of antiretroviral agents in HIV-1 infected adults and adolescents. Available at: http://AIDSinfo.nih.gov/guidelines.
18. Yeni PG, Hammer SM, Hirsch MS, et al. Treatment for Adult HIV Infection. 2004 Recommendations of the International AIDS Society-USA Panel. *JAMA* 2004;292:251-65.
19. Perelson AS, Essunger P, Cao Y, et al. Decay characteristics of HIV-1 infected compartments during combination therapy. *Nature* 1997;387(6629):188-91.
20. Interim Technical Update. Technical and Operational Considerations for Implementing HIV Viral Load Testing July 2014. WHO.
21. Mulder J, McKinney N, Christopher C, et al. Rapid and simple PCR assay for quantitation of human immunodeficiency virus type 1 RNA in plasma: application to acute retroviral infection. *J Clin Microbiol* 1994;32:292-300.
22. Dewar RL, Highbarger HC, Sarmiento MD, et al. Application of branched DNA signal amplification to monitor human immunodeficiency virus type 1 burden in human plasma. *J Inf Diseases* 1994;170:1172-9.
23. Van Gemen B, Kievits T, Schukkink R, et al. Quantification of HIV-1 RNA in plasma using NASBA™ during HIV-1 primary infection. *J Virol Methods* 1993;43:177-87.
24. Yen-Lieberman B, Brambilla D, Jackson B, et al. Evaluation of a quality assurance program for quantitation of human immunodeficiency virus type 1 RNA in plasma by the AIDS clinical trials group virology laboratories. *J Clin Microbiol* 1996;34:2695-701.
25. Holmes H, Davis C, Heath A, et al. An international collaborative study to establish the 1st international standard for HIV-1 RNA for use in nucleic acid-based techniques. *J Virol Methods* 2001;92:141-50.
26. Davis C, Heath A, Best S, et al. Calibration of HIV-1 working reagents for nucleic acid amplification techniques against the 1st international standard for HIV-1 RNA. *J Virol Meth* 2003;107:37-44.
27. Myers TW, Gelfand DH. Reverse Transcription and DNA Amplification by a *Thermus thermophilus* DNA Polymerase. *Biochem* 1991;30:7661-6.
28. Myers G, Korber B, Wain-Hobson S, et al. Human retroviruses and AIDS 1994: A compilation and analysis of nucleic acid and amino acid sequences. Los Alamos National Laboratory, Theoretical Biology and Biophysics (T10). Los Alamos, New Mexico:1994.
29. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; December 2009. [Also available online. *Type*> www.cdc.gov, *search*>BMBL5>look up sections III and IV.]

30. US Department of Labor, Occupational Safety and Health Administration. 29 CFR Part 1910.1030. *Bloodborne Pathogens*.
31. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline—Third Edition*. CLSI Document M29-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
32. World Health Organization. *Laboratory Biosafety Manual*. 3rd ed. Geneva, Switzerland: World Health Organization; 2004.
33. Ginocchio C, Wang X, Kaplan M, et al. Effects of specimen collection, processing, and storage conditions on stability of human immunodeficiency virus type 1 RNA levels in plasma. *J Clin Microbiol* 1997;35(11):2886-93.
34. Sebire K, McGavin K, Land S, et al. Stability of human immunodeficiency virus RNA in blood specimens as measured by a commercial PCR-based assay. *J Clin Microbiol* 1998;36(2):493-8.
35. National Committee for Clinical Laboratory Standards. *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline - NCCLS document EP6-A,* NCCLS: Wayne, PA, 2002.
36. National Committee for Clinical Laboratory Standards. *Preliminary Evaluation of Quantitative Clinical Laboratory Methods; Approved Guideline – Second Edition. NCCLS Document EP10-A2.* NCCLS: Wayne, PA, 2002.
37. National Committee for Clinical Laboratory Standards. *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline-Second Edition NCCLS document EP9-A2*, NCCLS Wayne, PA, 2002.

TECHNICAL ASSISTANCE

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

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. US patents #5,677,124, #5,919,625, #5,939,262 and patents pending.

Abbott Molecular Inc. is the legal manufacturer of the:

Abbott RealTi*m*e HIV-1 Amplification Reagent Kit (List No. 02G31-010)

Abbott RealTi*m*e HIV-1 Control Kit (List No. 2G31-80)

Abbott RealTi*m*e HIV-1 Calibrator Kit (List No. 2G31-70).

The Abbott RealTi*m*e HIV-1 Amplification Reagent Kit is imported into the European Union by Abbott Diagnostics GmbH, located at Max-Planck-Ring 2, 65205 Wiesbaden, Germany.

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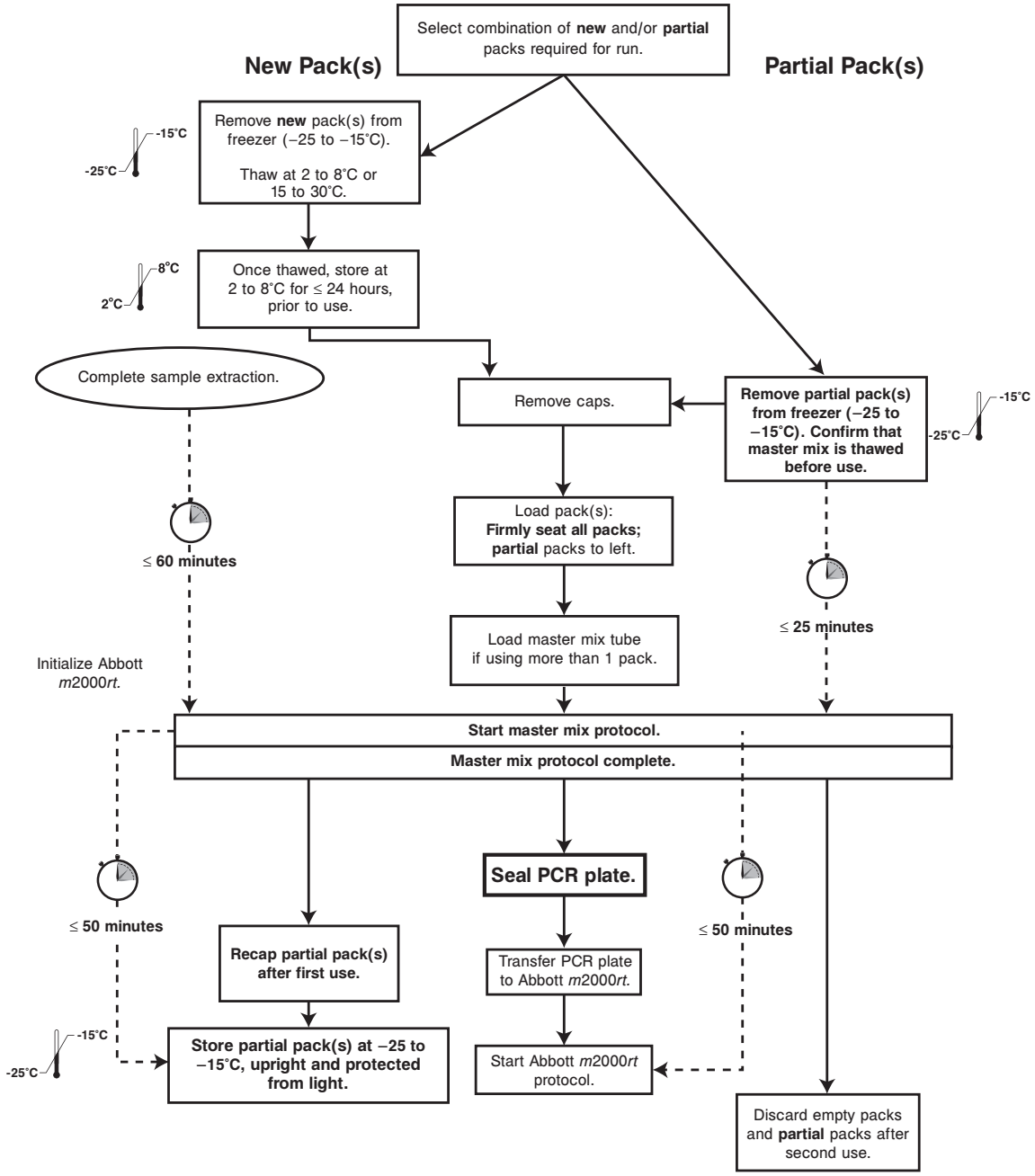
Abbott

APPENDIX 1. OVERVIEW OF THE ABBOTT REALTI*M*E HIV-1 AMPLIFICATION REAGENT EXTENDED USE FEATURE

The amplification reagent extended use feature allows for the use of an amplification reagent pack and internal control (IC) a total of 2 times. Amplification reagent packs that have not yet been used to prepare master mix are referred to as **new** amplification reagent packs. Amplification reagent packs that have been used once and contain prepared master mix are referred to as **partial** amplification reagent packs. Refer to the instructions provided in this manual for additional details.

Storage Conditions (Amplification Reagent Pack and Internal Control Vials)		
Pack Type	**Storage Temperature**	**Storage Time**
New Packs	-25 to -15°C	Until date shown on label
New IC	-25 to -15°C	Until date shown on label
Partial Packs	-25 to -15°C (protected from light)	Up to 7 days after initial use
Partial IC	-25 to -15°C	Up to 14 days after initial use



- Amplification reagent packs eligible for extended use must have a 6-digit serial number above the barcode.
- **Partial** amplification reagent packs can only be used a second time on the same instrument as the initial use. Using them on a different instrument will generate a processing error, which may delay the run.
- **Partial** and **new** amplification reagent packs may be used together. All amplification reagent packs used on the instrument for a run must have the same lot number.

APPENDIX 2. OPTIONAL UNG PROCEDURE FOR PROTOCOLS I, II, AND III

The uracil-N-glycosylase (UNG) procedure is to be used in conjunction with the Abbott RealTi*m*e HIV-1 assay as an optional contamination control for customer laboratories that are currently using or have previously used amplification technologies that incorporate uracil into the amplification product.

REAGENTS

Uracil-N-glycosylase (UNG), List No. 06L87-02 (1 tube, 112 µL, 1U/µL)

Description

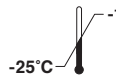
Uracil DNA glycosylase (uracil-N-glycosylase) removes uracil residues from the sugar moiety of single- and double-stranded DNA without destroying the phosphodiester backbone, preventing its use as a hybridization target or as a template for DNA polymerases. Uracil DNA glycosylase will not remove uracil from RNA.

Active Ingredients

- Uracil-N-glycosylase (UNG; < 0.1%)
- Tween 20 (< 0.1%)

Storage and Handling

The product is shipped on dry ice.



Store at −25˚ to −15˚C.

UNG Limited License

The product is authorized for use by the purchaser only for contamination control as indicated in the accompanying protocol. No rights are conveyed for use in reactions that incorporate dUTP or any patents owned or controlled by Life Technologies.

OPTIONAL UNG PROCEDURE FOR ASSAY PROTOCOL I: USING ABBOTT *m*1000 SYSTEM OR THE MANUAL SAMPLE PREPARATION METHOD AND ABBOTT *m*2000*rt* INSTRUMENT

NOTE: The step numbering from Protocol I is maintained. Starting from Step 11, execute the following:

Amplification Area

11. Switch on and initialize the Abbott *m*2000*rt* instrument.

NOTE: The Abbott *m*2000*rt* instrument requires 15 minutes to warm up.

12. Create the Abbott *m*2000*rt* test order. Refer to the Operating Instructions section of the Abbott *m*2000*rt* Operations Manual. From the Protocol screen, select the appropriate application file corresponding to the sample volume being tested.
    - Enter calibrator (needed if a calibration curve has not been stored on the Abbott *m*2000*rt*) and control lot-specific values in the test order for accurate calibration and control evaluation. Lot-specific values are specified in each Abbott RealTi*m*e HIV-1 Calibrator and Control Kit Card.

Reagent Preparation Area

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

NOTE: Change gloves before handling the amplification reagents.

13. Prepare the amplification master mix.
    - Each Amplification Reagent Pack supports up to 24 reactions.
    - Prior to opening the amplification reagents, ensure that the contents of the vials are at the bottom by tapping the vials in an upright position 5 to 10 times on the bench to bring the liquid to the bottom of the vials.
    - Use a **PIPETTE DEDICATED FOR REAGENT USE ONLY** to add 27 µL of UNG to the Thermostable rTth Polymerase Enzyme bottle (Reagent 3).
    - Prepare the master mix by using a **PIPETTE DEDICATED FOR REAGENT USE ONLY** to add 271 µL of the Activation Reagent (Reagent 1) and 949 µL of the HIV-1 Oligonucleotide Reagent (Reagent 2) together in the Thermostable rTth Polymerase Enzyme bottle (Reagent 3).
    - If performing 25 to 48 reactions, prepare a second amplification master mix with a second Amplification Reagent Pack.

    NOTE: The Abbott *m*2000*rt* protocol (step 20) must be initiated within 50 minutes of the addition of Activation Reagent into the first rTth Enzyme Reagent bottle (step 13). This 50 minutes includes 10 minutes incubation at room temperature (step 19, below).

14. Pipette the contents of the master mix from the enzyme bottle(s) into a single-use RNase/DNase-free tube and vortex to mix.
15. Place an Abbott 96-Well Optical Reaction Plate in a StrataCooler 96 or Eppendorf PCR Cooler stored as indicated in the instruction manual. Using a **DEDICATED PIPETTE**, dispense 50-µL aliquots of the amplification master mix into the Abbott 96-Well Optical Reaction Plate. A calibrated repeat pipettor may be used. Visually verify that 50 µL has been dispensed into each well.
16. Transfer the Abbott 96-Well Optical Reaction Plate on the StrataCooler 96 or Eppendorf PCR Cooler to the Sample Preparation Area.

Sample Preparation Area

17. In the Sample Preparation Area, transfer 50 µL of sample eluate to the Abbott 96-Well Optical Reaction Plate on the StrataCooler 96 or Eppendorf PCR Cooler. **Use a separate pipette tip for each sample eluate transfer**. During the transfer of each sample, mix the reaction by pipetting up and down 3 to 5 times. Visually verify that 100 µL has been dispensed into each well.
18. Seal the Abbott 96-Well Optical Reaction Plate according to the instructions in the Abbott *m*2000*rt* Operations Manual.
19. Remove the Abbott 96-Well Optical Reaction Plate from the StrataCooler 96 or Eppendorf PCR Cooler to the Abbott Splash-Free Support Base. Centrifuge the Abbott 96-Well Optical Reaction Plate in the Abbott Splash-Free Support Base at 5000*g* for 5 minutes. Incubate at room temperature (15 to 30˚C) for 10 minutes. Centrifugation may take place during the 10-minute room temperature incubation. Following room temperature incubation, transfer the Abbott 96-Well Optical Reaction Plate on the Abbott Splash-Free Support Base to the Amplification Area.

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

Amplification Area

20. Place the Abbott 96-Well Optical Reaction Plate in the Abbott *m*2000*rt* instrument. From the Protocol screen, select the appropriate application file corresponding to the sample volume being tested. Initiate the Abbott RealTi*m*e HIV-1 protocol as described in the Abbott *m*2000*rt* Operations Manual, Operating Instructions section.

ASSAY PROTOCOL II: OPTIONAL UNG PROCEDURE WITH PLASMA SAMPLES PREPARED FOR AMPLIFICATION USING THE ABBOTT *m*2000*sp*

NOTE: The step numbering from Protocol II is maintained. Starting from Step 11, execute the following:

The Abbott *m*2000*sp* 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.

11. Load the amplification reagents and the master mix tube (if needed) on the Abbott *m*2000*sp* worktable after sample preparation is completed. The following table shows the number of amplification reagent packs needed based on the number of reactions.
    If only 1 amplification reagent pack is being used, no master mix tube is required.

Amplification Reagent Pack Requirements[a]			
1 to 24 Reactions	**25 to 48 Reactions**	**49 to 72 Reactions**	**73 to 96 Reactions**
1 if **new**; up to 4 with **partial** packs	2 if **new**; up to 4 with **partial** packs	3 if **new**; up to 4 with **partial** packs	4 **new** or **partial** packs

[a] Refer to the Abbott *m*2000*sp* Operations Manual (List No. 9K20-06 or higher) for instructions on inventory management to determine the maximum number of reactions that can be tested with the partial packs selected.

- Partial amplification reagent packs can only be used on the same Abbott *m*2000*sp* instrument used for the reagent pack's initial preparation. Using an amplification reagent pack for a second time on a different instrument will result in an error, which may delay the run.

- Partial and new amplification reagent packs may be used together.

IMPORTANT: Partial amplification reagent packs should be stored at – 25 to – 15°C until immediately before the second use. Confirm that master mix is thawed before placing partial pack(s) on the Abbott *m*2000*sp* worktable. Once removed from – 25 to – 15°C, partial amplification reagent packs being used a second time must be used within 25 minutes or discarded. This applies to cumulative room temperature exposure, including instances where packs are removed from storage, but not used.

- Ensure that the contents of new amplification reagent packs are at the bottom of the vials prior to opening the amplification reagents by tapping the vials in an upright position on the bench 5 to 10 times.
- Do not tap partial amplification reagent packs being used a second time. Tapping may result in loss of master mix volume in the cap.
- Remove caps. If a new amplification reagent pack will be stored for a second use, the vials will need to be recapped for storage. If planning to reuse the original caps to recap the reagent vials, save the original caps. If planning to use fresh caps to recap the reagent vials, original caps may be discarded.
- Use a **PIPETTE DEDICATED FOR REAGENT USE ONLY** to add specified volume of 1 U/µL UNG (List No. 06L87-02) to the reagent vial in position 3 of **new** and **partial** amplification reagent packs.
- Use the table below to determine the volume of UNG to add to the reagent vial in position 3 of new and partial amplification reagent packs. The reagent vial in position 3 of a **new** reagent pack contains the Thermostable rTth polymerase enzyme. The reagent vial in position 3 of a **partial** reagent pack contains master mix.

NOTE: The volume of UNG added to the reagent vial in position 3 depends upon the number of tests remaining in the reagent pack, and not the number of samples being run. Refer to the Abbott *m*2000*sp* Operations Manual for instructions pertaining to amplification reagent pack inventory management and how to determine the number of tests remaining in a reagent pack.

- **Manually mix by pipetting gently up and down for all partial packs. Do not mix new packs.**
- **Partial amplification packs are loaded to the left of new amplification reagent packs on the Abbott *m*2000*sp* worktable.**
- **Ensure that amplification reagent packs are firmly seated on the instrument.**

Volume of UNG to Add to the Reagent Vial in Position 3 of Each Amplification Reagent Pack

Tests Remaining in the Pack	Add This Volume of UNG (µL)
1	5
2	6
3	7
4	8
5	9
6	10
7	11
8	12
9	13
10	13
11	14
12	15
13	16
14	17
15	18
16	19
17	20
18	21
19	22
20	23
21	24
22	25
23	26
24 (**new** pack)	27

12. Select the appropriate deep well plate that matches the corresponding sample preparation extraction. Initiate the Abbott *m*2000*sp* Master Mix Addition protocol. Follow the instructions as described in the Abbott *m*2000*sp* Operations Manual, Operating Instructions section.

    NOTE: The operator should not manually fill any empty/unfilled wells in the Abbott 96-Well Optical Reaction Plate.

    - After sample extraction is complete, the Abbott *m*2000*sp* automatically fills any empty wells in the Abbott 96-Well Optical Reaction Plate when there are greater than 48 samples processed within a run. Plate fill is not performed for runs containing 48 samples or fewer.
    - If prompted by the instrument, Reagent Carrier 2 should remain in place, minimally containing the reagent vessel for *m*Elution Buffer (Reagent Carrier 2, location 6). If this reagent vessel has been unloaded, place a new reagent vessel with the *m*Elution Buffer label into Reagent Carrier 2, location 6. System fluid will be added to the reagent vessel and used to fill empty wells. Once this process is complete, the system will continue with the master mix addition.

    NOTE: System instructions for use of the automated plate-filling feature are found in the Abbott *m*2000*sp* Operations Manual (List No. 9K20 version 6 or higher), section 5, Operating Instructions, Sample Extraction – Closed Mode.

    - **The Abbott *m*2000*rt* protocol (step 16) must be started within 50 minutes of the initiation of the Master Mix Addition protocol (step 12).**

    NOTE: If the run is aborted for any reason subsequent to step 12, a new 96-well Optical Reaction Plate must be used if the Abbott *m*2000*sp* Master Mix Addition Protocol (step 12) will be repeated.

13. Switch on and initialize the Abbott *m*2000*rt* instrument in the amplification area.

    NOTE: The Abbott *m*2000*rt* 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 *m*2000*sp* instrument has completed addition of samples and master mix according to the Abbott *m*2000*sp* Operations Manual, Operating Instructions section.
15. Keep the sealed optical reaction plate to the Abbott Splash-Free Support Base and incubate at room temperature (15 to 30°C) for 10 minutes. Following room temperature incubation, transfer the Abbott 96-Well Optical Reaction Plate on the Abbott Splash-Free Support Base to the Abbott *m*2000*rt* instrument.
16. Place the Abbott 96-Well Optical Reaction Plate in the Abbott *m*2000*rt* instrument. From the Protocol screen, select the appropriate application file corresponding to the sample volume being tested. Initiate the Abbott RealTi*m*e HIV-1 protocol as described in the Abbott *m*2000*rt* Operations Manual, Operating Instructions section.

    NOTE: Test order transfer through the use of CD-ROM or network connection with export and import features of the *m*2000*sp* and *m*2000*rt* software is recommended. If creating the Abbott *m*2000*rt* test order manually, enter sample IDs in the corresponding PCR tray locations according to the "Wells for Selected Plate" grid, found on the detail screen of the "PCR Plate Results" on the Abbott *m*2000*sp*. See Section 5 of the Abbott *m*2000*sp* Operations Manual.

17. If a prepared partial amplification reagent pack is to be used a second time, cap the 3 reagent vials with the saved caps or new caps (List No. 3N20-01) and promptly store the reagents at – 25 to – 15°C, protected from light, and in an upright position. Discard any amplification reagent packs that are exhausted or have been used twice.

IMPORTANT: Amplification reagents that will be used a second time must be stored at – 25 to – 15°C within 50 minutes of the initiation of the master mix addition protocol.

ASSAY PROTOCOL III: OPTIONAL UNG PROCEDURE WITH DBS SAMPLES PREPARED FOR AMPLIFICATION USING THE ABBOTT *m*2000*sp*

NOTE: The step numbering from Protocol III is maintained. Starting from Step 22, execute the following:

The Abbott *m*2000*sp* Master Mix Addition protocol (step 23) must be initiated within 1 hour after completion of Sample Preparation.

NOTE: Change gloves before handling the amplification reagents.

22. Load the amplification reagents and the master mix tube (if needed) on the Abbott *m*2000*sp* worktable after sample preparation is completed. The following table shows the number of amplification reagent packs needed based on the number of reactions.
If only 1 amplification reagent pack is being used, no master mix tube is required.

Amplification Reagent Pack Requirements[a]			
1 to 24 Reactions	**25 to 48 Reactions**	**49 to 72 Reactions**	**73 to 96 Reactions**
1 if **new**; up to 4 with **partial** packs	2 if **new**; up to 4 with **partial** packs	3 if **new**; up to 4 with **partial** packs	4 **new** or **partial** packs

a Refer to the Abbott *m*2000*sp* Operations Manual (List No. 9K20 version 6 or higher) for instructions on inventory management to determine the maximum number of reactions that can be tested with the partial packs selected.

- Partial amplification reagent packs can only be used on the same Abbott *m*2000*sp* instrument used for the reagent pack's initial preparation. Using an amplification reagent pack for a second time on a different instrument will result in an error, which may delay the run.
- Partial and new amplification reagent packs may be used together.

IMPORTANT: Partial amplification reagent packs should be stored at −25 to −15°C until immediately before the second use. Confirm that master mix is thawed before placing partial pack(s) on the Abbott *m*2000*sp* worktable. Once removed from −25 to −15°C, partial amplification reagent packs being used a second time must be used within 25 minutes or discarded. This applies to cumulative room temperature exposure, including instances where packs are removed from storage, but not used.

- Ensure that the contents of new amplification reagent packs are at the bottom of the vials prior to opening the amplification reagents by tapping the vials in an upright position on the bench 5 to 10 times.
- Do not tap partial amplification reagent packs being used a second time. Tapping may result in loss of master mix volume in the cap.
- Remove caps. If a new amplification reagent pack will be stored for a second use, the vials will need to be recapped for storage. If planning to reuse the original caps to recap the reagent vials, save the original caps. If planning to use fresh caps to recap the reagent vials, original caps may be discarded.
- Use a **PIPETTE DEDICATED FOR REAGENT USE ONLY** to add specified volume of 1 U/µL UNG (List No. 06L87-02) to the reagent vial in position 3 of **new** and **partial** amplification reagent packs.
- Use the table below to determine the volume of UNG to add to the reagent vial in position 3 of **new** and **partial** amplification reagent packs. The reagent vial in position 3 of a **new** reagent pack contains the Thermostable rTth polymerase enzyme. The reagent vial in position 3 of a **partial** reagent pack contains master mix.

NOTE: The volume of UNG added to the reagent vial in position 3 depends upon the number of tests remaining in the reagent pack, and not the number of samples being run. Refer to the Abbott *m*2000*sp* Operations Manual for instructions pertaining to amplification reagent pack inventory management and how to determine the number of tests remaining in a reagent pack.

- **Manually mix by pipetting gently up and down for all partial packs. Do not mix new packs.**
- **Partial amplification packs are loaded to the left of new amplification reagent packs on the Abbott *m*2000*sp* worktable.**
- **Ensure that amplification reagent packs are firmly seated on the instrument.**

Volume of UNG to Add to the Reagent Vial in Position 3 of Each Amplification Reagent Pack	
Tests Remaining in the Pack	Add This Volume of UNG (µL)
1	5
2	6
3	7
4	8
5	9
6	10
7	11
8	12
9	13
10	13
11	14
12	15
13	16
14	17
15	18
16	19
17	20
18	21
19	22
20	23
21	24
22	25
23	26
24 (**new** pack)	27

23. Select the appropriate deep well plate that matches the corresponding sample preparation extraction. Initiate the Abbott *m*2000*sp* Master Mix Addition protocol. Follow the instructions as described in the Abbott *m*2000*sp* Operations Manual, Operating Instructions section.

NOTE: The operator should not manually fill any empty/unfilled wells in the Abbott 96-Well Optical Reaction Plate.

- After sample extraction is complete, the Abbott *m*2000*sp* automatically fills any empty wells in the Abbott 96-Well Optical Reaction Plate with *m*Elution buffer when there are greater than 48 samples processed within a run. Plate fill is not performed for runs containing 48 samples or fewer.
- If prompted by the instrument, Reagent Carrier 2 should remain in place, minimally containing the reagent vessel for *m*Elution Buffer (Reagent Carrier 2, location 6). If this reagent vessel has been unloaded, place a new reagent vessel with the *m*Elution Buffer label into Reagent Carrier 2, location 6. System fluid will be added to the reagent vessel and used to fill empty wells. Once this process is complete, the system will continue with the master mix addition.

NOTE: System instructions for use of the automated plate-filling feature are found in the Abbott *m*2000*sp* Operations Manual (List No. 9K20 version 6 or higher), section 5, Operating Instructions, Sample Extraction – Closed Mode.

- **The Abbott *m*2000*rt* protocol (step 27) must be started within 50 minutes of the initiation of the Master Mix Addition protocol (step 23)**.

NOTE: If the run is aborted for any reason subsequent to step 23, a new 96-well Optical Reaction Plate must be used if the Abbott *m*2000*sp* Master Mix Addition Protocol (step 23) will be repeated.

24. Switch on and initialize the Abbott *m*2000*rt* instrument in the amplification area.

NOTE: The Abbott *m*2000*rt* requires 15 minutes to warm-up.

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

25. Seal the Abbott 96-Well Optical Reaction Plate after the Abbott *m*2000*sp* instrument has completed addition of samples and master mix according to the Abbott *m*2000*sp* Operations Manual, Operating Instructions section.
26. Keep the sealed optical reaction plate to the Abbott Splash-Free Support Base and incubate at room temperature (15 to 30°C) for 10 minutes. Following room temperature incubation, transfer the Abbott 96-Well Optical Reaction Plate on the Abbott Splash-Free Support Base to the Abbott *m*2000*rt* instrument.

27. Place the Abbott 96-Well Optical Reaction Plate in the Abbott *m*2000*rt* instrument. From the Protocol screen, select the HIV-1 DBS Viral Load application file. Initiate the Abbott RealTi*m*e HIV-1 protocol as described in the Abbott *m*2000*rt* Operations Manual, Operating Instructions section.

    NOTE: Test order transfer through the use of CD-ROM or network connection with export and import features of the *m*2000*sp* and *m*2000*rt* software is recommended. If creating the Abbott *m*2000*rt* test order manually, enter sample IDs in the corresponding PCR tray locations according to the "Wells for Selected Plate" grid, found on the detail screen of the "PCR Plate Results" on the Abbott *m*2000*sp*. See Section 5 of the Abbott *m*2000*sp* Operations Manual.

28. If a prepared partial amplification reagent pack is to be used a second time, cap the 3 reagent vials with the saved caps or new caps (List No. 3N20-01) and promptly store the reagents at −25 to −15°C, protected from light, and in an upright position. Discard any amplification reagent packs that are exhausted or have been used twice.

IMPORTANT: Amplification reagents that will be used a second time must be stored at −25 to −15°C within 50 minutes of the initiation of the master mix addition protocol.

Abbott RealTi*me*

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IN VITRO TEST

REF 2G31-70

51-602103/R6

HIV-1 Calibrators

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

Notice to User

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

Intended Use

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

Intended User

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

Contents

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

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

NOTE: Changes Highlighted

Standardization

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

Precautions

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

CAUTION: This preparation contains human sourced and/or potentially infectious components. Components sourced from human blood have been tested and found to be nonreactive by FDA-licensed tests for antibody to HCV, antibody to HIV-1, antibody to HIV-2, and HBsAg. The material is also tested and found to be negative by FDA-licensed PCR methods for HIV-1 RNA and HCV RNA. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. These reagents and human specimens should be handled as if infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories,[2] OSHA Standards on Bloodborne Pathogens,[3] CLSI Document M29-A3,[4] and other appropriate biosafety practices.[5] Therefore all human sourced materials should be considered infectious.

These precautions include, but are not limited to, the following:

- Wear gloves when handling specimens or reagents.
- Do not pipette by mouth.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where these materials are handled.
- Clean and disinfect spills of specimens by including the use of a tuberculocidal disinfectant such as 1.0% sodium hypochlorite or other suitable disinfectant.[2]
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state and federal regulations.[5]

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

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


Consult instructions for use

Store at ≤ -10°C

Shipping Conditions

Ship on dry ice.

BIBLIOGRAPHY

1. Yen-Lieberman B, Brambilla D, Jackson B, et al. Evaluation of a quality assurance program for quantitation of human immunodeficiency virus type 1 RNA in plasma by the AIDS clinical trials group virology laboratories. *J Clin Microbiol.* 1996;34:2695-701.
2. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; December 2009. [Also available online. *Type*> www.cdc.gov, *search*>BMBL5>*look up* sections III and IV.]
3. US Department of Labor, Occupational Safety and Health Administration. 29 CFR Part 1910.1030. *Bloodborne Pathogens*.
4. Clinical and Laboratory Standards Institute. P*rotection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline—Third Edition*. CLSI Document M29-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
5. World Health Organization. *Laboratory Biosafety Manual*. 3rd ed. Geneva, Switzerland: World Health Organization; 2004.

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.

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


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Des Plaines, IL 60018 USA

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www.abbottmolecular.com

May 2020

Abbott

Abbott RealTi*me*

en

IN VITRO TEST

REF 2G31-80

51-602108/R6

HIV-1 Controls

Key to symbols used	
REF	List Number
IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
	Expiration Date
CONTROL –	Negative Control
CONTROL L	Positive Control Low
CONTROL H	Positive Control High
-10°C	Store at ≤ -10°C
	Consult instructions for use
	Warning
	CAUTION: Handle human sourced materials as potentially infectious. Consult instructions for use. (Infection Risk)
EC REP	Authorized Representative
	Manufacturer

Notice to User

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

Intended Use

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

Intended User

The intended users for The Abbott RealTi*me* HIV-1 Controls are laboratory and healthcare professionals.

Contents

1. CONTROL – **Abbott RealTi*me* HIV-1 Negative Control (List No. 2G31Z) (8 vials, 1.8 mL per vial)**. Negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, HBV DNA, and anti-HCV. Preservatives: 0.1% ProClin® 300 and 0.15% ProClin 950.
2. CONTROL L **Abbott RealTi*me* HIV-1 Low Positive Control (List No. 2G31W) (8 vials, 1.8 mL per vial)**. Noninfectious Armored RNA® with HIV-1 sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, HBV DNA, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.
3. CONTROL H **Abbott RealTi*me* HIV-1 High Positive Control (List No. 2G31X) (8 vials, 1.8 mL per vial)**. Noninfectious Armored RNA with HIV-1 sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, HBV DNA, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

NOTE: Changes Highlighted

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

Precautions

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

CAUTION: This preparation contains human sourced and/or potentially infectious components. Components sourced from human blood have been tested and found to be nonreactive by FDA-licensed tests for antibody to HCV, antibody to HIV-1, antibody to HIV-2, and HBsAg. The material is also tested and found to be negative by FDA-licensed PCR methods for HIV-1 RNA and HCV RNA. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. These reagents and human specimens should be handled as if infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories,[1] OSHA Standards on Bloodborne Pathogens,[2] CLSI Document M29-A3,[3] and other appropriate biosafety practices.[4] Therefore all human sourced material should be considered infectious.

These precautions include, but are not limited to, the following:

- Wear gloves when handling specimens or reagents.
- Do not pipette by mouth.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where these materials are handled.
- Clean and disinfect spills of specimens by including the use of a tuberculocidal disinfectant such as 1.0% sodium hypochlorite or other suitable disinfectant.[1]
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and federal regulations.[4]

Components of the Abbott RealTi*me* HIV-1 Control Kit (List No. 2G31-80) contain the following components:

- 2-Methyl-2H-isothiazol-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)
- 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. 220-239-6)(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.


Consult instructions for use

Store at ≤ -10°C

Shipping Conditions

Ship on dry ice.

BIBLIOGRAPHY

1. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; December 2009. [Also available online. *Type*> www.cdc.gov, *search*>BMBL5>*look up* sections III and IV.]
2. US Department of Labor, Occupational Safety and Health Administration. 29 CFR Part 1910.1030. *Bloodborne Pathogens*.
3. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline—Third Edition*. CLSI Document M29-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
4. World Health Organization. *Laboratory Biosafety Manual*. 3rd ed. Geneva, Switzerland: World Health Organization; 2004.

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.

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


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www.abbottmolecular.com

May 2020

Abbott

Abbott RealTi*me* HIV-1

en

REF 2G31

51-602100/R15

REF 2G31

51-602100/R15

NOTE: Changes Highlighted

Key to Symbols Used	
REF	Reference Number
LOT	Lot Number
IVD	In Vitro Diagnostic Medical Device
	Use By
CONTROL –	Negative Control
CONTROL L	Low Positive Control
CONTROL H	High Positive Control
CAL A	Calibrator A
CAL B	Calibrator B
INTERNAL CONTROL	Internal Control
AMPLIFICATION REAGENT PACK	Amplification Reagent Pack
	Upper Limit of Temperature
	Consult instructions for use
	Caution
	Warning
	Manufacturer
EC REP	Authorized Representative in the European Community

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

NOTICE TO USER

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

CUSTOMER SERVICE

INTERNATIONAL: CALL YOUR ABBOTT REPRESENTATIVE

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

NAME

Abbott RealTi*me* HIV-1

INTENDED USE

The Abbott RealTi*me* HIV-1 assay is an in vitro reverse transcription-polymerase chain reaction (RT-PCR) assay for the quantitation of Human Immunodeficiency Virus type 1 (HIV-1) in human plasma from HIV-1 infected individuals. The Abbott RealTi*me* HIV-1 assay is intended for use in conjunction with clinical presentation and other laboratory markers as an indicator of disease prognosis and for use as an aid in assessing viral response to antiretroviral treatment as measured by changes in plasma HIV-1 RNA levels. This assay is not intended to be used as a screening test for HIV-1 or as a diagnostic test to confirm the presence of HIV-1 infection.

INTENDED USER

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

SUMMARY AND EXPLANATION OF THE TEST

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

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

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

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The Abbott RealTi*me* HIV-1 assay consists of 3 reagent kits:

- Abbott RealTi*me* HIV-1 Amplification Reagent Kit
- Abbott RealTi*me* HIV-1 Control Kit
- Abbott RealTi*me* HIV-1 Calibrator Kit

The Abbott RealTi*me* HIV-1 assay uses RT-PCR[26] to generate amplified product from the RNA genome of HIV-1 in clinical specimens. An RNA sequence that is unrelated to the HIV-1 target sequence is introduced into each specimen at the beginning of sample preparation. This unrelated RNA sequence is simultaneously amplified by RT-PCR, and serves as an internal control (IC) to demonstrate that the process has proceeded correctly for each sample. The amount of HIV-1 target sequence that is present at each amplification cycle is measured through the use of fluorescent-labeled oligonucleotide probes on the Abbott *m*2000*rt* instrument. The probes do not generate signal unless they are specifically bound to the amplified product. The amplification

cycle at which fluorescent signal is detected by the Abbott *m*2000*rt* is proportional to the log of the HIV-1 RNA concentration present in the original sample.

Sample Preparation

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

The Abbott *m*Sample Preparation System (4 × 24 Preps) uses magnetic particle technology to capture nucleic acids and washes the particles to remove unbound sample components. The bound nucleic acids are eluted and transferred to output tubes or a 96 deep-well plate. The nucleic acids are then ready for amplification. The IC is taken through the entire sample preparation procedure along with the calibrators, controls, and specimens.

Two automated instrument systems, the Abbott *m*2000*sp* or the Abbott *m*1000 System can be used to prepare samples for the Abbott RealTi*m*e HIV-1 assay. The Abbott *m*2000*sp* provides automated sample eluate transfer and reaction assembly in the Abbott 96-Well Optical Reaction Plate, while the Abbott *m*1000 System requires manual sample eluate transfer and reaction assembly.

Alternatively, samples can be prepared manually using the Abbott *m*Sample Preparation System, followed by manual reaction assembly.

Reagent Preparation and Reaction Plate Assembly

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

Abbott *m*1000 System users and manual sample preparation method users manually combine the Abbott RealTi*m*e HIV-1 amplification reagent components to create the amplification master mix and transfer aliquots of the master mix and sample eluates to the reaction plate. The plate is ready, after manual application of the optical seal and centrifugation, for transfer to the Abbott *m*2000*rt*.

Amplification

During the amplification reaction on the Abbott *m*2000*rt*, the target RNA is converted to cDNA by the reverse transcriptase activity of the thermostable rTth DNA polymerase. First, the HIV-1 and IC reverse primers anneal to their respective targets and are extended during a prolonged incubation period. After a denaturation step, in which the temperature of the reaction is raised above the melting point of the double-stranded cDNA:RNA product, a second primer anneals to the cDNA strand and is extended by the DNA polymerase activity of the rTth enzyme to create a double-stranded DNA product.

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

The target sequence for the Abbott RealTi*m*e HIV-1 assay is in the *pol* integrase region of the HIV-1 genome. This region is highly conserved.[27] The primers are designed to hybridize to the *pol* integrase region with the fewest possible mismatches among various subtypes.

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

Detection

During the read cycles of amplification on the Abbott *m*2000*rt*, the temperature is lowered further to allow fluorescent detection of amplification products as the HIV-1 and IC probes anneal to their targets (real-time fluorescence detection). The HIV-1 probe has a fluorescent moiety that is covalently linked to the 5′ end. A short oligonucleotide (quencher oligonucleotide) is complementary to the 5′ end of the HIV-1 probe and has a quencher molecule at its 3′ end. In the absence of HIV-1 target, the HIV-1 probe fluorescence is quenched through hybridization to the quencher oligonucleotide. In the presence of the HIV-1 target sequence, the HIV-1 probe preferentially hybridizes to the target sequence, dissociating from the quencher oligonucleotide, allowing fluorescent detection.

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

The HIV-1 and IC specific probes are each labeled with a different fluorophore, thus allowing for simultaneous detection of both amplified products at each cycle. The amplification cycle at which fluorescent signal is detected by the Abbott *m*2000*rt* is proportional to the log of the HIV-1 RNA concentration present in the original sample.

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 RealTi*m*e HIV-1 assay. Refer to the **SPECIAL PRECAUTIONS** section of this package insert.

REAGENTS

Abbott RealTi*m*e HIV-1 Amplification Reagent Kit (List No. 2G31-90)

1. **INTERNAL CONTROL** Abbott RealTi*m*e 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, HIV RNA, HCV RNA, HBV DNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin® 300 and 0.15% ProClin 950.
2. **AMPLIFICATION REAGENT PACK** Abbott RealTi*m*e HIV-1 Amplification Reagent Pack (List No. 2G31) (4 packs, 24 tests/pack)
   - 1 bottle (0.141 mL) Thermostable rTth Polymerase Enzyme (2.9 to 3.5 Units/µL) in buffered solution.
   - 1 bottle (1.10 mL) HIV-1 Oligonucleotide Reagent. < 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 RealTi*m*e HIV-1 Control Kit (List No. 2G31-80)

1. **CONTROL –** Abbott RealTi*m*e HIV-1 Negative Control (List No. 2G31Z) (8 vials, 1.8 mL per vial) Negative human plasma tested and found to be nonreactive for HBsAg, HIV RNA, HCV RNA, HBV DNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.
2. **CONTROL L** Abbott RealTi*m*e HIV-1 Low Positive Control (List No. 2G31W) (8 vials, 1.8 mL per vial) Noninfectious Armored RNA with HIV-1 sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HIV RNA, HCV RNA, HBV DNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.
3. **CONTROL H** Abbott RealTi*m*e HIV-1 High Positive Control (List No. 2G31X) (8 vials, 1.8 mL per vial). Noninfectious Armored RNA with HIV-1 sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HIV RNA, HCV RNA, HBV DNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

Abbott RealTi*m*e HIV-1 Calibrator Kit (List No. 2G31-70)

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

WARNINGS AND PRECAUTIONS

IVD

For In Vitro Diagnostic Use

This assay is not intended to be used as a screening test for HIV-1 or as a diagnostic test to confirm the presence of HIV-1 infection.

Safety Precautions

Refer to the Abbott *m*1000 Operating Manual, Safety Section, the Manual Sample Preparation for Abbott RealTi*m*e RNA Assays Procedure, Handling Precaution Section, or Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals, Hazard Section, for instructions on safety precautions.

CAUTION: This preparation contains human sourced and/or potentially infectious components. Components sourced from human blood have been tested and found to be nonreactive by FDA-licensed tests for antibody to 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,[28] OSHA Standards on Bloodborne Pathogens,[29] CLSI Document M29-A3,[30] and other appropriate biosafety practices.[31] Therefore all human sourced materials should be considered infectious.

These precautions include, but are not limited to, the following:

- Wear gloves when handling specimens or reagents.
- Do not pipette by mouth.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where these materials are handled.
- Clean and disinfect spills of specimens by including the use of a tuberculocidal disinfectant such as 1.0% sodium hypochlorite or other suitable disinfectant.[28]
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and federal regulations.[31]

Components of the Abbott RealTi*m*e HIV-1 Amplification Reagent Kit (List No. 2G31-90), the Abbott RealTi*m*e HIV-1 Calibrator Kit (List No. 2G31-70), and the Abbott RealTi*m*e HIV-1 Control Kit (List No. 2G31-80) contain the following components:

- 2-Methyl-2H-isothiazol-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)
- 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. 220-239-6)(3:1)

The following warnings apply:

Warning

H317	May cause an allergic skin reaction.
P261	Avoid breathing mist/vapours/spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves/protective clothing/ eye protection.
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 before reuse.
P501	Dispose of contents/container in accordance with local regulations.

SPECIAL PRECAUTIONS

Handling Precautions

The Abbott RealTi*m*e HIV-1 assay is only for use with plasma specimens that have been handled and stored in capped tubes as described in the **SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE** section.

During preparation of samples, compliance with good laboratory practices is essential to minimize the risk of cross-contamination between samples and the inadvertent introduction of ribonucleases (RNases) into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with RNA.

Amplification technologies such as PCR are sensitive to accidental introduction of product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the Abbott RealTi*m*e reagents used in the amplification step become contaminated by accidental introduction of even a few molecules of amplification product. Measures to reduce the risk of contamination in the laboratory include physically separating the activities involved in performing PCR in compliance with good laboratory practices.

Work Areas

Use 3 dedicated areas within the laboratory for performing the Abbott RealTi*m*e HIV-1 assay with the Abbott *m*1000 System or manual sample preparation using the Abbott *m*Sample Preparation System and Abbott *m*2000*rt*:

- The **Reagent Preparation Area** is dedicated to combining the Abbott RealTi*m*e HIV-1 amplification reagent components to create the amplification master mix and transferring aliquots of the master mix to the reaction plate. Laboratory coats, pipettes, pipette tips, and vortexers 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.**
- The **Sample Preparation Area** is dedicated to processing samples (specimens, Abbott RealTi*m*e HIV-1 Controls, and Calibrators), and to adding processed samples, controls, and calibrators to the Abbott 96-Well Optical Reaction Plate. **All reagents used in the Sample Preparation Area should remain in this dedicated area at all times. Laboratory coats, pipettes, pipette tips, and vortexers used in the Sample Preparation Area must remain in this area and not be moved to either the Reagent Preparation Area or the Amplification Area. Do not bring amplification product into the Sample Preparation Area.**
- The **Amplification Area** is dedicated to the amplification and detection of amplified product. Laboratory coats and equipment used in the **Amplification Area** must remain in this area and not be moved to either the **Reagent Preparation Area** or the **Sample Preparation Area**.

Only 2 dedicated areas, Sample Preparation Area and Amplification Area, are recommended when the Abbott *m*2000*sp* and Abbott *m*2000*rt* are used.

Components contained within a kit are intended to be used together. Do not mix components from different kit lots. For example, do not use the negative control from control kit lot X with the positive controls from control kit lot Y.

Do not use kits or reagents after the dates shown on kit labels.

Work area and instrument platforms must be considered potential sources of contamination. Change gloves after contact with potential contaminants (specimens, eluates, and/or amplified product) before handling unopened reagents, negative control, positive controls, calibrators, or specimens. Refer to the Abbott *m*1000 Operating Manual and the Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals for instrument cleaning procedures.

If the Abbott *m*1000 System or Abbott *m*2000*sp* instrument run is aborted, dispose of all commodities and reagents according to the Abbott *m*1000 Operating Manual or the Abbott *m*2000*sp* Operations Manual. If the Abbott *m*2000*sp* master mix addition protocol is aborted, seal the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott *m*2000*sp* Operations Manual, Hazards section, along with the gloves used to handle the plate.

If the Abbott *m*2000*rt* instrument run is interrupted or aborted, seal the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott *m*2000*rt* Operations Manual along with the gloves used to handle the plate.

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

NOTE: Autoclaving the sealed Reaction Plate will not degrade the amplified product and may contribute to the release of the amplified product by opening the sealed plate. The laboratory area can become contaminated with amplified product if the waste materials are not carefully handled and contained.

Aerosol Containment

To reduce the risk of nucleic acid contamination due to aerosols formed during manual pipetting, aerosol barrier pipette tips must be used for all manual pipetting. The pipette tips must be used only 1 time. Clean and disinfect spills of specimens and reagents as stated in the Abbott *m*1000 Operating Manual or the Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals.

Contamination and Inhibition

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

- Wear appropriate personal protective equipment at all times.
- Use powder-free gloves.
- Change gloves after having contact with potential contaminants (such as specimens, eluates, and/or amplified product).
- To reduce the risk of nucleic acid contamination due to aerosols formed during pipetting, pipettes with aerosol barrier tips must be used for all pipetting. The length of the tip should be sufficient to prevent contamination of the pipette barrel. While pipetting, care should be taken to avoid touching the pipette barrel to the inside of the sample tube or container. The use of extended aerosol barrier pipette tips is recommended.
- Change aerosol barrier pipette tips between ALL manual liquid transfers.
- The Abbott *m*Sample Preparation System (4 × 24 Preps) reagents are single use only. Use new reagent troughs or vessels, reaction vessels, and newly opened reagents for every new Abbott RealTi*m*e HIV-1 assay run. At the end of each run, discard all remaining reagents from the worktable as stated in the Abbott *m*1000 Operating Manual or the Abbott *m*2000*sp* Operations Manual and the Abbott *m*Sample Preparation System (4 × 24 Preps) product information sheet.

STORAGE INSTRUCTIONS

Abbott RealTi*m*e HIV-1 Amplification Reagent Kit (List No. 2G31-90)

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

Abbott RealTi*m*e HIV-1 Control Kit (List No. 2G31-80)

The Abbott RealTi*m*e HIV-1 Negative and Positive Controls must be stored at – 10°C or colder.

Abbott RealTi*m*e HIV-1 Calibrator Kit (List No. 2G31-70)

The Abbott RealTi*m*e HIV-1 Calibrator A and Calibrator B must be stored at – 10°C or colder.

SHIPPING CONDITIONS

- Abbott RealTi*m*e HIV-1 Amplification Reagent Kit: Ship on dry ice.
- Abbott RealTi*m*e HIV-1 Control Kit: Ship on dry ice.
- Abbott RealTi*m*e HIV-1 Calibrator Kit: Ship on dry ice.

INDICATION OF INSTABILITY OR DETERIORATION OF REAGENTS

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

INSTRUMENT PROCEDURE

The nucleic acid testing (NAT) software must be installed on the Abbott *m*1000 System prior to performing the assay. For detailed information on NAT software installation, refer to the Abbott *m*1000 Operating Manual, Putting into Operation section.

The Abbott RealTi*m*e HIV-1 application files must be installed on the Abbott *m*2000*sp* and Abbott *m*2000*rt* systems from the Abbott RealTi*m*e HIV-1 *m*2000 ROW System Combined Application CD-ROM prior to performing the assay. For detailed information on application file installation, refer to the Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals, Operating Instructions section.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE

Specimen Collection and Storage

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

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

Separate plasma from cells by centrifugation.

After centrifugation, plasma may be removed from cells. Plasma specimens may be stored at 15 to 30°C for up to 24 hours or at 2 to 8°C for up to 5 days. Plasma specimens may be stored at -20 +/- 10°C for up to 60 days.

If longer storage is required, plasma specimens must be kept at – 70°C or lower.[32,33] Multiple freeze-thaw cycles should be avoided. If frozen, thaw plasma specimens at 15 to 30°C or at 2 to 8°C. Once thawed, if plasma specimens are not being processed immediately, they can be stored at 2 to 8°C for up to 6 hours.

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

Specimen Transport

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

ABBOTT REALTI*M*E HIV-1 ASSAY PROCEDURE

This Abbott RealTi*m*e HIV-1 package insert contains 2 assay protocols:

- Samples prepared for amplification using the Abbott *m*1000 System or the manual sample preparation method follow **ASSAY PROTOCOL I.**
- Samples prepared for amplification using the Abbott *m*2000*sp* instrument follow **ASSAY PROTOCOL II**.

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

Materials Provided

- Abbott RealTi*m*e HIV-1 Amplification Reagent Kit (List No. 2G31-90)

Materials Required But Not Provided

- Abbott RealTi*m*e HIV-1 Calibrator Kit (List No. 2G31-70)
- Abbott RealTi*m*e HIV-1 Control Kit (List No. 2G31-80)

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

For Abbott *m*1000 System
Sample Preparation Area

- Abbott *m*1000 System
- Abbott *m*Sample Preparation System (4 × 24 Preps) (List No. 04J70-24)
- Reaction Vessels
- Calibrated precision pipettes capable of delivering 20 to 1000 µL
- 20 µL to 1000 µL aerosol barrier pipette tips for precision pipettes
- 11.6 to 16 mm Sample Tubes
- 200 µL and 1000 µL disposable tips
- Abbott 96 Deep-Well Plate (List No. 04J71-30)
- Vortex Mixer
- Abbott Optical Adhesive Covers (List No. 04J71-75)
- Abbott Adhesive Cover Applicators
- Abbott Splash-Free Support Base (List No. 09K31-01)
- Reagent Troughs
- 1.5 mL Output Tubes
- Centrifuge capable of 5000*g*

For Abbott *m*2000*sp* Instrument
Sample Preparation Area

- Abbott *m*2000*sp* instrument
- Abbott *m*Sample Preparation System (4 × 24 Preps) (List No. 04J70-24)
- 5 mL Reaction Vessels
- Calibrated precision pipettes capable of delivering 20 to 1000 µL
- 20 µL to 1000 µL aerosol barrier pipette tips for precision pipettes
- 11.5 to 16 mm Sample Tubes
- 200 µL and 1000 µL disposable tips
- Vortex Mixer
- Abbott Optical Adhesive Covers (List No. 04J71-75)
- Abbott Adhesive Cover Applicators
- Abbott Splash-Free Support Base (List No. 09K31-01)
- Master Mix Vial
- 200 mL Reagent Vessels
- Abbott 96-Deep-Well Plate (List No. 04J71-30)
- Abbott RealTi*m*e HIV-1 *m*2000 ROW System Combined Application CD-ROM (List No. 1L68)
- Abbott 96-Well Optical Reaction Plate (List No. 04J71-70)
- Centrifuge capable of 2000*g*

For Abbott *m*1000 System

Reagent Preparation Area

- PCR cooler, either Strata-Cooler® 96 Benchtop Cooler or Eppendorf® PCR-Cooler
- Abbott 96-Well Optical Reaction Plate (List No. 04J71-70)
- Calibrated precision pipettes capable of delivering 20 to 1000 µL
- 20 µL to 1000 µL aerosol barrier pipette tips for precision pipettes
- Single-use RNase/DNase-free tube or container
- Vortex Mixer

For Abbott *m*2000*rt* Instrument

Amplification Area

- Abbott *m*2000*rt* instrument
- Abbott RealT*ime* HIV-1 *m*2000 ROW System Combined Application CD-ROM (List No. 1L68)
- Abbott *m*2000*rt* Optical Calibration Kit (List No. 04J71-93)

Other Materials

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

†Note: These 3 items are used in the procedure for **Monitoring the Laboratory for the Presence of Contamination**. Refer to the **QUALITY CONTROL PROCEDURES** section of this package insert.

Procedural Precautions

Read the instructions in this package insert carefully before processing samples.

The Abbott RealT*ime* HIV-1 Calibrators, Internal Control, Negative Control, Low Positive Control, and High Positive Control vials are intended for single-use only and should be discarded after use.

Use aerosol barrier pipette tips or disposable pipettes only one time when pipetting specimens, IC, or amplification reagents. To prevent contamination to the pipette barrel while pipetting, care should be taken to avoid touching the pipette barrel to the inside of the sample tube or container. The use of extended aerosol barrier pipette tips is recommended.

Monitoring procedures for the presence of amplification product can be found in the **QUALITY CONTROL PROCEDURES** section in this package insert.

To reduce the risk of nucleic acid contamination, clean and disinfect spills of specimens by including the use of a tuberculocidal disinfectant such as 1.0% sodium hypochlorite or other suitable disinfectant.

The Abbott RealT*ime* HIV-1 Calibrators and Controls must be prepared in conjunction with the specimens to be tested. The use of the Abbott RealT*ime* HIV-1 Controls and Calibrators is integral to the performance of the Abbott RealT*ime* HIV-1 assay. Refer to the **QUALITY CONTROL PROCEDURES** section of this package insert for details.

ASSAY PROTOCOL I: ABBOTT *m*1000 SYSTEM OR THE MANUAL SAMPLE PREPARATION METHOD AND ABBOTT *m*2000*rt* INSTRUMENT

For a detailed description of how to perform an Abbott *m*1000 System and Abbott *m*2000*rt* instrument protocol, refer to the Abbott *m*1000 Operating Manual, Operation section and the Abbott *m*2000*rt* Operations Manual, Operating Instructions section.

Laboratory personnel must be trained to operate the Abbott *m*1000 System and the Abbott *m*2000*rt* instrument. The operator must have a thorough knowledge of the software applications and must follow good laboratory practices.

1. Thaw assay controls and IC at 15 to 30°C or at 2 to 8°C. Thaw calibrators at 15 to 30°C or at 2 to 8°C only if performing a calibration run; see **QUALITY CONTROL PROCEDURES** section of this package insert.
   - Once thawed, assay controls, IC, and calibrators can be stored at 2 to 8°C for up to 24 hours before use.
   - Vortex each assay calibrator and each control 3 times for 2 to 3 seconds before use. Ensure that the contents of each vial are at the bottom after vortexing by tapping the vials on the bench to bring liquid to the bottom of the vial.
2. Thaw amplification reagents at 15 to 30°C or at 2 to 8°C and store at 2 to 8°C until required for the amplification master mix procedure.
   - Once thawed, the amplification reagents can be stored at 2 to 8°C for up to 24 hours if not used immediately.

NOTE: Use 1 bottle of *m*Lysis Buffer, 1 vial of IC, and 1 Abbott RealT*ime* HIV-1 Amplification Reagent Pack to support up to 24 reactions. Use a second set of reagents to support 25 to 48 reactions. A maximum of 48 reactions can be performed per run using an Abbott *m*1000 instrument.

Sample Preparation Area

For sample preparation using the Abbott *m*1000 System, follow steps 3 through 10. For the manual sample preparation method refer to the Extraction Protocol Section of the Manual Sample Preparation for Abbott RealT*ime* RNA Assays Procedure (List No. 06L73).

3. Gently invert the Abbott *m*Sample Preparation bottles to ensure a homogeneous solution. If crystals are observed in any of the reagent bottles upon opening, allow the reagent to equilibrate at room temperature until the crystals disappear. Do not use the reagents until the crystals have dissolved.
4. Vortex each IC 3 times for 2 to 3 seconds before use.
5. Use a calibrated precision **PIPETTE DEDICATED FOR INTERNAL CONTROL USE ONLY** to add 500 µL of IC to each bottle of *m*Lysis Buffer. Mix by gently inverting the container 5 to 10 times to minimize foaming.
6. **A total of 48 samples can be processed in each run**. A negative control, a low positive control, and a high positive control are included in each run, therefore allowing a maximum of 45 specimens to be processed per run.
   - The Abbott RealT*ime* HIV-1 assay minimum sample volume and associated rack requirements on the Abbott *m*1000 System are:

		Abbott RealT*ime* HIV-1 Minimum Sample Volume Assay Application		
Rack	**Tube Diameter[a]**	**0.2 mL**	**0.5 mL**	**1.0 mL**
13 mm	11.6 mm - 14.0 mm	0.7 mL	1.0 mL	1.5 mL
16 mm	15.0 mm - 16.0 mm	1.0 mL	1.3 mL	1.8 mL

[a] Refers to sample tube outer diameter

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

     NOTE: For every stored specimen, the following actions must be done in the order described: vortex the specimen first and follow with centrifugation. If these actions are not performed in this order, then invalid results may occur.
   - Vortex each specimen 3 times for 2 to 3 seconds.
   - Centrifuge specimens at 2000*g* for 5 minutes before loading onto the Abbott *m*1000 worktable. Aliquot each specimen into clean tubes or vials if necessary. Refer to the Abbott *m*1000 Operating Manual for tube sizes. Avoid touching the inside of the cap when opening tubes.
7. Place the calibrators (if applicable), low and high positive controls, the negative control, and the patient specimens into the Abbott *m*1000 sample rack. Follow directions for performing a user-defined protocol, as described in the Abbott *m*1000 Operating Manual, Operation section.
8. Place the Reaction Vessels into the Abbott *m*1000 1 mL subsystem carrier.
9. Load the Abbott *m*Sample Preparation System reagents and the 1.5 mL Output Tubes on the Abbott *m*1000 System worktable as described in the Abbott *m*1000 Operating Manual, Operation section.
10. Initiate the Abbott *m*1000 protocol as described in the Abbott *m*1000 Operating Manual, Operation section. From the Protocol screen, select the appropriate application file corresponding to the sample volume being tested.
    - **The assembly of the amplification master mix and sample eluates into the Abbott 96-Well Optical Reaction Plate (step 17) must be initiated within 1 hour after completion of Sample Preparation.**

Amplification Area

11. Switch on and initialize the Abbott *m*2000*rt* instrument.

    NOTE: The Abbott *m*2000*rt* instrument requires 15 minutes to warm up.

12. Create the Abbott *m*2000*rt* test order. Refer to the Operating Instructions section of the Abbott *m*2000*rt* Operations Manual. From the Protocol screen, select the appropriate application file corresponding to the sample volume being tested.
    - Enter calibrator (needed if a calibration curve has not been stored on the Abbott *m*2000*rt*) and control lot specific values in the test order for accurate calibration and control evaluation. Lot-specific values are specified in each Abbott RealTi*m*e HIV-1 Calibrator and Control Kit Card.

Reagent Preparation Area

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

13. Prepare the amplification master mix.
    - Each Amplification Reagent Pack supports up to 24 reactions.
    - Prior to opening the amplification reagents, ensure that the contents of the vials are at the bottom by tapping the vials in an upright position on the bench to bring the liquid to the bottom of the vials.
    - Prepare the master mix by using a **PIPETTE DEDICATED FOR REAGENT USE ONLY** to add 271 µL of the HIV-1 Activation Reagent (Reagent 1) and 949 µL of the HIV-1 Oligonucleotide Reagent (Reagent 2) together in the Thermostable rTth DNA Polymerase Enzyme bottle (Reagent 3).
    - If performing 25 to 48 reactions, prepare a second amplification master mix with a second Amplification Reagent Pack.
    - **The Abbott *m*2000*rt* protocol (step 20) must be initiated within 40 minutes of the addition of Activation Reagent into the first rTth Enzyme Reagent bottle (step 13).**
14. Pipette the contents of the master mix from the enzyme bottle(s) into a single-use RNase/DNase-free tube and vortex to mix.
15. Place an Abbott 96-Well Optical Reaction Plate in a PCR cooler stored as indicated in the PCR cooler instruction manual. Using a **DEDICATED PIPETTE**, dispense 50 µL aliquots of the amplification master mix into the Abbott 96-Well Optical Reaction Plate. A calibrated repeat pipettor may be used. Visually verify that 50 µL has been dispensed into each well.
16. Transfer the Abbott 96-Well Optical Reaction Plate on the PCR cooler to the Sample Preparation Area.

Sample Preparation Area

17. In the Sample Preparation Area, transfer 50 µL of sample eluate to the Abbott 96-Well Optical Reaction Plate on the PCR cooler. **Use a separate pipette tip for each sample eluate transfer**. During the transfer of each sample, mix the reaction by pipetting up and down 3 to 5 times. Visually verify that 100 µL has been dispensed into each well.
18. Seal the Abbott 96-Well Optical Reaction Plate according to the instructions in the Abbott *m*2000*rt* Operations Manual.
19. Remove the Abbott 96-Well Optical Reaction Plate from the PCR cooler and place in the Abbott Splash-Free Support Base. Centrifuge the Abbott 96-Well Optical Reaction Plate in the Abbott Splash-Free Support Base at 5,000*g* for 5 minutes. Transfer to the Amplification Area.

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

Amplification Area

20. Place the Abbott 96-Well Optical Reaction Plate in the Abbott *m*2000*rt* instrument. From the Protocol screen, select the appropriate application file corresponding to the sample volume being tested. Initiate the Abbott RealTi*m*e HIV-1 protocol, as described in the Abbott *m*2000*rt* Operations Manual, Operating Instructions section.

POST PROCESSING PROCEDURES

1. Clean the PCR cooler as described in the PCR cooler instruction manual and return to the Reagent Preparation Area.
2. Remove the 1.5 mL Output Tubes from the worktable and dispose of according to the Abbott *m*1000 Operating Manual.
3. Place the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose of according to the Abbott *m*2000*rt* Operations Manual along with the gloves used to handle the plate.
4. Clean the Splash-Free Support Base before next use, according to the Abbott *m*2000*rt* Operations Manual.
5. For manual sample preparation method users, refer to the Clean Up Section of the Manual Sample Preparation for Abbott RealTi*m*e RNA Assays Procedure (List No. 06L73).

ASSAY PROTOCOL II: ABBOTT *m*2000*sp* INSTRUMENT AND ABBOTT *m*2000*rt* INSTRUMENT

For a detailed description of how to perform an Abbott *m*2000*sp* instrument and Abbott *m*2000*rt* instrument protocol, refer to the Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals, Operating Instructions sections. The 96-sample capability requires Abbott *m*2000*sp* Software Version 2.0 or higher. Please follow Abbott *m*2000*sp* Operations Manual (List 09K20-02) and addendum or addenda.

Laboratory personnel must be trained to operate the Abbott *m*2000*sp* and Abbott *m*2000*rt* instruments. The operator must have a thorough knowledge of the applications run on the instruments and must follow good laboratory practices.

1. Thaw assay controls and IC at 15 to 30°C or at 2 to 8°C. Thaw calibrators at 15 to 30°C or at 2 to 8°C only if performing a calibration run; see **QUALITY CONTROL PROCEDURES** section of this package insert.
    - Once thawed, assay controls, IC, and calibrators can be stored at 2 to 8°C for up to 24 hours before use.
    - Vortex each assay calibrator and each control 3 times for 2 to 3 seconds before use. Ensure that the contents of each vial are at the bottom after vortexing by tapping the vials on the bench to bring liquid to the bottom of the vial.
2. Thaw amplification reagents at 15 to 30°C or at 2 to 8°C and store at 2 to 8°C until required for the amplification master mix procedure.
    - Once thawed, the amplification reagents can be stored at 2 to 8°C for up to 24 hours if not used immediately.

    NOTE: Use 1 bottle of *m*Lysis Buffer, 1 vial of IC, and 1 Abbott RealTi*m*e HIV-1 Amplification Reagent Pack to support up to 24 reactions. Use a second set of reagents to support 25 to 48 reactions, a third set of reagents to support 49 to 72 reactions, and a fourth set of reagents to support 73 to 96 reactions WITH THE EXCEPTION OF *m*MICROPARTICLES. USE ONLY 2 BOTTLES OF *m*MICROPARTICLES WHEN PROCESSING 25 TO 96 SAMPLES.
3. Gently invert the Abbott *m*Sample Preparation bottles to ensure a homogeneous solution. If crystals are observed in any of the reagent bottles upon opening, allow the reagent to equilibrate at room temperature until the crystals disappear. Do not use the reagents until the crystals have dissolved.
4. Vortex each IC 3 times for 2 to 3 seconds before use.
5. Use a calibrated precision **PIPETTE DEDICATED FOR INTERNAL CONTROL USE ONLY** to add 500 µL of IC to each bottle of *m*Lysis Buffer. Mix by gently inverting the container 5 to 10 times to minimize foaming.
6. **A total of** 96 **samples can be processed in each run**, **with the exception of the 1.0 ml Assay Application**. A negative control, a low positive control, and a high positive control are included in each run, therefore allowing a maximum of 93 specimens to be processed per run. For the 1.0 ml Assay Application, a total of 48 samples can be processed in each run, allowing a maximum of 45 specimens to be processed per run.
    - The Abbott RealTi*m*e HIV-1 assay minimum sample volume and associated rack requirements on the Abbott *m*2000*sp* are:

		Abbott RealTi*m*e HIV-1 Minimum Sample Volume Assay Application			
Rack	**Tube Diameter**[a]	**0.2 mL**	**0.5 mL**	**0.6 mL**	**1.0 mL**
13 mm	11.5 - 14.0 mm	0.4 - 0.8 mL	0.7 - 1.2 mL	0.8 - 1.3 mL	1.2 - 1.7 mL
16 mm	14.5 - 16.0 mm	0.4 - 1.0 mL	0.8 - 1.4 mL	0.9 - 1.5 mL	1.3 - 1.9 mL

[a] Refers to sample tube outer diameter. Minimum sample volume varies with tube geometry and size. Refer to the Abbott *m*2000*sp* Operations Manual and **QUICK REFERENCE GUIDE FOR SAMPLE TUBE SIZES AND VOLUMES** for recommended sample input volume.

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

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

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

7. Place the low and high positive controls, the negative control, the calibrators, if applicable, and the patient specimens into the Abbott *m*2000*sp* sample rack.
8. Place the 5 mL Reaction Vessels into the Abbott *m*2000*sp* 1 mL subsystem carrier.
9. Load the Abbott *m*Sample Preparation System reagents and the Abbott 96 Deep-Well Plate on the Abbott *m*2000*sp* worktable as described in the Abbott *m*2000*sp* Operations Manual, Operating Instructions section.
10. From the Protocol screen, select the appropriate application file corresponding to the sample volume being tested. Initiate the sample extraction protocol as described in the Abbott *m*2000s*p* Operations Manual, Operating Instruction section.
    - Enter calibrator (needed if a calibration curve has not been stored on the Abbott *m*2000*rt*) and control lot specific values in the **Sample Extraction: Worktable Setup, Calibrator and Control** fields. Lot-specific values are specified in each Abbott RealTi*m*e HIV-1 Calibrator and Control Kit Card.
    - **The Abbott *m*2000*sp* 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.
11. Load the amplification reagents and the master mix vial on the Abbott *m*2000*sp* 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.
    - A second Amplification Reagent Pack is required if performing 25 to 48 reactions.
    - A third Amplification Reagent Pack is required if performing 49 to 72 reactions.
    - A fourth Amplification Reagent Pack is required if performing 73 to 96 reactions.
12. Select the appropriate deep-well plate that matches the corresponding sample preparation extraction. Initiate the Abbott *m*2000*sp* Master Mix Addition protocol. Follow the instructions as described in the Abbott *m*2000*sp* Operations Manual, Operating Instructions section.

    NOTE: The operator should not manually fill any empty/unfilled wells in the Abbott 96-Well Optical Reaction Plate.
    - After sample extraction is complete, the Abbott *m*2000*sp* automatically fills any empty wells in the Abbott 96-Well Optical Reaction Plate when there are greater than 48 samples processed within a run. Plate fill is not performed for runs containing 48 samples or fewer.
    - If prompted by the instrument, Reagent Carrier 2 should remain in place, minimally containing the reagent vessel for *m*Elution Buffer (Reagent Carrier 2, location 6). If this reagent vessel has been unloaded, place a new reagent vessel with the *m*Elution Buffer label into Reagent Carrier 2, location 6. System fluid will be added to the reagent vessel and used to fill empty wells. Once this process is complete, the system will continue with the master mix addition.

      NOTE: System instructions for use of the automated plate-filling feature are found in the Abbott *m*2000*sp* Operations Manual (List No. 9K20-04 or higher), section 5, Operating Instructions, Sample Extraction—Closed Mode.
    - **The Abbott *m*2000*rt* protocol (step 16) must be started within 50 minutes of the initiation of the Master Mix Addition protocol (step 12).**

      NOTE: If the run is aborted for any reason subsequent to step 12, a new 96-well PCR plate must be used if the Abbott *m*2000*sp* Master Mix Addition Protocol (step 12) will be repeated.
13. Switch on and initialize the Abbott *m*2000*rt* instrument in the Amplification Area.

    NOTE: The Abbott *m*2000*rt* 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 *m*2000*sp* instrument has completed addition of samples and master mix according to the Abbott *m*2000*sp* Operations Manual, Operating Instructions section.
15. Place the sealed optical reaction plate into the Abbott Splash-Free Support Base for transfer to the Abbott *m*2000*rt* instrument.
16. Place the Abbott 96-Well Optical Reaction Plate in the Abbott *m*2000*rt* instrument. From the Protocol screen, select the appropriate application file corresponding to the sample volume being tested. Initiate the Abbott RealTi*m*e HIV-1 protocol, as described in the Abbott *m*2000*rt* Operations Manual, Operating Instructions section.

    NOTE: If creating the Abbott *m*2000*rt* test order manually, enter sample IDs in the corresponding PCR tray locations according to the "Wells for Selected Plate" grid, found on the detail screen of the "PCR Plate Results" on the Abbott *m*2000*sp*. See Section 5 of the Abbott *m*2000*sp* Operations Manual.

POST PROCESSING PROCEDURES

1. Remove the Abbott 96 Deep-Well Plate from the worktable and dispose of according to the Abbott *m*2000*sp* Operations Manual.
2. Place the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott *m*2000*rt* Operations Manual along with the gloves used to handle the plate.
3. Clean the Abbott Splash-Free Support Base before next use, according to the Abbott *m*2000*rt* Operations Manual.

QUALITY CONTROL PROCEDURES

Abbott *m*2000*rt* Optical Calibration

Refer to the Calibration Procedures section in the Abbott *m*2000*rt* Operations Manual for a detailed description of how to perform an Abbott *m*2000*rt* Optical Calibration.

Optical calibration of the Abbott *m*2000*rt* instrument is required for the accurate measurement and discrimination of dye fluorescence during the Abbott RealTi*m*e HIV-1 assay.

The following Abbott *m*2000*rt* Optical Calibration Plates are used to calibrate the Abbott *m*2000*rt* instrument for the Abbott RealTi*m*e HIV-1 assay:

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

Assay Calibration

For a detailed description of how to perform an assay calibration refer to the Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals, Operating Instructions sections.

A calibration curve is required to quantitate the HIV-1 RNA concentration of specimens and controls. Two assay calibrators are run in replicates of 3 to generate a calibration curve (HIV-1 concentration versus the threshold cycle [C_T] at which a reactive level of fluorescent signal is detected). The calibration curve slope and intercept are calculated and stored on the instrument. The concentration of HIV-1 RNA in a sample is calculated from the stored calibration curve. Results are automatically reported on the Abbott *m*2000*rt* workstation.

Follow the procedure for sample extraction, master mix addition, amplification and detection protocols as stated in the Abbott *m*1000 Operating Manual or Abbott *m*2000*sp* Operations Manual, and the Abbott *m*2000*rt* Operations Manual.

Once an Abbott RealTi*m*e HIV-1 calibration is accepted and stored, it may be used for 6 months. During this time, all subsequent samples may be tested without further calibration unless:

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

- Pure Dye optical re-calibration of the Abbott RealTi*m*e HIV-1 assay-specific dyes (FAM, VIC, or ROX) is performed per the Calibration Procedures section of the Abbott *m*2000*rt* Operations Manual.

Detection of Inhibition

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

A defined, consistent quantity of IC is introduced into each specimen, calibrator, and control at the beginning of sample preparation and measured on the Abbott *m*2000*rt* instrument to demonstrate proper specimen processing and assay validity. The IC is comprised of an RNA sequence unrelated to the HIV-1 target sequence.

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

An error control flag is displayed when a specimen or control fails to meet this specification. Refer to the Abbott *m*2000*rt* Operations Manual for an explanation of the corrective actions for the error control flag. Specimens whose IC C_T value exceeds the established range must be retested starting with sample preparation.

Negative and Positive Controls

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

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

An error control flag is displayed when a control result is out of range. Refer to the Abbott *m*2000*rt* Operations Manual for an explanation of the corrective actions for the error control flag. If negative or positive controls are out of range, all of the specimens and controls from that run must be reprocessed, beginning with sample preparation.

The presence of HIV-1 must not be detected in the negative control. HIV-1 detected in the negative control is indicative of contamination by other samples or by amplified product introduced during sample preparation or during preparation of the Abbott 96-Well Optical Reaction Plate. To avoid contamination, clean the Abbott *m*1000 System or Abbott *m*2000*sp* instrument and the Abbott *m*2000*rt* instrument and repeat sample processing for controls and specimens following the **Procedural Precautions**. If negative controls are persistently reactive, contact your Abbott representative.

Monitoring the Laboratory for the Presence of Contamination

It is recommended that this test be done at least once a month to monitor laboratory surfaces and equipment for contamination by amplification product. It is very important to test all areas that may have been exposed to processed specimens, controls, and calibrators, and/or amplification product. This includes routinely handled objects such as pipettes, the Abbott *m*1000 System, the Abbott *m*2000*sp* and Abbott *m*2000*rt* function keys, laboratory bench surfaces, microcentrifuges, and centrifuge adaptors.

1. Add 0.8 mL RNase-free water to a 1.7 mL molecular biology grade microcentrifuge tube.
2. Saturate the cotton tip of an applicator (Puritan or equivalent) in the RNase-free water from the microcentrifuge tube.
3. Using the saturated cotton tip of the applicator, wipe the area to be monitored using a sweeping motion. Place the applicator into the microcentrifuge tube.
4. Swirl the cotton tip in RNase-free water 10 times, and then press the applicator along the inside of the tube so that the liquid drains back into the solution at the bottom of the microcentrifuge tube. Discard the applicator.
5. Pipette 0.5 mL of *m*Wash 1 buffer to a clean tube using the pipette dedicated for Internal Control use.
6. Add 20 µL of the *m*Wash 1 buffer to each microcentrifuge tube.
7. Cap the microcentrifuge tube.
8. Test this sample according to the assay procedure section of this package insert.
   - Transfer liquid from the microcentrifuge tube to a 5 mL Reaction Vessel.
   - Bring the volume to 1.5 mL with RNase-free water.
9. The presence of contamination is indicated by the detection of HIV-1 nucleic acid in the swab samples.
10. If HIV-1 nucleic acid is detected on equipment, follow the cleaning and decontaminating guidelines given in that equipment's operations manual. If HIV-1 nucleic acid is detected on surfaces, clean the contaminated areas with 1.0% (v/v) sodium hypochlorite solution, followed by 70% ethanol or water.

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

RESULTS

Calculation

The concentration of viral HIV-1 RNA in a sample or control is calculated from the stored calibration curve. The Abbott *m*2000*rt* instrument automatically reports the results on the Abbott *m*2000*rt* workstation. Assay results can be reported in copies/mL, log [copies/mL], International Units (IU)/mL, or log [IU/mL]; (1 IU = 0.58 copies, 1 copy = 1.74 IU).

INTERPRETATION OF RESULTS

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

[a] 40 Copies/mL

[b] 75 Copies/mL

[c] 150 Copies/mL

[d] ULQ = upper limit of quantitation

LIMITATIONS OF THE PROCEDURE

- **FOR IN VITRO DIAGNOSTIC USE**
- Optimal performance of this test requires appropriate specimen collection, storage, and transport to the test site (refer to the **SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE** section of this package insert).
- Human plasma specimens (collected in ACD-A or EDTA tubes) may be used with the Abbott RealTi*m*e HIV-1 assay. The use of other anticoagulants has not been validated with the Abbott RealTi*m*e HIV-1 assay.
- Use of the Abbott RealTi*m*e HIV-1 assay is limited to personnel who have been trained in the procedures of a molecular diagnostic assay and/or the Abbott *m*1000 System, the Abbott *m*2000*sp,* and the Abbott *m*2000*rt* instruments.
- The instruments and assay procedures reduce the risk of contamination by amplification product. However, nucleic acid contamination from the calibrators, positive controls, or specimens must be controlled by good laboratory practices and careful adherence to the procedures specified in this package insert.
- As with any diagnostic test, results from the Abbott RealTi*m*e HIV-1 assay should be interpreted in conjunction with other clinical and laboratory findings. A specimen with a result of "Not Detected" cannot be presumed to be negative for HIV-1 RNA.

SPECIFIC PERFORMANCE CHARACTERISTICS

The performance characteristics were determined using the Abbott RealTi*m*e HIV-1 assay with Abbott *m*2000*sp* sample preparation and 1.0 mL sample volume, unless otherwise specified.

Limit of Detection (LOD)

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

Limit of Detection, 1.0 mL Sample Volume

The LOD of the Abbott RealTi*m*e HIV-1 assay is 40 copies/mL with the 1.0 mL sample volume procedure.

The LOD was determined by testing dilutions of a viral standard from the Virology Quality Assurance (VQA) Laboratory of the AIDS Clinical Trial Group. Dilutions were made in HIV-1 negative human plasma. Testing was performed with 3 lots of amplification reagents on 3 Abbott *m*2000 Systems. The results, representative of the analytical sensitivity performance of the Abbott RealTi*m*e HIV-1 assay, are summarized in **Table 1**.

Table 1.

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

[a] One replicate generated an invalid replicate error message and was deleted from the data analysis.

Probit analysis of the data determined that the concentration of HIV-1 RNA detected with 95% probability was 25 copies/mL (95% CI 20 to 33).

Limit of Detection, 0.6 mL Sample Volume

The LOD of the Abbott RealTi*m*e HIV-1 assay is 40 copies/mL with the 0.6 mL sample volume procedure.

The LOD for the 0.6 mL sample volume procedure was determined as described for the 1.0 mL sample volume procedure. The results, representative of the analytical sensitivity performance of the Abbott RealTi*m*e HIV-1 assay, are summarized in **Table 2**.

Table 2.

Conc. (Copies/mL)	Number Tested	Number Detected	Percent Detected
100	57	57	100
75	57	56	98
60	57	57	100
50	57	54	95
40	57	54	95
30	57	55	96
20	57	44	77
10	57	27	47
5	57	13	23

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

Limit of Detection, 0.5 mL Sample Volume

The LOD of the Abbott RealTi*m*e HIV-1 assay is 75 copies/mL with the 0.5 mL sample volume procedure.

The LOD for the 0.5 mL sample volume procedure was determined as described for the 1.0 mL sample volume procedure. The results, representative of the analytical sensitivity performance of the Abbott RealTi*m*e HIV-1 assay, are summarized in **Table 3**.

Table 3.

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

[a] One replicate generated an invalid replicate error message and was deleted from the data analysis.

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

Limit of Detection, 0.2 mL Sample Volume

The LOD of the Abbott RealTi*m*e HIV-1 assay is 150 copies/mL with the 0.2 mL sample volume procedure.

The LOD for the 0.2 mL sample volume procedure was determined as described for the 1.0 mL sample volume procedure. The results, representative of the analytical sensitivity performance of the Abbott RealTi*m*e HIV-1 assay, are summarized in **Table 4**.

Table 4.

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

[a] Eight replicates were invalid due to an instrument error and were deleted from the data analysis.

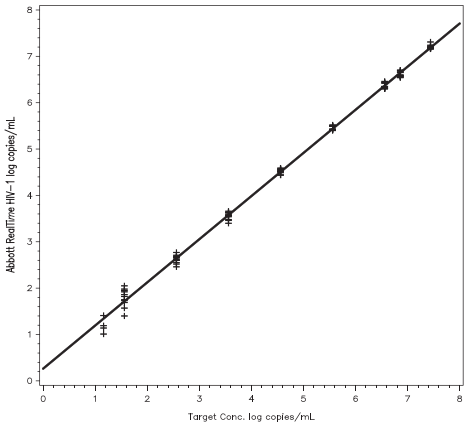
Probit analysis of the data determined that the concentration of HIV-1 RNA detected with 95% probability was 119 copies/mL (95% CI 102 to 150).

Linear Range

The upper limit of quantitation (ULQ) for the Abbott RealTi*m*e HIV-1 assay is 10 million copies/mL, and the lower limit of quantitation is equivalent to the LOD (40 copies/mL for the 1.0 mL and 0.6 mL sample volume procedure, 75 copies/mL for the 0.5 mL sample volume procedure, and 150 copies/mL for the 0.2 mL sample volume procedure).

A 9-member panel prepared by diluting armored HIV-1 RNA from 7.44 log copies/mL to 1.16 log copies/mL in HIV-1 negative human plasma was tested. Linearity analysis was performed following the NCCLS EP6-A guideline.[34] The results, representative of the Abbott RealTi*m*e HIV-1 assay linearity, are shown in **Figure 1**.

Figure 1.



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

Precision

The precision of the Abbott RealTi*m*e HIV-1 assay was evaluated for the 1.0 mL sample volume procedure using the Abbott *m*1000 and Abbott *m*2000*sp* sample preparation systems and the manual sample preparation method. The Abbott RealTi*m*e HIV-1 assay is designed to achieve an inter-assay standard deviation (SD) of less than or equal to 0.25 log copies of HIV-1 RNA per mL for samples containing HIV-1 concentrations from 500 to 5 million copies/mL. A 7-member HIV-1 RNA panel was prepared by diluting an HIV-1 viral stock (panel members 1 through 3) and armored HIV-1 RNA (panel members 4 through 7) in negative human plasma. For the precision studies with the Abbott *m*1000 and the Abbott *m*2000*sp*, the panel members were tested in replicates

of 5 in a total of 15 runs on 3 instrument systems, with 3 lots of amplification reagents. For the precision study using the manual sample preparation method, panel members were tested in replicates of 2 for the first run on each instrument and replicates of 3 for each subsequent run for a total of 15 runs on 3 Abbott *m*2000*rt* instruments with 3 lots of amplification reagents. Precision analysis was performed following the NCCLS EP10-A2 guideline.[35] Within-run, between-run, and inter-assay (within-run and between-run) standard deviations were determined. The results, representative of the precision of the Abbott RealTi*m*e HIV-1 assay, are summarized in **Tables 5**, **6**, and **7**.

Table 5.

Precision with the Abbott *m*1000 System

Panel Member	n	Conc. Mean (copies/mL)	Conc. Mean (log copies/mL)	Within-Run SD Component	Between-Run SD Component	Inter-Assay SD[a]
1	75	57	1.75	0.21	0.00	0.21
2	75	573	2.76	0.08	0.00	0.08
3	75	5,000	3.70	0.05	0.02	0.06
4	73[b,c]	35,751	4.55	0.03	0.01	0.04
5	75	315,065	5.50	0.07	0.03	0.07
6	74[b]	2,947,538	6.47	0.05	0.04	0.07
7	75	5,347,285	6.73	0.04	0.05	0.07

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

[b] Two replicates were inhibited and were deleted from the data analysis.

[c] HIV-1 RNA was not detected in 1 replicate.

Table 6.

Precision with the Abbott *m*2000 System

Panel Member	n	Conc. Mean (copies/mL)	Conc. Mean (log copies/mL)	Within-Run SD Component	Between-Run SD Component	Inter-Assay SD[a]
1	74[b]	72	1.86	0.18	0.07	0.19
2	75	652	2.81	0.08	0.00	0.08
3	75	5,417	3.73	0.04	0.02	0.05
4	75	39,458	4.60	0.04	0.03	0.05
5	74[c]	358,587	5.55	0.03	0.03	0.04
6	75	3,102,654	6.49	0.03	0.02	0.04
7	75	5,953,879	6.77	0.04	0.04	0.05

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

[b] HIV-1 RNA was not detected in 1 replicate.

[c] One replicate was inhibited and was deleted from the data analysis.

Table 7.

Precision with Manual Sample Preparation Method

Panel Member	n	Conc. Mean (copies/mL)	Conc. Mean (log copies/mL)	Within-Run SD Component	Between-Run SD Component	Inter-Assay SD[a]
1	40[b]	46	1.66	0.21	0.07	0.22
2	41[c]	471	2.67	0.11	0.09	0.14
3	42	4,474	3.65	0.05	0.10	0.11
4	42	34,503	4.54	0.02	0.06	0.07
5	42	362,283	5.56	0.04	0.08	0.09
6	42	3,597,099	6.56	0.03	0.04	0.05
7	42	6,552,825	6.82	0.05	0.05	0.07

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

[b] HIV-1 RNA was not detected in 2 replicates.

[c] One replicate was inhibited and deleted from the data analysis.

Potentially Interfering Substances

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

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

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

Drugs at concentrations in excess of the peak plasma or serum levels were tested in 5 pools. No interference in the performance of the Abbott RealTi*m*e HIV-1 assay was observed in the presence of the following drug pools for all positive and negative samples tested:

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

Specificity

The target specificity of the Abbott RealTi*m*e HIV-1 assay is greater than or equal to 99.5% after resolution.

The specificity of the Abbott RealTi*m*e HIV-1 assay was evaluated by testing 187 HIV-1 seronegative plasma specimens. The specimens were tested on 3 Abbott *m*2000 instrument systems with 3 lots of amplification reagents. HIV-1 RNA was not detected, resulting in 100% (187/187) specificity (95% CI 98.05 to 100.00) in this representative study.

The specificity of the assay was further evaluated by testing 70 specimens that had been either obtained from individuals diagnosed or screened for an autoimmune disorder or serologically characterized as positive for the following markers: systemic lupus erythematosus (SLE), anti-nuclear antibodies (ANA), rheumatoid factor (RF), HBsAg, anti-HTLV-I/II, anti-HCV, and anti-HIV-2. HIV-1 RNA was not detected in any of the specimens tested. The results demonstrated that the presence of an autoimmune disorder or serologic markers for autoimmune disease or viral pathogens other than HIV-1 did not affect the Abbott RealTi*m*e HIV-1 assay.

Cross-Reactivity

The following viruses and microorganisms were evaluated for potential cross-reactivity in the Abbott RealTi*m*e HIV-1 assay. Purified nucleic acid or viral lysate from each microorganism or virus was added to HIV-1 RNA negative samples and samples that contained 10,000 copies/mL HIV-1 RNA.

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

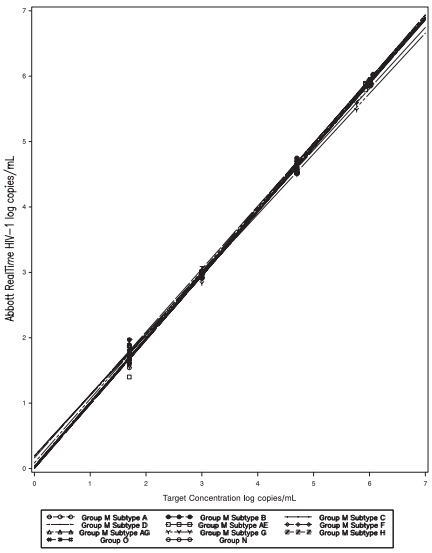
No interference in the performance of the Abbott RealTi*m*e HIV-1 assay was observed in the presence of the potential cross-reactants for all positive and negative samples tested.

Detection of HIV-1 Subtypes and Groups

The performance of the Abbott RealTi*m*e HIV-1 assay with HIV-1 subtypes/groups was evaluated by analysis of purified RNA transcripts from Group M (subtypes A, B, C, D, CRF01-AE, F, CRF02-AG, G, and H), Group O, and Group N, and by testing 10 clinical specimens of each Group M subtype (A, B, C, D, CRF01-AE, F, CRF02-AG, and G), and 10 specimens from Group O.

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

Figure 2.



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

A total of 90 clinical specimens, 10 of each Group M subtype (A, B, C, D, CRF01-AE, F, CRF02-AG, G) and Group O, were tested with the Abbott RealTi*m*e HIV-1 assay and by 2 other HIV-1 quantitative assays referred to as Comparator 1 and Comparator 2. The results are summarized in **Table 8**.

Table 8.

Group/ Subtypes	n	RealTi*m*e Detected	Comparator 1 Detected[a]	Comparator 2 Detected[a]
M/Subtype A	10	10	10 (1)	10 (1)
M/Subtype B	10	10	10 (0)	10 (0)
M/Subtype C	10	10	10 (0)	10 (0)
M/Subtype D	10	10	10 (0)	10 (0)
M/Subtype AE	10	10	10 (0)	10 (0)
M/Subtype F	10	10	10 (0)	10 (0)
M/Subtype AG	10	10	10 (3)	10 (1)
M/Subtype G	10	10	10 (2)	10 (1)
Group O	10	10	0 (NA)	7 (7)

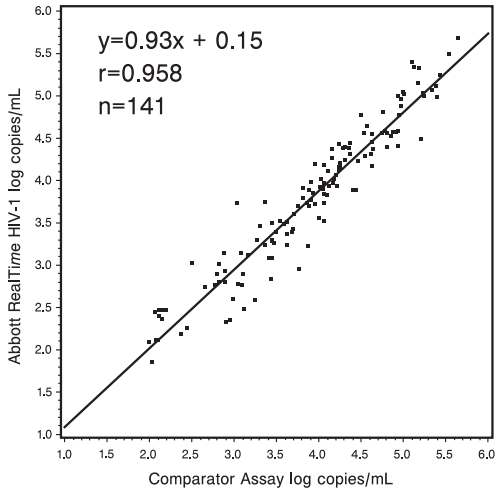
[a] The numbers in parentheses are the number of specimens that had lower quantitation values by more than 1.00 log copies/mL when compared to Abbott RealTi*m*e HIV-1 assay.

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

Correlation

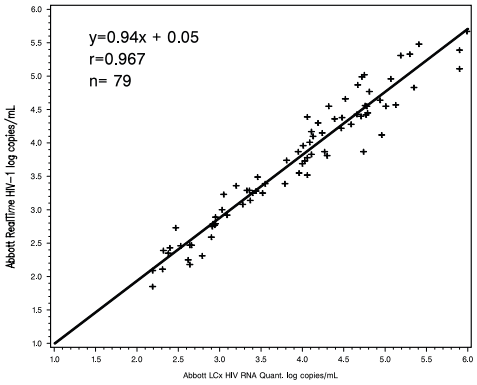
Method comparison analysis was performed following NCCLS EP9-A2.[36] Specimens from 141 HIV-1 infected patients were tested with the Abbott RealTi*m*e HIV-1 assay and a comparator assay. The correlation plot is shown in **Figure 3**.

Figure 3.



Specimens from 79 HIV-1 infected patients (a subset of the 141 tested) were tested with the Abbott LCx HIV RNA Quantitative assay. The correlation plot is shown in **Figure 4**.

Figure 4.



BIBLIOGRAPHY

1. Barre-Sinoussi F, Chermann JC, Rey F, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* 1983;220:868-71.
2. Popovic M, Sarngadharan MG, Read E, et al. Detection, isolation and continuous production of cytopathic retroviruses (HTLV-I) from patients with AIDS and pre-AIDS. *Science* 1984;224:497-500.
3. Gallo RC, Salahuddin SZ, Popovic M, et al. Frequent detection and isolation of cytopathic retroviruses (HTLV-I) from patients with AIDS and at risk for AIDS. *Science* 1984;224:500-3.
4. Curran JW, Jaffe HW, Hardy AM, et al. Epidemiology of HIV infection and AIDS in the United States. *Science* 1988;239:610-16.
5. Daar ES, Moudgil T, Meyer RD, Ho DD. Transient high levels of viremia in patients with primary human immunodeficiency virus type 1 infection. *New Engl J Med* 1991;324:961-4.
6. Clark SJ, Saag MS, Decker WD. High titers of cytopathic virus in plasma of patients with symptomatic primary HIV-1 infection. *New Engl J Med* 1991;324:954-60.
7. Albert J, Abrahamsson B, Nagy K, et al. Rapid development of isolate-specific neutralizing antibodies after primary HIV-1 infection and consequent emergence of virus variants which resist neutralization by autologous sera. *AIDS* 1990;4:107-12.

8. Horsburgh CR Jr, Ou CY, Jason J, et al. Duration of human immunodeficiency virus infection before detection of antibody. *Lancet* 1989;334:637-40.
9. Pantaleo G, Graziosi C, Fauci AS. New concepts in the immunopathogenesis of human immunodeficiency virus (HIV) infection. *New Engl J Med* 1993;328:327-35.
10. Ho DD, Neumann AU, Perelson AS, et al. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* 1995;373:123-6.
11. Wei X, Ghosh SK, Taylor ME, et al. Viral dynamics in human immunodeficiency virus type 1 infection. *Nature* 1995;373:117-22.
12. Mellors JW, Rinaldo CR JR, Gupta P, et al. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science* 1996;272:1167-70.
13. Mellors JW, Munoz A, Giorgi JV, et al. Plasma viral load and $CD4^+$ lymphocytes as prognostic markers of HIV-1 infection. *Ann Intern Med* 1997;126(12):946-54.
14. Chene G, Sterne JA, May M, et al. Prognostic importance of initial response in HIV-1 infected patients starting potent antiretroviral therapy: analysis of prospective studies. *Lancet* 2003;362:679-86.
15. Egger M, May M, Chene G, et al. Prognosis of HIV-1 infected drug patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies. *Lancet* 2002;360:119-29.
16. Wood E, Hogg RS, Yip B, et al. Higher baseline levels of plasma human immunodeficiency virus type 1 RNA are associated with increased mortality after initiation of triple-drug antiretroviral therapy. *J Infect Dis* 2003;188:1421-5.
17. US Department of Health and Human Services. 2004 guidelines for the use of antiretroviral agents in HIV-1 infected adults and adolescents. Available at: http://AIDSinfo.nih.gov/guidelines.
18. Yeni PG, Hammer SM, Hirsch MS, et al. Treatment for Adult HIV Infection. 2004 Recommendations of the International AIDS Society-USA Panel. *JAMA* 2004;292:251-65.
19. Perelson AS, Essunger P, Cao Y, et al. Decay characteristics of HIV-1 infected compartments during combination therapy. *Nature* 1997;387(6629):188-91.
20. Mulder J, McKinney N, Christopher C, et al. Rapid and simple PCR assay for quantitation of human immunodeficiency virus type 1 RNA in plasma: application to acute retroviral infection. *J Clin Microbiol* 1994;32:292-300.
21. Dewar RL, Highbarger HC, Sarmiento MD, et al. Application of branched DNA signal amplification to monitor human immunodeficiency virus type 1 burden in human plasma. *J Inf Diseases* 1994;170:1172-9.
22. Van Gemen B, Kievits T, Schukkink R, et al. Quantification of HIV-1 RNA in plasma using NASBA™ during HIV-1 primary infection. *J Virol Methods* 1993;43:177-87.
23. Yen-Lieberman B, Brambilla D, Jackson B, et al. Evaluation of a quality assurance program for quantitation of human immunodeficiency virus type 1 RNA in plasma by the AIDS clinical trials group virology laboratories. *J Clin Microbiol* 1996;34:2695-701.
24. Holmes H, Davis C, Heath A, et al. An international collaborative study to establish the 1st international standard for HIV-1 RNA for use in nucleic acid-based techniques. *J Virol Methods* 2001;92:141-50.
25. Davis C, Heath A, Best S, et al. Calibration of HIV-1 working reagents for nucleic acid amplification techniques against the 1st international standard for HIV-1 RNA. *J Virol Meth* 2003;107:37-44.
26. Myers TW, Gelfand DH. Reverse Transcription and DNA Amplification by a *Thermus thermophilus* DNA Polymerase. *Biochem* 1991;30:7661-6.
27. Myers G, Korber B, Wain-Hobson S, et al. Human retroviruses and AIDS 1994: A compilation and analysis of nucleic acid and amino acid sequences. Los Alamos National Laboratory, Theoretical Biology and Biophysics (T10). Los Alamos, New Mexico:1994.
28. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories.* 5th ed. Washington, DC: US Government Printing Office; December 2009. [Also available online. Type> www.cdc.gov, search>BMBL5>*look up* sections III and IV.]
29. US Department of Labor, Occupational Safety and Health Administration. 29 CFR Part 1910.1030. *Bloodborne Pathogens*.
30. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline—Third Edition.* CLSI Document M29-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
31. World Health Organization. Laboratory *Biosafety Manual.* 3rd ed. Geneva, Switzerland: World Health Organization; 2004.
32. Ginocchio C, Wang X, Kaplan M, et al. Effects of specimen collection, processing, and storage conditions on stability of human immunodeficiency virus type 1 RNA levels in plasma. *J Clin Microbiol* 1997;35(11):2886-93.
33. Sebire K, McGavin K, Land S, et al. Stability of human immunodeficiency virus RNA in blood specimens as measured by a commercial PCR-based assay. *J Clin Microbiol* 1998;36(2):493-8.
34. National Committee for Clinical Laboratory Standards. *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline - NCCLS document EP6-A,* NCCLS: Wayne, PA, 2002.
35. National Committee for Clinical Laboratory Standards. *Preliminary Evaluation of Quantitative Clinical Laboratory Methods; Approved Guideline – Second Edition. NCCLS Document EP10-A2.* NCCLS: Wayne, PA, 2002.
36. National Committee for Clinical Laboratory Standards. *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline-Second Edition NCCLS document EP9-A2*, NCCLS Wayne, PA, 2002.

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