WHO Prequalification of Diagnostics Programme
PUBLIC REPORT

Product: Abbott RealTi*m*e HIV-1 Qualitative (*m*2000sp)
Number: PQDx 0084-027-00

The **Abbott RealTi*m*e HIV-1 Qualitative (*m*2000sp)** with product code **4N66-90**, manufactured by **Abbott Molecular Inc.**, 1300 East Touhy Avenue, Des Plaines, IL 60018, United States of America, **CE-marked** regulatory version, was accepted for the WHO list of prequalified diagnostics and was listed on 30 May 2013. This version of the product is intended to be used in conjunction with the following instruments/reagents: **9K14-02, 9K15-01, 4N66-80, 4N66-03 or higher, 6K12-24 and 4N66-66** (optional).

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

	Date	Outcome
Status on PQ list	30-May-2013	listed
Dossier assessment	20-May-2013	MR
Inspection status	12 -14-Sep-2018	MR
Laboratory evaluation	FT	MR

MR: Meets Requirements
FT: Fast-Tracked

Report amendments and/or product changes

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

Version	Summary of amendment	Date of report amendment
2.0	Implemented software change.	1-Jun-2018
3.0	1. The Notified Body number on the Abbott RealTime HIV-1 Quantitative and Qualitative kit labels and package inserts were updated to reflect the new notified body Polskie Centrum Badan I Certyfikacji S.A. (PCBC) Notified Body number of 1434.	16-Dec-2019

	2. The word "abbott" were aligned to the center of the Abbott logo (where applicable). 3. Label and Package Insert revision numbers were updated accordingly. List 04N66 4. Update on manufacturing site inspection commitments	
4.0	Updated Abbott's European Authorized Representative (EC Rep) legal entity name from Abbott GmbH & Co. KG to Abbott GmbH. Labeling changes to comply with the labeling requirements for product registered under IVDR.	1-Nov-2021

Intended use:

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

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

Assay principle:

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

Test kit contents:

Item	Product code(s)
Abbott RealTime HIV-1 Qualitative Amplification Reagent Kit (to reference the new assay CD-ROM version 3)	4N66-90 (4 packs x 24 tests/pack)

Materials required but not provided:

Item	Product code(s)
Instrumentation:	
Abbott *m*2000*sp* Instrument with software version 4.0 or higher	9K14-02
Abbott *m*2000*rt* Instrument with software version 3.0 or higher	9K15-01
Reagents:	
Abbott *m*Sample Preparation System$_{DNA}$	6K12-24
Abbott RealTi*m*e HIV-1 Internal Control	2G31Y
Abbott RealTime HIV-1 Qualitative Control Kit	4N66-80
Software:	
Abbott RealTi*m*e HIV‑1 Qualitative m2000 System ROW Combined Application CD‑ROM, version 3.0	04N66-003 or higher
Optional:	
Abbott RealTi*m*e HIV-1 UNG Protocol	4N66-66
Consumables required:	
Abbott *m*2000*rt* Optical Calibration kit	4J71-93
5 mL Reaction Vessels	4J71-20
Calibrated Pipettes capable of delivering 20‑1000 µL	4J71-10
200 µL Disposable Tips	4J71-17
Abbott Optical Adhesive Covers	4J71-75
Abbott Adhesive Cover Applicator	9K32-01
Abbott Splash-Free Support Base	9K31-01
Master Mix Tube	4J71-80
13 mm Sample Racks	4J72-82
200 mL Reagent Vessels	4J71-60
Abbott 96-Deep-Well Plate	4J71-30
Abbott 96-Well Optical Reaction Plate	4J71-70
Bulk *m*LysisDNA Buffer (for DBS processing only)	2N77-01
Aerosol Barrier Pipette Tips for 20-1000 µL Pipettes	n/a
1000 µL Disposable Tips	n/a
Vortex Mixer	n/a
USP Grade 190-200 Proof Ethanol (95%-100% Ethanol). Do not use ethanol that contains denaturants	n/a
Centrifuge capable of 2000g	n/a
50 mL Tubes (NUNC or equivalent) (for DBS processing only) (optional)	n/a

Storage:

Item	Temperature (°C)
Abbott RealTi*m*e HIV-1 Qualitative Control Kit	at -10°C or colder
Abbott RealTi*m*e HIV-1 Qualitative Amplification Reagent Kit	at -10°C or colder
Abbott mSample Preparation System$_{DNA}$	at 15-30°C
Uracil-N-glycosylase (UNG) required for the Abbott RealTi*m*e HIV-1 UNG Protocol (4N66-66)	at -15 to -25°C

Maximum shelf-life upon manufacture:

Item	Duration
Abbott RealTi*m*e HIV-1 Qualitative Amplification Reagent Kit 4N66-90:	
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 Qualitative Control Kit 4N66-80:	
Negative Control 2G31Z	18 months
High Positive Control 2G31X	18 months
The Abbott *m*Sample Preparation System$_{DNA}$	18 months

Prioritization for prequalification

Based on the established eligibility criteria, Abbott RealTime 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 Qualitative (*m*2000*sp*) as per the Instructions for compilation of a product dossier (PQDx_018 v1). The information submitted in the product dossier was reviewed by WHO staff and external experts (assessors) appointed by WHO in accordance with the internal report on the screening and assessment of a product dossier (PQDx_009 v2). Based on the product dossier screening and assessment findings, a recommendation was made to accept the product dossier for Abbott RealTi*m*e HIV-1 Qualitative (*m*2000*sp*) for prequalification.

Commitments for prequalification:
The manufacturer has amended and submitted additional documentation as per the product dossier assessment findings. No further amendments are required.

Manufacturing site inspection

An inspection was performed at the site of manufacture (1300 East Touhy Avenue, 60018 Des Plaines, IL, USA) of the Abbott RealTime HIV-1 Qualitative (m2000sp) between 12 -14 September 2018 as described in 'Information for manufacturers on WHO prequalification inspection procedures for the sites of manufacture of diagnostics (PQDx_014 v4)'.

The inspection found that the manufacturer had a well-established quality management system and manufacturing practices in place that would ensure the manufacture of a product of consistent quality. The manufacturer's responses to the nonconformities noted at the time of the inspection were accepted on 12 February 2019.

Product performance evaluation

Given the regulatory version of the product submitted for prequalification and the quality of the data submitted as part of the product dossier to support the claims for its intended use, the Abbott RealTi*m*e HIV-1 Qualitative (*m*2000*sp*) assay was found eligible to undergo the WHO fast track[1] procedure. Subsequently, the product was not required to undergo a laboratory evaluation.

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

Labelling

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

1. Labels

1.1 Abbott RealTi*m*e HIV-1 Qualitative Amplification Reagent Kit label(04N66-90)

(de) Abbott RealTime HIV-1 Qualitative ist ein In-vitro-Amplifikationsassay zum qualitativen Nachweis von Nukleinsäuren des Human Immunodeficiency Virus Typ 1 (HIV-1) in Humanplasma und getrockneten Blutproben (Dried Blood Spots - DBS). Abbott RealTime HIV-1 Qualitative ist als Hilfsmittel bei der Diagnose einer HIV-1-Infektion des Kindern und Erwachsenen vorgesehen.
Abbott RealTime HIV-1 Qualitative ist nicht zur Verwendung als Screenmittel für HIV-1 bei Blutspendern vorgesehen.
Inhalt:
1. **INTERNAL CONTROL** Abbott RealTime HIV-1 Internal Control (4 Fläschchen, 1,2 ml pro Fläschchen). Nicht-infektiöse Armored RNA mit internen Kontrollsequenzen in negativem Humanplasma. Negatives Humanplasma, getestet und nicht reaktiv für HBsAg, anti-HIV-1/HIV-2, anti-HCV, HIV RNA, HCV RNA und HBV DNA. Konservierungsmittel: 0,1 % ProClin 300 und 0,15 % ProClin 950.
2. **AMPLIFICATION REAGENT PACK** Abbott RealTime HIV-1 Qualitative Amplifikation Reagent Pack (4 Packungen, 24 Tests/Packung). Jede Reagenzienpackung enthält:
   - 1 Fläschchen (0,141 ml) Thermostables rTth Polymerase Enzym (2,9 bis 3,5 Units/µl) in gepuffertes 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 (1,40 ml) Aktivierungsreagenz. 30 mmol/l Mangankhloridlösung. 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) Abbott RealTime HIV-1 Qualitative est un dosage d'amplification *in vitro* pour la détection qualitative des acides nucléiques du virus de l'immunodéficience humaine de type 1 (VIH-1) à partir de plasma humain et de sangs de buvards. Abbott RealTime HIV-1 Qualitative est destiné à être utilisé comme aide dans le diagnostic d'une infection par le VIH-1 chez les adultes et les enfants.
Abbott RealTime HIV-1 Qualitative n'est pas destiné à être utilisé comme test de dépistage du VIH-1 dans des échantillons provenant de dons.
Composition :

1. **INTERNAL CONTROL** Abbott RealTime HIV-1 Internal Control (4 flacons de 1,2 ml chacun). Armored RNA (ARN encapsulé) non infectieux contenant des séquences de contrôle interne dans du plasma humain négatif. Le plasma humain négatif a été testé et trouvé non réactif pour l'AgHBs, les anticorps anti-VIH-1/VIH-2 et anti-VHC, l'ARN VIH, l'ARN VHC et l'ADN VHB. Conservateurs : ProClin 300 à 0,1 % et ProClin 950 à 0,15 %.
2. **AMPLIFICATION REAGENT PACK** Abbott RealTime HIV-1 Qualitative Amplification Reagent Pack (4 boîtes, 24 tests/boîte). Chaque coffret 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 VIH-1. < 0,1 % d'oligonucléotides synthétiques (4 amorces, 2 sondes et 1 oligonucléotide désactivateur) et < 0,3 % de dNTPs dans une solution tampon avec un fluorochrome de référence. Conservateurs : ProClin 300 à 0,1 % et ProClin 950 à 0,15 %.
   - 1 flacon (1,40 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 de Ambion.
Abbott RealTime est une marque commerciale d'Abbott Laboratories.

(es) Abbott RealTime HIV-1 Qualitative es un ensayo de amplificación *in vitro* para la detección cualitativa de ácidos nucleicos del virus de la inmunodeficiencia humana de tipo 1 (VIH-1) en muestras de plasma y sangre humanas secas. El ensayo Abbott RealTime HIV-1 Qualitative se utiliza como ayuda en el diagnóstico de la infección por VIH-1 en niños y adultos.
El ensayo Abbott RealTime HIV-1 Qualitative no debe utilizarse como un ensayo de cribado de donantes para el VIH-1.
Contenido:

1. **INTERNAL CONTROL** Abbott RealTime HIV-1 Internal Control (control interno) (4 viales, 1,2 ml cada uno). 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 el HBsAg, ni para el RNA del VIH, el RNA del VHC ni el DNA del VHB, ni reactividad de anticuerpos anti-VIH-1/VIH-2 ni anti-VHC. Conservantes: ProClin 300 al 0,1 % y ProClin 950 al 0,15 %.
2. **AMPLIFICATION REAGENT PACK** Abbott RealTime HIV-1 Qualitative Amplification Reagent Pack (envase de reactivos de amplificación) (4 envases, 24 tests/envase). Cada envase de reactivos contiene:
   - 1 frasco (0,141 ml) de enzima de polimerasa termoestable rTth (2,9 a 3,5 unidades/µl) en solución tamponada.
   - 1 frasco (1,10 ml) de reactivo de oligonucleótidos del VIH-1. < 0,1 % de oligonucleótidos sintéticos (4 cebadores, 2 sondas y 1 oligonucleótido extintor de fluorescencia) y < 0,3% de dNTPs en solución tamponada con un colorante de referencia. Conservantes: ProClin 300 al 0,1 % y ProClin 950 al 0,15%.
   - 1 frasco (0,40 ml) de reactivo de activación. Solución de cloruro de manganeso 30 mM. Conservantes: ProClin 300 al 0,1% y ProClin 950 al 0,15%.

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Armored RNA es una marca comercial registrada de Ambion.
Abbott RealTime es una marca comercial de Abbott Laboratories.

(it) L'Abbott RealTime HIV-1 Qualitative è un dosaggio di amplificazione *in vitro* per la rilevazione qualitativa del virus dell'immunodeficienza umana di tipo 1 (HIV-1) dell'acido nucleico proveniente da campioni di plasma e spot di sangue essiccato umano (DBS, dried blood spots). L'Abbott RealTime HIV-1 Qualitative serve da ausilio nella diagnosi dell'infezione da HIV-1 in soggetti pediatrici e adulti.
L'Abbott RealTime HIV-1 Qualitative non è previsto come test di screening di donatori per l'HIV-1.
Contenuto:

1. **INTERNAL CONTROL** Abbott RealTime HIV-1 Internal Control (4 provette, 1,2 ml ciascuna). Armored RNA non infettivo con sequenze di controllo interno in plasma umano negativo. Plasma umano negativo analizzato e risultato non reattivo per l'HBsAg, per gli anticorpi anti-HIV-1/HIV-2 e anti-HCV, per l'HIV RNA, l'HCV RNA e l'HBV DNA. Conservanti: ProClin 300 allo 0,1% e ProClin 950 allo 0,15%.
2. **AMPLIFICATION REAGENT PACK** Abbott RealTime HIV-1 Qualitative Amplification Reagent Pack (4 confezioni, 24 test/confezione). Ogni confezione del reagenti contiene:
   - 1 flacone (0,141 ml) di Termostabile rTth Polymerase Enzyme (enzima termostabile rTth, da 2,9 a 3,5 unità/µl) in soluzione tamponata.
   - 1 flacone (1,10 ml) di HIV-1 Oligonucleotide Reagent (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 di tampone con un colorante di riferimento. Conservanti: ProClin 300 allo 0,1% e ProClin 950 allo 0,15%.
   - 1 flacone (0,40 ml) Activation Reagent (reagente di attivazione). 30 ml di soluzione di cloruro di manganese. Conservanti: ProClin 300 allo 0,1% e ProClin 950 allo 0,15%.

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Armored RNA è un marchio commerciale registrato di Ambion.
Abbott RealTime è un marchio commerciale di Abbott Laboratories.

Abbott RealTime HIV-1 Qualitative

Amplification Reagent Kit

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

1. **INTERNAL CONTROL** Abbott RealTime HIV-1 Internal Control (4 vials, 1.2 mL per vial). Noninfectious Armored RNA with internal control sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, anti-HIV-1/HIV-2, anti-HCV, HIV RNA, HCV RNA, and HBV DNA. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.
2. **AMPLIFICATION REAGENT PACK** Abbott RealTime HIV-1 Qualitative Amplification Reagent Pack (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.

ProClin is a registered trademark of Rohm and Haas.
Armored RNA is a registered trademark of Ambion.
Abbott RealTime is a trademark of Abbott Laboratories.

Caution: Consult Instructions For Use (Infection Risk)

REF 4N66-90

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Product of USA , Produkt aus den USA, Produit des Etats-Unis,
Producto de EE.UU., Prodotto degli USA, Fabricado nos EUA

Abbott RealTime HIV-1 Qualitative

Amplification Reagent Kit

(pt) O Abbott RealTime HIV-1 Qualitative é um ensaio de amplificação *in vitro* para a detecção qualitativa de ácidos nucleicos do vírus da imunodeficiência humana tipo 1 (HIV-1) em amostras de plasma e de sangue seco humano. O ensaio Abbott RealTime HIV-1 Qualitative destina-se a ser utilizado como meio auxiliar no diagnóstico da infecção por HIV-1 em adultos e crianças.
O ensaio Abbott RealTime HIV-1 Qualitative não se destina a ser utilizado no rastreio do HIV-1 em amostras de dadores.
Conteúdo:

1. **INTERNAL CONTROL** Abbott RealTime HIV-1 Internal Control (4 frascos, 1,2 ml por frasco). Armored RNA não infeccioso com sequências de controlo interno em plasma humano negativo. Plasma humano negativo testado e considerado não reactivo para HBsAg, anticorpos anti-HIV-1/HIV-2 e anti-HCV, ARN do HIV, ARN do HCV e ADN do HBV. Conservantes: 0,1% de ProClin 300 e 0,15% de ProClin 950.
2. **AMPLIFICATION REAGENT PACK** Abbott RealTime HIV-1 Qualitative Amplification Reagent Pack (4 embalagens, 24 testes/embalagem). Cada embalagem de reagentes contém:
   - 1 Frasco (0,141 ml) de enzima rTth polimerase termoestável (2,9 a 3,5 unidades/µl) erm solução tamponada.
   - 1 Frasco (1,10 ml) de reagente de oligonucleótidos 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. 30 mM de solução de cloreto de manganésio. Conservantes: 0,1% de ProClin 300 e 0,15% de ProClin 950.

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Abbott RealTime é uma marca comercial de Abbott Laboratories.

H317
P261, P280, P272, P302+P352, P333+P313, P362+364, P501

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Abbott

1.2 HIV-1 Qualitative Control Kit label (04N66-80)

Abbott RealTime HIV-1 Qualitative

Control Kit

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

Contents:

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

ProClin is a registered trademark of Rohm and Haas.

Armored RNA is a registered trademark of Ambion.

Abbott RealTime is a trademark of Abbott Laboratories.

Caution: Consult Instructions For Use (Infection Risk)

REF 4N66-80

IVD

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REF

(pt) Para utilização em diagnóstico *in vitro*. Os Abbott RealTime HIV-1 Qualitative Controls destinam-se à validação do processamento do ensaio Abbott RealTime HIV-1 Qualitative quando utilizado para a detecção qualitativa de ácidos nucleicos do vírus da imunodeficiência humana tipo 1 (HIV-1) em amostras de plasma e sangue seco humano.

Conteúdo:

1. **CONTROL −** Abbott RealTime HIV-1 Negative Control (12 frascos, 1,8 ml por frasco). Plasma humano negativo testado e considerado não reactivo para HBsAg, anticorpos anti-HIV-1/HIV-2 e anti-HCV, ARN do HIV, ARN do HCV e ADN do HBV. Conservantes: 0,1% de ProClin 300 e 0,15% de ProClin 950.
2. **CONTROL H** Abbott RealTime HIV-1 High Positive Control (12 frascos, 1,8 ml por frasco). Armored RNA não infeccioso com sequências de HIV-1 em plasma humano negativo. Plasma humano negativo testado e considerado não reactivo para HBsAg, anticorpos anti-HIV-1/HIV-2 e anti-HCV, ARN do HIV, ARN do HCV e ADN do HBV. Conservantes: 0,1% de ProClin 300 e 0,15% de ProClin 950.

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Abbott RealTime é uma marca comercial de Abbott Laboratories.

Product of USA, Produkt aus den USA, Produit des Etats-Unis, Producto de EE.UU., Prodotto degli USA, Fabricado nos EUA

(de) Zur Verwendung als In-vitro-Diagnostikum. Die Abbott RealTime HIV-1 Qualitative Controls dienen zur Sicherstellung der Testgültigkeit des Abbott RealTime HIV-1 Qualitative Assays beim qualitativen Nachweis von Nukleinsäuren des Human Immundefizienz Virus Typ 1 (HIV-1) in Humanplasma und getrockneten Blutproben (Dried Blood Spots - DBS).

Inhalt:

1. **CONTROL −** Abbott RealTime HIV-1 Negative Control (12 Fläschchen, 1,8 ml pro Fläschchen). Negatives Humanplasma, getestet und nicht reaktiv für HBsAg, anti-HIV-1/HIV-2, anti-HCV, HIV RNA, HCV RNA und HBV DNA. Konservierungsmittel: 0,1 % ProClin 300 und 0,15 % ProClin 950.
2. **CONTROL H** Abbott RealTime HIV-1 High Positive Control (12 Fläschchen, 1,8 ml pro Fläschchen). Nicht-infektiöse Armored RNA mit HIV-1-Sequenzen in negativem Humanplasma. Negatives Humanplasma, getestet und nicht reaktiv für HBsAg, anti-HIV-1/HIV-2, anti-HCV, HIV RNA, HCV RNA und HBV DNA. 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 Abbott RealTime HIV-1 Qualitative Controls sont utilisés pour établir la validité du dosage Abbott RealTime HIV-1 Qualitative lors de la détection qualitative des acides nucléiques du virus de l'immunodéficience humaine de type 1 (VIH-1) à partir de plasma humain et de sangs de buvards.

Composition :

1. **CONTROL −** Abbott RealTime HIV-1 Negative Control (12 flacons de 1,8 ml chacun). Plasma humain négatif testé et trouvé non réactif pour l'AgHBs, les anticorps anti-VIH-1/VIH-2 et anti-VHC, l'ARN VIH, l'ARN VHC et l'ADN VHB. Conservateurs : ProClin 300 à 0,1 % et ProClin 950 à 0,15 %.
2. **CONTROL H** Abbott RealTime HIV-1 High Positive Control (12 flacons de 1,8 ml chacun). Armored RNA (ARN encapsulé) 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, les anticorps anti-VIH-1/VIH-2 et anti-VHC, l'ARN VIH, l'ARN VHC et l'ADN VHB. 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 Abbott RealTime HIV-1 Qualitative Controls se utilizan para establecer la validez del procesamiento con el ensayo Abbott RealTime HIV-1 Qualitative, en la detección cualitativa de ácidos nucleicos del virus de la inmunodeficiencia humana de tipo 1 (VIH-1) en muestras de plasma y sangre humanas secas.

Contenido:

1. **CONTROL −** Abbott RealTime HIV-1 Negative Control (control negativo) (12 viales, 1,8 ml cada uno). Plasma humano negativo analizado y sin reactividad para el HBsAg, ni para el RNA del VIH, el RNA del VHC ni el DNA del VHB, ni reactividad de anticuerpos anti-VIH-1/VIH-2, ni anti-VHC. Conservantes: ProClin 300 al 0,1% y ProClin 950 al 0,15%.
2. **CONTROL H** Abbott RealTime HIV-1 High Positive Control (control positivo alto) (12 viales, 1,8 ml cada uno). Armored RNA no infeccioso con secuencias de VIH-1 en plasma humano negativo. El plasma humano negativo ha sido analizado y no se ha encontrado reactividad para el HBsAg, ni para el RNA del VIH, el RNA del VHC ni el DNA del VHB, ni reactividad de anticuerpos anti-VIH-1/VIH-2 ni anti-VHC. 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*. Gli Abbott RealTime HIV-1 Qualitative Controls vengono utilizzati per stabilire la validità dell'analisi con il dosaggio Abbott RealTime HIV-1 Qualitative nella rilevazione qualitativa del virus dell'immunodeficienza umana di tipo 1 (HIV-1) dell'acido nucleico proveniente da campioni di plasma e spot di sangue essiccato umano (DBS, dried blood spots).

Contenuto:

1. **CONTROL −** Abbott RealTime HIV-1 Negative Control (12 provette, 1,8 ml ciascuna). Plasma umano negativo analizzato e risultato non reattivo per l'HBsAg, per gli anticorpi anti-HIV-1/HIV-2 e anti-HCV, per l'HIV RNA, l'HCV RNA e l'HBV DNA. Conservanti: ProClin 300 allo 0,1% e ProClin 950 allo 0,15%.
2. **CONTROL H** Abbott RealTime HIV-1 High Positive Control (12 provette, 1,8 ml ciascuna). Armored RNA non infettivo con sequenze di HIV-1 in plasma umano negativo. Plasma umano negativo analizzato e risultato non reattivo per l'HBsAg, per gli anticorpi anti-HIV-1/HIV-2 e anti-HCV per l'HIV RNA, l'HCV RNA e l'HBV DNA. 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 Qualitative

Control Kit

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

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

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

www.abbottmolecular.com

Abbott

1.3 Negative Control Vial label (2G31Z)

Top Edge

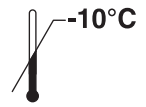
REF 2G31Z

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

1.4 High Positive Control Vial Label (2G31X)

Top Edge

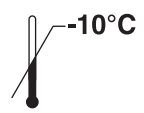
++

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

1.6 Abbott RealTi*me* HIV-1 Internal Control Vial Label (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[2]

[2] 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 Qualitative

en

REF 4N66-90

51-608381/R7

Abbott RealTi*me*
HIV-1 Qualitative
REF 4N66-90
51-608381/R7

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

Key to Symbols Used

REF	Reference Number
IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
	Use By
	Upper Limit of Temperature
CONTROL –	Negative Control
CONTROL H	High Positive Control
	Consult instructions for use
	Caution
	Warning
INTERNAL CONTROL	Internal Control
AMPLIFICATION REAGENT PACK	Amplification Reagent Pack
EC REP	Authorized Representative in the European Community
	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.

CUSTOMER SERVICE INTERNATIONAL: CALL YOUR ABBOTT REPRESENTATIVE

This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.
See **REAGENTS** section for a full explanation of symbols used in reagent component naming.

NAME

Abbott RealTi*me* HIV-1 Qualitative

INTENDED USE

The Abbott RealTi*me* HIV-1 Qualitative is an in vitro amplification assay for the qualitative detection of Human Immunodeficiency Virus Type 1 (HIV-1) nucleic acids from human plasma and dried blood spots (DBS).

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

INTENDED USER

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

SUMMARY AND EXPLANATION OF THE TEST

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

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The Abbott RealTi*me* HIV-1 Qualitative assay consists of 2 reagent kits:
- Abbott RealTi*me* HIV-1 Qualitative Amplification Reagent Kit
- Abbott RealTi*me* HIV-1 Qualitative Control Kit

The Abbott RealTi*me* HIV-1 Qualitative assay uses PCR[18] to generate amplified product from the HIV-1 nucleic acids in clinical specimens. The presence of HIV-1 target sequence is indicated by the fluorescent signal generated through the use of fluorescent-labeled oligonucleotide probes on the Abbott *m*2000*rt* instrument. The probes do not generate a signal unless they are specifically bound to the amplified product.
An RNA sequence that is unrelated to the HIV-1 target sequence is introduced into each specimen at the beginning of sample preparation. This unrelated RNA sequence is simultaneously amplified, and serves as an internal control (IC) to demonstrate that the process has proceeded correctly for each sample.

Sample Preparation

The purpose of sample preparation is to extract, concentrate, and purify the target nucleic acids for amplification. The Abbott *m*Sample Preparation System$_{DNA}$ uses magnetic particle technology to capture target nucleic acids and washes the particles to remove unbound sample components. The bound nucleic acids are eluted and are then ready for amplification. The IC is taken through the entire sample preparation procedure along with the controls and specimens. The Abbott *m*2000*sp* automated sample preparation system can be used to prepare samples for the Abbott RealTi*me* HIV-1 Qualitative assay. Alternatively, samples can be prepared manually.
NOTE: One Abbott *m*Sample Preparation System$_{DNA}$ kit is sufficient to complete 4 × 24 (96) sample preparations.

Reagent Preparation and Reaction Plate Assembly

The Abbott *m*2000*sp* combines the Abbott RealTi*m*e HIV-1 Qualitative 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*. After manual application of the optical seal, the plate is ready for transfer to the Abbott *m*2000*rt*. Alternatively, manual sample preparation method users combine the Abbott RealTi*m*e HIV-1 Qualitative amplification reagent components manually to create the amplification master mix and transfer aliquots of the master mix and the nucleic acid samples to the Abbott 96-Well Optical Reaction Plate. After manual application of the optical seal, the plate is ready for transfer to the Abbott *m*2000*rt*.

Amplification

During the amplification reaction on the Abbott *m*2000*rt*, target DNA is amplified by the DNA polymerase activity of the thermostable rTth DNA polymerase. If target RNA is present, it is first converted to cDNA by the reverse transcriptase activity of the enzyme and subsequently amplified. Amplification of HIV-1 and IC targets takes place simultaneously in the same reaction. The target sequence for the Abbott RealTi*m*e HIV-1 Qualitative assay is in the *pol* integrase region of the HIV-1 genome. This region is highly conserved.[19] The IC 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 to allow fluorescent detection of amplification products as the HIV-1 and IC probes anneal to their respective targets (real-time fluorescent detection). The HIV-1 probe has a fluorescent moiety that is covalently linked to the 5´ end. A short quencher oligonucleotide is complementary to the 5´ end of the HIV-1 probe and has a quencher moiety at its 3´ end. In the absence of HIV-1 target, the HIV-1 probe fluorescence is quenched through hybridization to the quencher oligonucleotide. In the presence of the HIV-1 target sequence, the HIV-1 probe preferentially hybridizes to the target sequence, dissociating from the quencher oligonucleotide and allowing fluorescent detection. The IC probe is a single stranded DNA oligonucleotide with a fluorophore at the 5´ end and a quencher at the 3´ end. In the absence of IC sequences, the IC probe adopts a series of random conformations, some of which bring the quencher close enough to the excited fluorophore to absorb its energy before it can be fluorescently emitted. When the IC probe binds to its complementary sequence in the target, the fluorophore and the quencher are held apart, allowing fluorescent emission and detection by the Abbott *m*2000*rt*. The HIV-1 and IC specific probes are each labeled with a different fluorophore, thus allowing simultaneous detection of both amplified products.

Assay Results

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

PREVENTION OF NUCLEIC ACID CONTAMINATION

The possibility of nucleic acid contamination is minimized because:

- Reverse transcription, PCR amplification, and oligonucleotide hybridization occur in a sealed Abbott 96-Well Optical Reaction Plate.
- Detection is carried out automatically without the need to open the Abbott 96-Well Optical Reaction Plate.
- Pipettes with aerosol barrier tips or disposable transfer pipettes are used for all pipetting. The disposable pipettes or pipette tips are discarded after use.
- Separate dedicated areas are used to perform the Abbott RealTi*m*e HIV-1 Qualitative assay. Refer to the **Contamination Precautions** section of this package insert.

REAGENTS

Abbott RealTi*m*e HIV-1 Qualitative Amplification Reagent Kit (List No. 04N66-90)

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, anti-HIV-1/HIV-2, anti-HCV, HIV RNA, HCV RNA, and HBV DNA. Preservatives: 0.1% ProClin® 300 and 0.15% ProClin 950.
2. **AMPLIFICATION REAGENT PACK** Abbott RealTi*m*e HIV-1 Qualitative Amplification Reagent Pack (List No. 4N66) (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 Qualitative Control Kit (List No. 4N66-80)

1. **CONTROL –** Abbott RealTi*m*e HIV-1 Negative Control (List No. 2G31Z) (12 vials, 1.8 mL per vial)
   Negative human plasma tested and found to be nonreactive for HBsAg, anti-HIV-1/HIV-2, anti-HCV, HIV RNA, HCV RNA, and HBV DNA. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.
2. **CONTROL H** Abbott RealTi*m*e HIV-1 High Positive Control (List No. 2G31X) (12 vials, 1.8 mL per vial)
   Noninfectious Armored RNA with HIV-1 sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, anti-HIV-1/HIV-2, anti-HCV, HIV RNA, HCV RNA, and HBV DNA. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

WARNINGS AND PRECAUTIONS

IVD In Vitro Diagnostic Medical Device

This assay is not intended to be used as a screening test for HIV-1.

Safety Precautions

Refer to the Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals, Hazards Section, for instructions on safety precautions.

⚠ **CAUTION**: This preparation contains human sourced and/or potentially infectious components. Components sourced from human blood have been tested and found to be nonreactive by FDA-licensed tests for antibody to HCV, antibody to HIV-1, antibody to HIV-2, and HBsAg. The material is also tested and found to be negative by FDA-licensed PCR methods for HIV-1 RNA and HCV RNA. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. These reagents and human specimens should be handled as if infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories,[20] OSHA Standards on Bloodborne Pathogens,[21] CLSI Document M29-A3,[22] and other appropriate biosafety practices.[23] Therefore all human sourced materials should be considered infectious.

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

- Wear gloves when handling specimens or reagents.
- Do not pipette by mouth.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where these materials are handled.
- Clean and disinfect spills of specimens by including the use of a tuberculocidal disinfectant such as 1.0% (v/v) sodium hypochlorite or other suitable disinfectant.[20]
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and federal regulations.[23]

Components of the Abbott RealTi*m*e HIV-1 Qualitative Control Kit (List No. 4N66-80) and the Abbott RealTi*m*e HIV-1 Qualitative Amplification Reagent Kit (List No. 4N66-90) 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.

Specimen Collection and Handling Precautions

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

Laboratory Precautions

- During preparation of samples, compliance with good laboratory practices is essential to minimize the risk of cross-contamination between samples as well as the inadvertent introduction of nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with RNA or DNA.
- Work area and instrument platforms must be considered potential sources of contamination. Change gloves after having contact with potential contaminants (such as RNases, DNases, specimens, eluates, and/or amplified product) before handling unopened reagents, negative control, positive control, or specimens. Refer to the Abbott *m*2000*sp* and *m*2000*rt* Operations Manuals and the instructions in the **POST PROCESSING PROCEDURES** section of this package insert for cleaning procedures.
- Wear appropriate personal protective equipment at all times.
- Use powder-free gloves.
- To reduce the risk of nucleic acid contamination due to aerosols formed during pipetting, pipettes with aerosol barrier tips or disposable transfer pipettes must be used for all pipetting. The length of the tip should be sufficient to prevent contamination of the pipette barrel. While pipetting, care should be taken to avoid touching the pipette barrel to the inside of the sample tube or container. The use of extended aerosol barrier pipette tips is recommended.
- Change aerosol barrier pipette tips between ALL manual liquid transfers.
- Clean and disinfect spills of specimens and reagents as stated in the Abbott *m*2000*sp* and *m*2000*rt* Operations Manuals and the instructions in the **POST PROCESSING PROCEDURES** section of this package insert.

Contamination Precautions

- Amplification reactions such as PCR are sensitive to accidental introduction of product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the reagents used 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.
- The use of 2 dedicated areas within the laboratory is recommended for performing the Abbott RealTi*m*e HIV-1 Qualitative assay with the automated Abbott *m*2000*sp* and *m*2000*rt*:
  - The **Sample Preparation Area** is dedicated to processing samples (specimens, Abbott RealTi*m*e HIV-1 Qualitative Controls) and to adding processed specimens and controls to the Abbott 96-Well Optical Reaction Plate. All reagents used in the Sample Preparation Area should remain in this dedicated area at all times. Laboratory coats, pipettes, pipette tips, and vortex mixers used in the Sample Preparation Area must remain in this area and not be moved to the Amplification Area. Do not bring amplification product into the Sample Preparation Area.
  - The **Amplification Area** is dedicated to the amplification and the detection of amplified product. Laboratory coats and equipment used in the Amplification Area must remain in this area and not be moved to the Sample Preparation Area.
- The use of 3 dedicated areas within the laboratory is recommended for performing the Abbott RealTi*m*e HIV-1 Qualitative assay with the manual sample preparation method and the Abbott *m*2000*rt*:
  - The **Reagent Preparation Area** is dedicated to combining the Abbott RealTi*m*e HIV-1 Qualitative amplification reagent components to create the amplification master mix and transferring aliquots of the master mix to the Abbott 96-Well Optical Reaction Plate. Laboratory coats, pipettes, and pipette tips used in the Reagent Preparation Area must remain in this area and not be moved to either the Sample Preparation Area or the Amplification Area. Do not bring target or amplification product into the Reagent Preparation Area.
  - The **Sample Preparation Area** is dedicated to processing samples (specimens, Abbott RealTi*m*e HIV-1 Qualitative Controls) and to adding processed specimens and controls to the Abbott 96-Well Optical Reaction Plate. All reagents used in the Sample Preparation Area should remain in this dedicated area at all times. Laboratory coats, pipettes, pipette tips, and vortex mixers used in the Sample Preparation Area must remain in this area and not be moved to either the Reagent Preparation Area or the Amplification Area. Do not bring amplification product into the Sample Preparation Area.
  - The **Amplification Area** is dedicated to the amplification and the detection of amplified product. Laboratory coats and equipment used in the Amplification Area must remain in this area and not be moved to either the Reagent Preparation Area or the Sample Preparation Area.
- If the Abbott *m*2000*sp* run is aborted, dispose of all commodities and reagents according to the Abbott *m*2000*sp* Operations Manual.
- If the manual sample preparation procedure is incorrectly performed or is interrupted at any point so that the timing of the steps exceeds the recommended timing per the protocol instructions, dispose of all commodities and reagents according to the instructions in the **POST PROCESSING PROCEDURES** section of this package insert.
- If the Abbott *m*2000*sp* master mix addition protocol is aborted after amplification reagents are added to the Abbott 96-Well Optical Reaction Plate, seal the Abbott 96-Well Optical Reaction Plate and put in a sealable plastic bag and dispose of according to the Abbott *m*2000*sp* Operations Manual, Hazards section, along with the gloves used to handle the plate.
- If manual preparation of the PCR reaction mix is aborted after amplification reagents are added to the Abbott 96-Well Optical Reaction Plate, seal the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose of according to laboratory guidelines, along with the gloves used to handle the plate.
- For all completed, interrupted or aborted Abbott *m*2000*rt* runs, dispose of the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag according to the Abbott *m*2000*rt* Operations Manual along with the gloves used to handle the plate.
- **Autoclaving the sealed Abbott 96-Well Optical Reaction Plate will not degrade the amplified product and may contribute to the release of the amplified product by opening the sealed plate. The laboratory area can become contaminated with amplified product if the waste materials are not carefully handled and contained.**
- Decontaminate and dispose of all specimens, reagents, and other potentially biohazardous or contaminated materials in accordance with local, state, and federal regulations.[20,23] All materials should be handled in a manner that minimizes the chance of potential contamination of the work area.

Contamination from External Deoxy-Uracil (dU)-Containing Amplified Product

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

REAGENT STORAGE AND HANDLING INSTRUCTIONS

Abbott RealTi*m*e HIV-1 Qualitative Amplification Reagent Kit (List No. 4N66-90)

The Abbott RealTi*m*e HIV-1 Qualitative Amplification Reagent Packs 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 Qualitative Amplification Reagent Pack that is in use from direct contact with specimens and controls. Reagents are shipped on dry ice.

Abbott RealTi*me* HIV-1 Qualitative Control Kit (List No. 4N66-80)

The Abbott RealTi*me* HIV-1 Qualitative Negative and Positive Control must be stored at −10°C or colder. Reagents are shipped 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 regarded as invalid and specimens must be retested.

INSTRUMENT METHODS

The Abbott RealTi*me* HIV-1 Qualitative assay is performed using the Abbott *m*2000*sp* or the manual sample preparation method for sample processing, and the Abbott *m*2000*rt* for amplification and detection. Refer to the appropriate Assay Protocol instructions in this package insert or to the Abbott *m*2000*sp* or *m*2000*rt* Operations Manuals for detailed operating procedures. The Abbott RealTi*me* HIV-1 Qualitative application files must be installed on the Abbott *m*2000*sp* and Abbott *m*2000*rt* systems from the Abbott RealTi*me* HIV-1 Qualitative *m*2000 System Combined Application CD-ROM (List No. 4N66-003 or higher) prior to performing the assay. For detailed information on application file installation, refer to the Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals, Operating Instructions section.

SPECIMEN COLLECTION AND HANDLING INSTRUCTIONS

Plasma Specimen Collection and Storage

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

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

After centrifugation, remove plasma from cells and pipette plasma into a separate tube for immediate testing or storage. Plasma specimens may be stored at 15 to 30°C for up to 24 hours or at 2 to 8°C for up to 5 days. If longer storage is required, plasma specimens may be stored at −10 to −30°C for up to 30 days, or at −70°C or lower.[24,25] Multiple freeze-thaw cycles should be avoided and should not exceed 3 freeze-thaw cycles. Thaw plasma specimens at 15 to 30°C or at 2 to 8°C. Once thawed, if plasma specimens are not being processed immediately, they can be stored at 2 to 8°C for up to 6 hours.

DBS Specimen Collection and Storage

DBS may be made on a Whatman 903 card (or equivalent) using blood obtained from a heel- or finger-stick or collected in a blood collection tube. Freshly drawn specimens (whole blood) may be held at 15 to 30°C for up to 6 hours or at 2 to 8°C for up to 24 hours. DBS are made by following these steps:

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

Specimen Transport

Ship plasma specimens frozen on dry ice. Ship DBS specimens under ambient conditions, packaged in bags with desiccant packs. For DBS specimens, total time under ambient conditions during transport and storage should not exceed 12 weeks. For domestic and international shipments, specimens should be packaged and labeled in compliance with applicable state, federal, and international regulations covering the transport of clinical, diagnostic, or biological specimens.

ABBOTT REALTI*ME* HIV-1 QUALITATIVE ASSAY PROCEDURE

This Abbott RealTi*me* HIV-1 Qualitative package insert contains 4 assay protocols:

ASSAY PROTOCOL I: PLASMA SPECIMENS USING ABBOTT *m*2000*sp* AUTOMATED SAMPLE PREPARATION SYSTEM

ASSAY PROTOCOL II: DBS SPECIMENS USING ABBOTT *m*2000*sp* AUTOMATED SAMPLE PREPARATION SYSTEM.

ASSAY PROTOCOL III: PLASMA SPECIMENS USING MANUAL SAMPLE PREPARATION METHOD

ASSAY PROTOCOL IV: DBS SPECIMENS USING MANUAL SAMPLE PREPARATION METHOD

Materials Provided

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

Materials Required But Not Provided

- Abbott RealTi*me* HIV-1 Qualitative Control Kit (List No. 4N66-80)
- Abbott RealTi*me* HIV-1 Qualitative *m*2000 System Combined Application CD-ROM (List No. 4N66-003 or higher)

SAMPLE PREPARATION AREA

Materials for Abbott *m*2000*sp* (ASSAY PROTOCOLS I and II)

- Abbott *m*2000*sp* instrument with software version 4.0 or higher
- Abbott *m*Sample Preparation System$_{DNA}$ (List No. 6K12-24)
  NOTE: One kit is sufficient to complete 96 sample preparations.
- 5 mL Reaction Vessels (List No. 4J71-20)
- Calibrated Pipettes capable of delivering 20 to 1000 µL
- Aerosol Barrier Pipette Tips for 20 to 1000 µL Pipettes
- 1000 µL Disposable Tips (List No. 4J71−10)
- 200 µL Disposable Tips (List No. 4J71-17)
- Vortex Mixer
- USP Grade 190 to 200 Proof Ethanol (95 to 100% Ethanol).
  Do not use ethanol that contains denaturants.
- Abbott Optical Adhesive Covers (List No. 4J71-75)
- Abbott Adhesive Cover Applicator (List No. 9K32-01)
- Abbott Splash-Free Support Base (List No. 9K31-01)
- Master Mix Tube (List No. 4J71-80)
- 13 mm Sample Racks (List No. 4J72-82)
- 200 mL Reagent Vessels (List No. 4J71-60)
- Abbott 96-Deep-Well Plate (List No. 4J71−30)
- Abbott 96-Well Optical Reaction Plate (List No. 4J71−70)
- Centrifuge capable of 2000*g*
- Bulk *m*Lysis$_{DNA}$ Buffer (List No. 2N77-01)
  (for DBS processing only)
- 50 mL Tubes (NUNC or equivalent) (optional)
  (for DBS processing only)

Materials for Manual Sample Preparation (ASSAY PROTOCOLS III and IV)

- Abbott *m*Sample Preparation System$_{DNA}$ (List No. 6K12-24)
  NOTE: One kit is sufficient to complete 96 sample preparations.
- Calibrated Pipettes capable of delivering 20 to 1000 µL
- Aerosol Barrier Pipette Tips for 20 to 1000 µL Pipettes
- 5 mL Reaction Vessels (List No. 4J71-20)
- 1.5 mL screw top microfuge tubes and caps
  (List No. 04J71-50 or equivalent)
- Magnetic stands for 5 mL Reaction Vessels
- Non-magnetic stands for 5 mL Reaction Vessels
- Magnetic stands for 1.5 mL tubes
- Non-magnetic stands for 1.5 mL tubes
- Dry heating block for 5 mL Reaction Vessels
  (capable of achieving 50°C)
- Dry heating block for 1.5 mL tubes (capable of achieving 75°C)
- Thermometer
- Timer
- Vortex Mixer
- USP Grade 190 to 200 Proof Ethanol (95 to 100% Ethanol).
  Do not use ethanol that contains denaturants.
- Liquid Container for waste (no bleach should be allowed to contact liquid waste).
  See the instructions in the **POST PROCESSING PROCEDURES** section of this package insert for warning and precaution information.
- Abbott Optical Adhesive Covers (List No. 4J71-75)
- Abbott Adhesive Cover Applicator (List No. 9K32-01)
- Abbott Splash-Free Support Base (List No. 9K31-01)
- Centrifuge capable of 2000*g*
- Centrifuge capable of 5000*g* (for centrifuging the Abbott 96-Well Optical Reaction Plate)
- Repeat pipettor capable of delivering 40 to 50 µL volumes (optional)
- Sterile repeat pipettor tips capable of delivering 40 to 50 µL volumes (optional)
- Disposable transfer pipettes (optional)
- 96-well polypropylene plate (optional)
- Bulk *m*Lysis$_{DNA}$ Buffer (List No. 2N77-01)
  (for DBS processing only)
- 50 mL Tubes (NUNC or equivalent) (optional)
  (for DBS processing only)

REAGENT PREPARATION AREA

Materials for Manual Sample Preparation (ASSAY PROTOCOLS III and IV)

- StrataCooler® 96 Benchtop Cooler or Eppendorf PCR Cooler
- Abbott 96-Well Optical Reaction Plate (List No. 4J71–70)
- Calibrated Pipettes capable of delivering 20 to 1000 µL
- Aerosol Barrier Pipette Tips for 20 to 1000 µL Pipettes
- Single-use RNase/DNase-free tube or container

NOTE: Magnetic stands for 5 mL Reaction Vessels, magnetic stands for 1.5 mL tubes, and Eppendorf PCR Cooler can be ordered as *m*2000 *m*Sample Preparation System Start Up Kit (List No. 02N28-03).

AMPLIFICATION AREA

Materials for Abbott *m*2000*sp* and Manual Sample Preparation (ASSAY PROTOCOLS I, II, III and IV)

- Abbott *m*2000*rt* instrument with software version 3.0 or higher
- Abbott *m*2000*rt* Optical Calibration Kit (List No. 4J71-93)

Other Materials (ASSAY PROTOCOLS I, II, III and IV)

- Biological safety cabinet approved for working with infectious materials
- Lab coat
- Powder-free disposable gloves
- Protective eyewear
- Solid waste container
- Sealable plastic bags
- RNase/DNase-free water (Eppendorf or equivalent)†
- Cotton Tip Applicators (Puritan or equivalent)†

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

Procedural Precautions

- Read the instructions in the package insert carefully before processing samples.
- The Abbott RealTi*m*e HIV-1 Qualitative reagents are intended to be used on the Abbott *m*2000*sp* or with the manual sample preparation method for sample processing and the Abbott *m*2000*rt* for amplification and detection.
- Do not use kits or reagents beyond expiration date.
- Control kit lots and amplification reagent kit lots can be used interchangeably. However, components contained within a kit are intended to be used together. For example, do not use the negative control from control kit lot X with the positive control from control kit lot Y.
- Amplification reagent components (enzyme, oligonucleotide reagent and activation reagent), Controls, and *m*Sample Preparation System$_{DNA}$ Reagents are for single-use only and should be discarded after use. Use new reagent vessels and new reaction vessels, for every new Abbott RealTi*m*e HIV-1 Qualitative assay run. At the end of each run, discard all these remaining reagents as stated in the Abbott *m*2000*sp* Operations Manual and the **POST PROCESSING PROCEDURES** section of this package insert.
- The amplification reagent kit and control kit can be thawed and refrozen up to 3 times before use.
- The Abbott RealTi*m*e HIV-1 Qualitative Controls must be processed with the specimens to be tested. The use of the Abbott RealTi*m*e HIV-1 Qualitative Controls is integral to the performance of the Abbott RealTi*m*e HIV-1 Qualitative assay. Refer to the **QUALITY CONTROL PROCEDURES** section in the package insert for details.
- Use only USP Grade 190 to 200 Proof Ethanol (95 to 100% Ethanol) to prepare the *m*Lysis$_{DNA}$, *m*Wash 1$_{DNA}$, and *m*Wash 2$_{DNA}$ sample preparation reagents. **Do not use ethanol that contains denaturants.**
- Replace any empty or partially used 200 µL and 1000 µL disposable tips on the Abbott *m*2000*sp* with full trays before every run. Refer to the Abbott *m*2000*sp* Operations Manuals, Operating Instructions section.
- Monitoring procedures for the presence of amplification product can be found in the **QUALITY CONTROL PROCEDURES** section in the package insert.
- To reduce the risk of nucleic acid contamination, clean and disinfect spills of specimens, reagents and controls by using a detergent solution followed by a tuberculocidal disinfectant such as 1.0% (v/v) sodium hypochlorite or other suitable disinfectant.

ASSAY PROTOCOL I: PLASMA SPECIMENS USING ABBOTT *m*2000*sp* AUTOMATED SAMPLE PREPARATION SYSTEM

For a detailed description of how to operate the Abbott *m*2000*sp* and *m*2000*rt* instruments, refer to the Abbott *m*2000*sp* and *m*2000*rt* Operations Manuals, Operating Instructions sections. Laboratory personnel must be trained to operate the Abbott *m*2000*sp* and *m*2000*rt* instruments. The operator must have a thorough knowledge of the applications run on the instruments and must follow good laboratory practices.

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

1. Thaw assay controls and IC at 15 to 30°C or at 2 to 8°C; see **QUALITY CONTROL PROCEDURES** section of the package insert.
   - Once thawed, assay controls and IC can be stored at 2 to 8°C for up to 24 hours before use.
   - Vortex each assay control 3 times for 2 to 3 seconds before use. Ensure that the contents of each vial are at the bottom after vortexing by tapping the vials on the bench to bring liquid to the bottom of the vial. Ensure bubbles or foam are not generated; if present, remove with a sterile pipette tip, using a new tip for each vial.
2. Thaw amplification reagents at 15 to 30°C or at 2 to 8°C.
   - Once thawed, the amplification reagents can be stored at 2 to 8°C for up to 24 hours until required for the amplification master mix procedure.
   - **For up to 24 reactions use:** 1 tube of positive control, 1 tube of negative control, 1 amplification reagent pack, 1 vial of IC, and 1 set of *m*Sample Preparation System$_{DNA}$ reagents.
   - **For 25 to 48 reactions use:** 1 tube of positive control, 1 tube of negative control, 2 amplification reagent packs, 2 vials of IC, 2 bottles of *m*Lysis$_{DNA}$ Buffer, and 1 bottle *m*Wash 1$_{DNA}$ Buffer, *m*Wash 2$_{DNA}$ Buffer, *m*Microparticle$_{DNA}$ and *m*Elution$_{DNA}$ Buffer.
   - **For 49 to 72 reactions use:** 1 tube of positive control, 1 tube of negative control, 3 amplification reagent packs, 3 vials of IC, 3 bottles of *m*Lysis$_{DNA}$ Buffer, 2 bottles of *m*Wash 1$_{DNA}$ Buffer and *m*Wash 2$_{DNA}$ Buffer, and 1 bottle of *m*Microparticle$_{DNA}$ and *m*Elution$_{DNA}$ Buffer.
   - **For 73 to 96 reactions use:** 1 tube of positive control, 1 tube of negative control, 4 amplification reagent packs, 4 vials of IC, 4 bottles of *m*Lysis$_{DNA}$ Buffer, 2 bottles of *m*Wash 1$_{DNA}$ Buffer and *m*Wash 2$_{DNA}$ Buffer, and 1 bottle of *m*Microparticle$_{DNA}$ and *m*Elution$_{DNA}$ Buffer.
3. Open the *m*Sample Preparation System$_{DNA}$ reagent pack(s). If crystals are observed in any of the reagent bottles upon opening, allow the reagent to equilibrate at room temperature until the crystals disappear. Do not use the reagents until the crystals have dissolved. Add USP Grade 190 to 200 Proof Ethanol (95 to 100% Ethanol) to the *m*Lysis$_{DNA}$, *m*Wash 1$_{DNA}$ and *m*Wash 2$_{DNA}$ bottles as indicated below. **Do not use ethanol that contains denaturants.**
   - Add 35 mL ethanol to each bottle of *m*Lysis$_{DNA}$ being used.
   - Add 23 mL ethanol to each bottle of *m*Wash 1$_{DNA}$ being used.
   - Add 70 mL ethanol to each bottle of *m*Wash 2$_{DNA}$ being used.
4. Vortex each IC vial 3 times for 2 to 3 seconds before use. Use a calibrated **PRECISION PIPETTE DEDICATED FOR INTERNAL CONTROL USE ONLY** to add 750 µL of IC to each bottle of *m*Lysis$_{DNA}$ Buffer.
5. Gently invert all the reagent bottles except *m*Microparticles$_{DNA}$ 5 to 10 times to ensure a homogeneous solution and pour the contents into the appropriate reagent vessels as indicated in the table below. Ensure bubbles or foam are not generated in the reagent vessels; if present, remove with a sterile pipette tip, using a new tip for each reagent vessel. For each bottle of *m*Lysis$_{DNA}$ Buffer being used, measure and gently transfer **95 mL** of the mixture of *m*Lysis$_{DNA}$ Buffer, Ethanol and IC into the appropriate reagent vessel(s) as indicated in the table.

Sample extraction reagents are distributed as follows:

Samples	1st Vessel	2nd Vessel	3rd Vessel	4th Vessel	5th Vessel	6th Vessel
1 to 24	1 *m*Lysis$_{DNA}$	NA	1 *m*Microparticle$_{DNA}$	1 *m*Wash1$_{DNA}$	1 *m*Wash2$_{DNA}$	1 *m*Elution$_{DNA}$
25 to 48	2 *m*Lysis$_{DNA}$	NA	1 *m*Microparticle$_{DNA}$	1 *m*Wash1$_{DNA}$	1 *m*Wash2$_{DNA}$	1 *m*Elution$_{DNA}$
49 to 72	2 *m*Lysis$_{DNA}$	1 *m*Lysis$_{DNA}$	1 *m*Microparticle$_{DNA}$	2 *m*Wash1$_{DNA}$	2 *m*Wash2$_{DNA}$	1 *m*Elution$_{DNA}$
73 to 96	2 *m*Lysis$_{DNA}$	2 *m*Lysis$_{DNA}$	1 *m*Microparticle$_{DNA}$	2 *m*Wash1$_{DNA}$	2 *m*Wash2$_{DNA}$	1 *m*Elution$_{DNA}$

NOTE: Immediately prior to initiation of the sample extraction protocol, vigorously mix or vortex the *m*Microparticles$_{DNA}$ until they are fully resuspended and pour the *m*Microparticles$_{DNA}$ into the appropriate 200 mL reagent vessel as indicated in the table.

6. A maximum of 96 samples can be processed per run. A negative control and a positive control are included in each run, therefore allowing a maximum of 94 specimens to be processed per run. Prepare the plasma specimens for processing by following these steps:
   - If frozen, thaw specimens at 15 to 30°C or at 2 to 8°C. Once thawed, specimens can be stored at 2 to 8°C for up to 6 hours if not processed immediately.
   - Vortex each specimen 3 times for 2 to 3 seconds.
   - **Centrifuge specimens at 2000*g* for 5 minutes** before loading on the Abbott *m*2000*sp* worktable. Aliquot each specimen into a 5 mL Reaction Vessel. Ensure that each newly aliquotted specimen has a minimal volume of 0.3 mL. Avoid touching the inside of the cap when opening tubes. Take care not to disturb contents of the tube while removing the tube from the centrifuge and that the bottom of the tube is not touched by the pipette tip.
7. Place the negative control, positive control, and the patient specimens into the Abbott *m*2000*sp* sample rack.

   NOTE: Use only 13 mm sample racks. Load specimens and controls into the 13 mm sample racks in consecutive positions.
   - Insert specimen and control tubes (uncapped) into sample racks carefully to avoid splashing. If used, bar codes on tube labels must face right for scanning. Ensure that each tube is placed securely in the sample rack so that the bottom of the tube reaches the inside bottom of the rack.
   - Load filled sample racks onto the Abbott *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.
8. Place the 5 mL Reaction Vessels into the Abbott *m*2000*sp* 1 mL subsystem carrier.
9. Load 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. Initiate the sample extraction protocol as described in the Abbott *m*2000*sp* Operations Manual, Operating Instruction section.

    NOTE: 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.

    NOTE: The plate-fill setup will automatically be enabled for batch sizes of 49 or greater. In such case, the reagent vessel for *m*Elution$_{DNA}$ Buffer (Reagent Carrier 2, location 6) should remain in place.
11. Load the amplification reagents and the master mix tube 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.
12. From the Protocol screen, select the appropriate deep well plate from the Run Master Mix Addition screen that matches the corresponding sample preparation extraction. Initiate the Abbott *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 Abbott *m*2000*rt* protocol (step 16) must be started within 50 minutes of the initiation of the Master Mix Addition protocol (step 12).
13. Switch on and initialize the Abbott *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 Abbott 96-well 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 and initiate the Abbott RealTi*m*e HIV-1 Qualitative assay protocol, as described in the Abbott *m*2000*rt* Operations Manual, Operating Instructions section. At the completion of the run, assay results are reported on the Abbott *m*2000*rt*. Refer to the **RESULTS** section of the package insert for further details.

ASSAY PROTOCOL II: DBS SPECIMENS USING ABBOTT *m*2000*sp* AUTOMATED SAMPLE PREPARATION SYSTEM

For a detailed description of how to operate the Abbott *m*2000*sp* and *m*2000*rt* instruments, refer to the Abbott *m*2000*sp* and *m*2000*rt* Operations Manuals, Operating Instructions sections. Laboratory personnel must be trained to operate the Abbott *m*2000*sp* and *m*2000*rt* instruments. The operator must have a thorough knowledge of the applications run on the instruments and must follow good laboratory practices.

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

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

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

   NOTE: Avoid direct contact of the cutting surface with DBS specimens. Clean the instrument used to cut DBS between specimens if necessary according to good laboratory practices.
2. Thaw assay controls and IC at 15 to 30°C or at 2 to 8°C; see **QUALITY CONTROL PROCEDURES** section of the package insert.
   - Once thawed, assay controls and IC can be stored at 2 to 8°C for up to 24 hours before use.
   - Vortex each assay control 3 times for 2 to 3 seconds before use. Ensure that the contents of each vial are at the bottom after vortexing by tapping the vials on the bench to bring liquid to the bottom of the vial. Ensure bubbles or foam are not generated; if present, remove with a sterile pipette tip, using a new tip for each vial.
3. Thaw amplification reagents at 15 to 30°C or at 2 to 8°C.
   - Once thawed, the amplification reagents can be stored at 2 to 8°C for up to 24 hours until required for the amplification master mix procedure.
   - **For up to 24 reactions use:** 1 tube of positive control, 1 tube of negative control, 1 amplification reagent pack, 1 vial of IC, and 1 set of *m*Sample Preparation System$_{DNA}$ reagents.
   - **For 25 to 48 reactions use:** 1 tube of positive control, 1 tube of negative control, 2 amplification reagent packs, 2 vials of IC, 2 bottles of *m*Lysis$_{DNA}$ Buffer, and 1 bottle *m*Wash 1$_{DNA}$ Buffer, *m*Wash 2$_{DNA}$ Buffer, *m*Microparticle$_{DNA}$ and *m*Elution$_{DNA}$ Buffer.
   - **For 49 to 72 reactions use:** 1 tube of positive control, 1 tube of negative control, 3 amplification reagent packs, 3 vials of IC, 3 bottles of *m*Lysis$_{DNA}$ Buffer, 2 bottles of *m*Wash 1$_{DNA}$ Buffer and *m*Wash 2$_{DNA}$ Buffer, and 1 bottle of *m*Microparticle$_{DNA}$ and *m*Elution$_{DNA}$ Buffer.
   - **For 73 to 96 reactions use:** 1 tube of positive control, 1 tube of negative control, 4 amplification reagent packs, 4 vials of IC, 4 bottles of *m*Lysis$_{DNA}$ Buffer, 2 bottles of *m*Wash 1$_{DNA}$ Buffer and *m*Wash 2$_{DNA}$ Buffer, and 1 bottle of *m*Microparticle$_{DNA}$ and *m*Elution$_{DNA}$ Buffer.
4. Open the *m*Sample Preparation System$_{DNA}$ reagent pack(s). If crystals are observed in any of the reagent bottles upon opening, allow the reagent to equilibrate at room temperature until the crystals disappear. Do not use the reagents until the crystals have dissolved. Add USP Grade 190 to 200 Proof Ethanol (95 to 100% Ethanol) to the *m*Lysis$_{DNA}$, *m*Wash 1$_{DNA}$, and *m*Wash 2$_{DNA}$ bottles as indicated below. **Do not use ethanol that contains denaturants.**

- Add 35 mL ethanol to each bottle of *m*Lysis$_{DNA}$ being used.
- Add 23 mL ethanol to each bottle of *m*Wash 1$_{DNA}$ being used.
- Add 70 mL ethanol to each bottle of *m*Wash 2$_{DNA}$ being used.

5. Vortex each IC vial 3 times for 2 to 3 seconds before use. Use a calibrated **PRECISION PIPETTE DEDICATED FOR INTERNAL CONTROL USE ONLY** to add 750 µL of IC to each bottle of *m*Lysis$_{DNA}$ Buffer.
6. Gently invert all the reagent bottles except *m*Microparticles$_{DNA}$ 5 to 10 times to ensure a homogenous solution and pour the contents into the appropriate reagent vessels as indicated in the table below. Ensure bubbles or foam are not generated in the reagent vessels; if present, remove with a sterile pipette tip, using a new tip for each reagent vessel. For each bottle of *m*Lysis$_{DNA}$ Buffer being used, measure and gently transfer **95 mL** of the mixture of *m*Lysis$_{DNA}$ Buffer, ethanol and IC into the appropriate reagent vessel(s) as indicated in the table.

Sample extraction reagents are distributed as follows:

Samples	1st Vessel	2nd Vessel	3rd Vessel	4th Vessel	5th Vessel	6th Vessel
1 to 24	1 *m*Lysis$_{DNA}$	NA	1 *m*Microparticle$_{DNA}$	1 *m*Wash1$_{DNA}$	1 *m*Wash2$_{DNA}$	1 *m*Elution$_{DNA}$
25 to 48	2 *m*Lysis$_{DNA}$	NA	1 *m*Microparticle$_{DNA}$	1 *m*Wash1$_{DNA}$	1 *m*Wash2$_{DNA}$	1 *m*Elution$_{DNA}$
49 to 72	2 *m*Lysis$_{DNA}$	1 *m*Lysis$_{DNA}$	1 *m*Microparticle$_{DNA}$	2 *m*Wash1$_{DNA}$	2 *m*Wash2$_{DNA}$	1 *m*Elution$_{DNA}$
73 to 96	2 *m*Lysis$_{DNA}$	2 *m*Lysis$_{DNA}$	1 *m*Microparticle$_{DNA}$	2 *m*Wash1$_{DNA}$	2 *m*Wash2$_{DNA}$	1 *m*Elution$_{DNA}$

NOTE: Immediately prior to initiation of the sample extraction protocol, vigorously mix or vortex the *m*Microparticles$_{DNA}$ until they are fully resuspended and pour the *m*Microparticles$_{DNA}$ into the appropriate 200 mL Reagent Vessel as indicated in the table.

7. Place the negative control, positive control, and the patient specimens into the Abbott *m*2000*sp* sample rack.

   NOTE: Use only 13 mm sample racks. Load specimens and controls into the 13 mm sample racks in consecutive positions.

   - Insert specimen and control tubes (uncapped) into sample racks carefully to avoid splashing. If used, bar codes on tube labels must face right for scanning. Ensure that each tube is placed securely in the sample rack so that the bottom of the tube reaches the inside bottom of the rack.
   - Load filled sample racks onto the Abbott *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.
8. Place the 5 mL Reaction Vessels into the Abbott *m*2000*sp* 1 mL subsystem carrier.
9. Load 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. Initiate the sample extraction protocol as described in the Abbott *m*2000*sp* Operations Manual, Operating Instruction section.

    NOTE: 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.

    NOTE: The plate-fill setup will automatically be enabled for batch sizes of 49 or greater. In such case, the reagent vessel for *m*Elution$_{DNA}$ Buffer (Reagent Carrier 2, location 6) should remain in place.
11. Load the amplification reagents and the master mix tube 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.
12. From the Protocol screen, select the appropriate deep well plate from the Run Master Mix Addition screen that matches the corresponding sample preparation extraction. Initiate the Abbott *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 Abbott *m*2000*rt* protocol (step 16) must be started within 50 minutes of the initiation of the Master Mix Addition protocol (step 12).
13. Switch on and initialize the Abbott *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 Abbott 96-Well 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 and initiate the Abbott RealTi*m*e HIV-1 Qualitative assay protocol, as described in the Abbott *m*2000*rt* Operations Manual, Operating Instructions section. At the completion of the run, assay results are reported on the Abbott *m*2000*rt*. Refer to the **RESULTS** section of the package insert for further details.

ASSAY PROTOCOL III: PLASMA SPECIMENS USING MANUAL SAMPLE PREPARATION METHOD

For a detailed description of how to operate the Abbott *m*2000*rt* instrument, refer to the Abbott *m*2000*rt* Operations Manual, Operating Instructions section. Laboratory personnel must be trained to operate the Abbott *m*2000*rt* instrument. The operator must have a thorough knowledge of the application run on the instrument and must follow good laboratory practices.

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

Reagent Preparation Area

Set Up

1. Thaw amplification reagents at 15 to 30˚C or at 2 to 8˚C.
   - Once thawed, the amplification reagents can be stored at 2 to 8˚C for up to 24 hours until required for the amplification master mix procedure.
   - **For up to 24 reactions use:** 1 tube of positive control, 1 tube of negative control, 1 amplification reagent pack, 1 vial of IC, and 1 set of *m*Sample Preparation System$_{DNA}$ reagents.
   - **For 25 to 48 reactions use:** 1 tube of positive control, 1 tube of negative control, 2 amplification reagent packs, 2 vials of IC, 2 bottles of *m*Lysis$_{DNA}$ Buffer, and 1 bottle *m*Wash 1$_{DNA}$ Buffer, *m*Wash 2$_{DNA}$ Buffer, *m*Microparticle$_{DNA}$ and *m*Elution$_{DNA}$ Buffer.
   - **For 49 to 72 reactions use:** 1 tube of positive control, 1 tube of negative control, 3 amplification reagent packs, 3 vials of IC, 3 bottles of *m*Lysis$_{DNA}$ Buffer, 2 bottles of *m*Wash 1$_{DNA}$ Buffer and *m*Wash 2$_{DNA}$ Buffer, and 1 bottle of *m*Microparticle$_{DNA}$ and *m*Elution$_{DNA}$ Buffer.
   - **For 73 to 96 reactions use:** 1 tube of positive control, 1 tube of negative control, 4 amplification reagent packs, 4 vials of IC, 4 bottles of *m*Lysis$_{DNA}$ Buffer, 2 bottles of *m*Wash 1$_{DNA}$ Buffer and *m*Wash 2$_{DNA}$ Buffer, and 1 bottle of *m*Microparticle$_{DNA}$ and *m*Elution$_{DNA}$ Buffer.

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

Sample Preparation Area

Set Up

2. A maximum of 96 samples can be processed per run. A negative control and a positive control are included in each run, therefore allowing a maximum of 94 plasma specimens to be processed per run. Prepare the plasma specimens for processing by following these steps:
   - If frozen, thaw specimens at 15 to 30˚C or at 2 to 8˚C. Once thawed, specimens can be stored at 2 to 8˚C for up to 6 hours if not processed immediately.
   - Vortex each specimen 3 times for 2 to 3 seconds.
   - **Centrifuge specimens at 2000*g* for 5 minutes** before adding to the reaction vessels. Avoid touching the inside of the cap when opening tubes. Take care not to disturb contents of the tube while removing the tube from the centrifuge and that the bottom of the tube is not touched by the pipette tip.
3. Thaw assay controls and IC at 15 to 30˚C or at 2 to 8˚C; see **QUALITY CONTROL PROCEDURES** section of the package insert.
   - Once thawed, assay controls and IC can be stored at 2 to 8˚C for up to 24 hours before use.

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

4. Open the *m*Sample Preparation System$_{DNA}$ reagent pack(s). If crystals are observed in any of the reagent bottles upon opening, allow the reagent to equilibrate at room temperature until the crystals disappear. Do not use the reagents until the crystals have dissolved. Add USP Grade 190 to 200 Proof Ethanol (95 to 100% Ethanol) to the *m*Lysis$_{DNA}$, *m*Wash 1$_{DNA}$, and *m*Wash 2$_{DNA}$ bottles as indicated below. **Do not use ethanol that contains denaturants.**
   - Add 35 mL ethanol to each bottle of *m*Lysis$_{DNA}$ being used.
   - Add 23 mL ethanol to each bottle of *m*Wash 1$_{DNA}$ being used.
   - Add 70 mL ethanol to each bottle of *m*Wash 2$_{DNA}$ being used.
5. Vortex each IC vial 3 times for 2 to 3 seconds before use. Use a calibrated **PRECISION PIPETTE DEDICATED FOR INTERNAL CONTROL USE ONLY** to add 750 µL of IC to each bottle of *m*Lysis$_{DNA}$ Buffer.
6. Gently invert all the reagent bottles except *m*Microparticles$_{DNA}$ 5 to 10 times to ensure a homogeneous solution prior to use.
7. Turn on the temperature controlled dry heating blocks.
   - Set the 5 mL Reaction Vessel block to 50˚C.
   - Set the 1.5 mL tubes block to 75˚C.

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

   WARNING: To avoid personal injury, follow manufacturer's instructions for dry heating block. To avoid burns, turn off the power and allow the heating blocks to cool to 35˚C or below before handling.
8. Label all necessary tubes.
   - One 5 mL Reaction Vessel per sample for the *m*Lysis$_{DNA}$ and *m*Wash 1$_{DNA}$ steps.
   - One 1.5 mL screw top microfuge tube per sample for the *m*Wash 2$_{DNA}$ and *m*Elution$_{DNA}$ Buffer steps.
   - One 1.5 mL screw top microfuge tube per sample or a 96-well polypropylene plate for the eluate.

***m*Lysis$_{DNA}$**

9. Resuspend *m*Microparticles$_{DNA}$ by vortexing or vigorously shaking until particles are in suspension and settled particles are no longer seen on the bottom of the bottle.
10. Place the 5 mL Reaction Vessels in a non-magnetic rack at room temperature. After the particles are resuspended, use a precision pipettor and a sterile 200 µL aerosol barrier pipette tip to add 40 µL of *m*Microparticles$_{DNA}$ to each reaction vessel. A calibrated repeat pipettor may be used.
11. Using a precision pipettor and a sterile 1000 µL aerosol barrier pipette tip, add 2.4 mL of *m*Lysis$_{DNA}$ to each reaction vessel in the non-magnetic rack.
12. Using a precision pipettor and a fresh, sterile 200 µL or 1000 µL aerosol barrier pipette tip for each sample, **add 0.2 mL of the controls and specimens to the reaction vessels.** Mix the sample-*m*Lysis$_{DNA}$ mixture by aspiration and dispense until a uniform suspension is obtained.

    NOTE: Aspirate and dispense liquid slowly to avoid foaming.
13. Place the reaction vessels in the 50˚C heating block, start the timer and incubate for 20 minutes.
14. Remove the reaction vessels from the 50˚C heating block. Using a fresh, sterile 1000 µL aerosol barrier pipette tip for each sample, mix the sample-*m*Lysis$_{DNA}$ mixture by aspiration and dispense until a uniform suspension is obtained.
15. Place the reaction vessels back in the 50˚C heating block, start the timer and incubate for 10 minutes.
16. After the incubation is complete, place the reaction vessels in a magnetic capture stand for 2 minutes to allow the particles to be captured on the side of the reaction vessels.
17. With the reaction vessels in the magnetic capture stand, use a fresh, sterile 1000 µL aerosol barrier pipette tip or disposable transfer pipette for each sample to carefully remove the lysate from each reaction vessel and discard the fluid into a liquid waste container. Remove the fluid as completely as possible. **DO NOT disturb or aspirate the captured magnetic particles.**
18. Remove the reaction vessels from the magnetic rack and transfer to a non-magnetic rack.

***m*Wash 1$_{DNA}$**

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

    NOTE: When adding wash buffers, dispense liquid slowly to avoid splashing.
20. Place the reaction vessels in the 50˚C heating block, start the timer and incubate for 5 minutes.
21. Remove the reaction vessels from the 50˚C heating block. Using a fresh, sterile 1000 µL aerosol barrier pipette tip for each sample, mix the sample-*m*Wash 1$_{DNA}$ mixture by aspiration and dispense until a uniform suspension is obtained.
22. Place the reaction vessels in a magnetic capture stand for 1 minute to allow the particles to be captured on the side of the tubes.
23. With the reaction vessels in the magnetic capture stand, use a fresh, sterile 1000 µL aerosol barrier pipette tip or disposable transfer pipette for each sample to carefully remove the *m*Wash 1$_{DNA}$ from each reaction vessel and discard the fluid into a liquid waste container. Remove the fluid as completely as possible. **DO NOT disturb or aspirate the captured magnetic particles.**
24. Remove the reaction vessels from the magnetic rack and transfer to a non-magnetic rack.

***m*Wash 2$_{DNA}$ First Wash**

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

    NOTE: When adding wash buffers, dispense liquid slowly to avoid splashing.
26. Transfer the wash fluid and particles to a labeled 1.5 mL screw top microfuge tube.
27. Place the tubes in a magnetic capture stand for 1 minute to allow the particles to be captured on the side of the tubes.
28. With the tubes in the magnetic capture stand, use a fresh, sterile 1000 µL aerosol barrier pipette tip for each sample to carefully remove the *m*Wash 2$_{DNA}$ from each tube and discard fluid into a liquid waste container. Remove the fluid as completely as possible. **DO NOT disturb or aspirate the captured magnetic particles.**
29. Remove the tubes from the magnetic rack and transfer to a non-magnetic rack.

***m*Wash 2$_{DNA}$ Second Wash**

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

    NOTE: When adding wash buffers, dispense liquid slowly to avoid splashing.
31. Place the tubes in a magnetic capture stand for 1 minute to allow the particles to be captured on the side of the tubes.
32. With the tubes in the magnetic capture stand, use a fresh, sterile 1000 µL aerosol barrier pipette tip for each sample to carefully remove the *m*Wash 2$_{DNA}$ from each tube and discard fluid into a liquid waste container. Remove the fluid as completely as possible. **DO NOT disturb or aspirate the captured magnetic particles.**
33. Remove the tubes from the magnetic rack and transfer to the 75˚C heating block and incubate for 10 minutes to allow for the evaporation of the ethanol.

***m*Elution$_{DNA}$ Buffer**

34. Remove the tubes from the 75˚C heating block. Using a precision pipettor and a fresh, sterile 200 µL aerosol barrier pipette tip for each sample, add 88 µL of *m*Elution$_{DNA}$ Buffer to the samples and resuspend the magnetic particles in the fluid by aspiration and dispense with the pipette tip. Wash the particles from the side of the tube, if necessary.
35. Place the tubes in the 75˚C heating block, start the timer and incubate for 5 minutes.
36. Remove the tubes from the 75˚C heating block. Using a fresh, sterile 200 µL aerosol barrier pipette tip for each sample, mix the sample-*m*Elution$_{DNA}$ Buffer mixture by aspiration and dispense until a uniform suspension is obtained.

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

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

Amplification Area

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

    NOTE: The Abbott *m*2000*rt* requires 15 minutes to warm-up.
41. 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.

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

Reagent Preparation Area

42. Place a new Abbott 96-Well Optical Reaction Plate in a StrataCooler 96 Benchtop Cooler or Eppendorf PCR Cooler stored as indicated in the StrataCooler 96 Benchtop Cooler instruction manual or Eppendorf PCR Cooler Instructions for Use, respectively. **DO NOT touch the surface or bottom of the plate. IF APPLICABLE:** For batch sizes between 49 and 95 (samples plus controls), prior to preparation of the amplification master mix, fill the wells that will be empty with distilled or deionized water using the following instructions:
    - Using a calibrated pipette, add 100 µl of distilled or deionized water to empty wells based on batch size:

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

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

43. Prepare the amplification master mix.

    NOTE: All reagent preparation must take place in the dedicated Reagent Preparation Area. Refer to the Contamination Precautions section of the package insert before preparing reagents. Change gloves before handling the amplification reagents.
    - Each amplification reagent pack supports up to 24 reactions.
    - Prior to opening the amplification reagents, ensure that the contents of the amplification reagent pack are at the bottom by tapping the amplification reagent pack in an upright position on the bench to bring the liquid to the bottom of the vials.
    - Identify the amplification reagents as follows:
        - Activation Reagent (Reagent 1): clear bottle, teal cap
        - Oligonucleotide Reagent (Reagent 2): black bottle, white cap
        - Thermostable rTth DNA Polymerase Enzyme (Reagent 3): clear bottle, white cap
    - Remove and discard caps.
    - Prepare the master mix by using a **PRECISION PIPETTE DEDICATED FOR REAGENT USE ONLY** to add 271 µL of the HIV-1 Activation Reagent (Reagent 1) and 949 µL of the HIV-1 Oligonucleotide Reagent (Reagent 2) to the Thermostable rTth DNA Polymerase Enzyme bottle (Reagent 3). Mix the Enzyme vial containing the reaction mixture (master mix) by gently pipetting up and down 5 to 7 times. Avoid creating foam.
    - If performing 25 to 48 reactions, prepare the amplification master mix from 2 amplification reagent packs.
    - If performing 49 to 72 reactions, prepare the amplification master mix from 3 amplification reagent packs.
    - If performing 73 to 96 reactions, prepare the amplification master mix from 4 amplification reagent packs.

    NOTE: The Abbott *m*2000*rt* protocol (step 50) must be started within 50 minutes of the addition of the activation reagent into the first enzyme bottle (step 43).
44. Pipette the contents of the master mix from the enzyme bottle(s) into a single-use RNase/DNase-free tube or container. Mix by gently pipetting up and down 5 to 7 times. Avoid creating foam.
45. Using a **PRECISION PIPETTE DEDICATED FOR REAGENT USE ONLY**, dispense 50 µL aliquots of the amplification master mix into each well of the Abbott 96-Well Optical Reaction Plate that will be used to run the samples and controls. A calibrated repeat pipettor may be used.
    - Add the master mix in an order starting with column 1 (from top to bottom), and moving to each consecutive column from left to right.
    - Visually verify that 50 µL has been dispensed into each well.
    - Transfer the Abbott 96-Well Optical Reaction Plate on the StrataCooler 96 Benchtop Cooler or Eppendorf PCR Cooler to the Sample Preparation Area.

Sample Preparation Area

46. Using a precision pipettor and a fresh, sterile 200 µL aerosol barrier pipette tip for each sample, transfer 50 µL of each eluted sample to the Abbott 96-Well Optical Reaction Plate on the StrataCooler 96 Benchtop Cooler or Eppendorf PCR Cooler. During the transfer of each sample, mix the reaction by pipetting up and down 3 to 5 times. Visually verify that a total of 100 µL has been dispensed into each well.
47. Seal the Abbott 96-Well Optical Reaction Plate according to the instructions in the Abbott *m*2000*rt* Operations Manual, Operating Instructions section.
48. Remove the Abbott 96-Well Optical Reaction Plate from the StrataCooler 96 Benchtop Cooler or Eppendorf PCR Cooler and place in the Abbott Splash-Free Support Base. **Centrifuge the Abbott 96-Well Optical Reaction Plate in the Abbott Splash-Free Support Base at 5000*g* for 5 minutes.**
49. Transfer the Abbott 96-Well Optical Reaction Plate in the Abbott Splash-Free Support Base to the Amplification Area.

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

Amplification Area

50. Place the Abbott 96-Well Optical Reaction Plate in the Abbott *m*2000*rt* instrument, select the test order created (step 41), and initiate the Abbott RealTi*m*e HIV-1 Qualitative assay protocol, as described in the Abbott *m*2000*rt* Operations Manual, Operating Instructions section. At the completion of the run, assay results are reported on the Abbott *m*2000*rt*. Refer to the **RESULTS** section of the package insert for further details.

ASSAY PROTOCOL IV: DBS SPECIMENS USING MANUAL SAMPLE PREPARATION METHOD

For a detailed description of how to operate the Abbott *m*2000*rt* instrument, refer to the Abbott *m*2000*rt* Operations Manual, Operating Instructions section. Laboratory personnel must be trained to operate the Abbott *m*2000*rt* instrument. The operator must have a thorough knowledge of the application run on the instrument and must follow good laboratory practices.

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

Reagent Preparation Area

Set Up

1. Thaw amplification reagents at 15 to 30°C or at 2 to 8°C.
    - Once thawed, the amplification reagents can be stored at 2 to 8°C for up to 24 hours until required for the amplification master mix procedure.
    - **For up to 24 reactions use:** 1 tube of positive control, 1 tube of negative control, 1 amplification reagent pack, 1 vial of IC, and 1 set of *m*Sample Preparation System$_{DNA}$ reagents.
    - **For 25 to 48 reactions use:** 1 tube of positive control, 1 tube of negative control, 2 amplification reagent packs, 2 vials of IC, 2 bottles of *m*Lysis$_{DNA}$ Buffer, and 1 bottle *m*Wash 1$_{DNA}$ Buffer, *m*Wash 2$_{DNA}$ Buffer, *m*Microparticle$_{DNA}$ and *m*Elution$_{DNA}$ Buffer.

- **For 49 to 72 reactions use:** 1 tube of positive control, 1 tube of negative control, 3 amplification reagent packs, 3 vials of IC, 3 bottles of *m*Lysis$_{DNA}$ Buffer, 2 bottles of *m*Wash 1$_{DNA}$ Buffer and *m*Wash 2$_{DNA}$ Buffer, and 1 bottle of *m*Microparticle$_{DNA}$ and *m*Elution$_{DNA}$ Buffer.
- **For 73 to 96 reactions use:** 1 tube of positive control, 1 tube of negative control, 4 amplification reagent packs, 4 vials of IC, 4 bottles of *m*Lysis$_{DNA}$ Buffer, 2 bottles of *m*Wash 1$_{DNA}$ Buffer and *m*Wash 2$_{DNA}$ Buffer, and 1 bottle of *m*Microparticle$_{DNA}$ and *m*Elution$_{DNA}$ Buffer.

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

Sample Preparation Area

Set Up

2. A maximum of 96 samples can be processed per run. A negative control and a positive control are included in each run, therefore allowing a maximum of 94 DBS specimens to be processed per run. Prepare the DBS specimens for processing by following these steps:
   - Prepare tubes with 1.7 mL Abbott *m*Lysis$_{DNA}$ Buffer (from the Bulk *m*Lysis$_{DNA}$ Buffer).
   - Cut out 2 entire DBS for each specimen from a Whatman 903 filter paper card (or equivalent). Each DBS should be approximately one-half-inch (12 millimeters) in diameter.
   - Place DBS in the tube containing the Abbott *m*Lysis$_{DNA}$ Buffer. Ensure that DBS are fully submerged in the *m*Lysis$_{DNA}$ Buffer.
   - Incubate at room temperature for 20 minutes with intermittent gentle mixing.

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

   NOTE: Avoid direct contact of the cutting surface with DBS specimens. Clean the instrument used to cut DBS between specimens if necessary according to good laboratory practices.
3. Thaw assay controls and IC at 15 to 30˚C or at 2 to 8˚C; see **QUALITY CONTROL PROCEDURES** section of the package insert.
   - Once thawed, assay controls and IC can be stored at 2 to 8˚C for up to 24 hours before use.
   - Vortex each assay control 3 times for 2 to 3 seconds before use. After vortexing ensure that the contents of each vial are at the bottom by tapping the vials on the bench to bring liquid to the bottom of the vial. Ensure bubbles or foam are not generated; if present, remove with a sterile pipette tip, using a new tip for each vial.
4. Open the *m*Sample Preparation System$_{DNA}$ reagent pack(s). If crystals are observed in any of the reagent bottles upon opening, allow the reagent to equilibrate at room temperature until the crystals disappear. Do not use the reagents until the crystals have dissolved. Add USP Grade 190 to 200 Proof Ethanol (95 to 100% Ethanol) to the *m*Lysis$_{DNA}$, *m*Wash 1$_{DNA}$. and *m*Wash 2$_{DNA}$ bottles as indicated below. **Do not use ethanol that contains denaturants.**
   - Add 35 mL ethanol to each bottle of *m*Lysis$_{DNA}$ being used.
   - Add 23 mL ethanol to each bottle of *m*Wash 1$_{DNA}$ being used.
   - Add 70 mL ethanol to each bottle of *m*Wash 2$_{DNA}$ being used.
5. Vortex each IC vial 3 times for 2 to 3 seconds before use. Use a calibrated **PRECISION PIPETTE DEDICATED FOR INTERNAL CONTROL USE ONLY** to add 750 µL of IC to each bottle of *m*Lysis$_{DNA}$ Buffer.
6. Gently invert all the reagent bottles except *m*Microparticles$_{DNA}$ 5 to 10 times to ensure a homogeneous solution prior to use.
7. Turn on the temperature controlled dry heating blocks.
   - Set the 5 mL Reaction Vessel block to 50˚C.
   - Set the 1.5 mL tubes block to 75˚C.

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

   WARNING: To avoid personal injury, follow manufacturer's instructions for dry heating block. To avoid burns, turn off the power and allow the heating blocks to cool to 35˚C or below before handling.
8. Label all necessary tubes.
   - One 5 mL Reaction Vessel per sample for the *m*Lysis$_{DNA}$ and *m*Wash 1$_{DNA}$ steps.
   - One 1.5 mL screw top microfuge tube per sample for the *m*Wash 2$_{DNA}$ and *m*Elution$_{DNA}$ Buffer steps.
   - One 1.5 mL screw top microfuge tube per sample or a 96 well polypropylene plate for the eluate.

***m*Lysis$_{DNA}$**

9. Resuspend *m*Microparticles$_{DNA}$ by vortexing or vigorously shaking until particles are in suspension and settled particles are no longer seen on the bottom of the bottle.
10. Place the 5 mL Reaction Vessels in a non-magnetic rack at room temperature. After the particles are resuspended, use a precision pipettor and a sterile 200 µL aerosol barrier pipette tip to add 40 µL of *m*Microparticles$_{DNA}$ to each reaction vessel. A calibrated repeat pipettor may be used.
11. Using a precision pipettor and a sterile 1000 µL aerosol barrier pipette tip, add 2.4 mL of *m*Lysis$_{DNA}$ to each reaction vessel in the non-magnetic rack.
12. Using a precision pipettor and a fresh, sterile 1000 µL aerosol barrier pipette tip for each sample, **add 1.0 mL of the controls and specimens to the reaction vessels. DO NOT transfer the filter paper.** Mix the sample-*m*Lysis$_{DNA}$ mixture by aspiration and dispense until a uniform suspension is obtained.

    NOTE: Aspirate and dispense liquid slowly to avoid foaming.
13. Place the reaction vessels in the 50˚C heating block, start the timer and incubate for 20 minutes.
14. Remove the reaction vessels from the 50˚C heating block. Using a fresh, sterile 1000 µL aerosol barrier pipette tip for each sample, mix the sample-*m*Lysis$_{DNA}$ mixture by aspiration and dispense until a uniform suspension is obtained.
15. Place the reaction vessels back in the 50˚C heating block, start the timer and incubate for 10 minutes.
16. After the incubation is complete, place the reaction vessels in a magnetic capture stand for 2 minutes to allow the particles to be captured on the side of the reaction vessels.
17. With the reaction vessels in the magnetic capture stand, use a fresh, sterile 1000 µL aerosol barrier pipette tip or disposable transfer pipette for each sample to carefully remove the lysate from each reaction vessel and discard the fluid into a liquid waste container. Remove the fluid as completely as possible. **DO NOT disturb or aspirate the captured magnetic particles.**
18. Remove the reaction vessels from the magnetic rack and transfer to a non-magnetic rack.

***m*Wash 1$_{DNA}$**

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

    NOTE: When adding wash buffers, dispense liquid slowly to avoid splashing.
20. Place the reaction vessels in the 50˚C heating block, start the timer and incubate for 5 minutes.
21. Remove the reaction vessels from the 50˚C heating block. Using a fresh, sterile 1000 µL aerosol barrier pipette tip for each sample, mix the sample-*m*Wash 1$_{DNA}$ mixture by aspiration and dispense until a uniform suspension is obtained.
22. Place the reaction vessels in a magnetic capture stand for 1 minute to allow the particles to be captured on the side of the tubes.
23. With the reaction vessels in the magnetic capture stand, use a fresh, sterile 1000 µL aerosol barrier pipette tip or disposable transfer pipette for each sample to carefully remove the *m*Wash 1$_{DNA}$ from each reaction vessel and discard the fluid into a liquid waste container. Remove the fluid as completely as possible. **DO NOT disturb or aspirate the captured magnetic particles.**
24. Remove the reaction vessels from the magnetic rack and transfer to a non-magnetic rack.

***m*Wash 2$_{DNA}$ First Wash**

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

    NOTE: When adding wash buffers, dispense liquid slowly to avoid splashing.
26. Transfer the wash fluid and particles to a labeled 1.5 mL screw top microfuge tube.
27. Place the tubes in a magnetic capture stand for 1 minute to allow the particles to be captured on the side of the tubes.

28. With the tubes in the magnetic capture stand, use a fresh, sterile 1000 µL aerosol barrier pipette tip for each sample to carefully remove the *m*Wash 2_{DNA} from each tube and discard fluid into a liquid waste container. Remove the fluid as completely as possible. **DO NOT disturb or aspirate the captured magnetic particles.**
29. Remove the tubes from the magnetic rack and transfer to a non-magnetic rack.

***m*Wash 2_{DNA} Second Wash**

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

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

31. Place the tubes in a magnetic capture stand for 1 minute to allow the particles to be captured on the side of the tubes.
32. With the tubes in the magnetic capture stand, use a fresh, sterile 1000 µL aerosol barrier pipette tip for each sample to carefully remove the *m*Wash 2_{DNA} from each tube and discard fluid into a liquid waste container. Remove the fluid as completely as possible. **DO NOT disturb or aspirate the captured magnetic particles.**
33. Remove the tubes from the magnetic rack and transfer to the 75˚C heating block and incubate for 10 minutes to allow for the evaporation of the ethanol.

***m*Elution$_{DNA}$ Buffer**

34. Remove the tubes from the 75˚C heating block. Using a precision pipettor and a fresh, sterile 200 µL aerosol barrier pipette tip for each sample, add 88 µL of *m*Elution$_{DNA}$ Buffer to the samples and resuspend the magnetic particles in the fluid by aspiration and dispense with the pipette tip. Wash the particles from the side of the tube, if necessary.
35. Place the tubes in the 75˚C heating block, start the timer and incubate for 5 minutes.
36. Remove the tubes from the 75˚C heating block. Using a fresh, sterile 200 µL aerosol barrier pipette tip for each sample, mix the sample-*m*Elution$_{DNA}$ Buffer mixture by aspiration and dispense until a uniform suspension is obtained.
37. Place the tubes back in the 75˚C heating block, start the timer and incubate for 5 minutes.
38. Remove the tubes from the 75˚C heating block and place in a magnetic capture stand for 1 minute to allow the particles to be captured on the side of the tubes.
39. With the tubes in the magnetic capture stand, use a fresh, sterile 200 µL aerosol barrier pipette tip for each sample to carefully remove the eluted sample from the tubes. **DO NOT disturb or aspirate the captured microparticles.** The eluted sample(s) can be placed into a fresh, labeled 1.5 mL screw top microfuge tube or a 96-well polypropylene plate.

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

Amplification Area

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

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

41. 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.

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

Reagent Preparation Area

42. Place a new Abbott 96-Well Optical Reaction Plate in a StrataCooler 96 Benchtop Cooler or Eppendorf PCR Cooler stored as indicated in the StrataCooler 96 Benchtop Cooler instruction manual or Eppendorf PCR Cooler Instructions for Use, respectively. **DO NOT touch the surface or bottom of the plate.**
    IF APPLICABLE: For batch sizes between 49 and 95 (samples plus controls), prior to preparation of the amplification master mix, fill the wells that will be empty with distilled or deionized water using the following instructions:
    - Using a calibrated pipette, add 100 µl of distilled or deionized water to empty wells based on batch size:

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

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

43. Prepare the amplification master mix.

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

    - Each amplification reagent pack supports up to 24 reactions.
    - Prior to opening the amplification reagents, ensure that the contents of the amplification reagent pack are at the bottom by tapping the amplification reagent pack in an upright position on the bench to bring the liquid to the bottom of the vials.
    - Identify the amplification reagents as follows:
        - Activation Reagent (Reagent 1): clear bottle, teal cap
        - Oligonucleotide Reagent (Reagent 2): black bottle, white cap
        - Thermostable rTth DNA Polymerase Enzyme (Reagent 3): clear bottle, white cap
    - Remove and discard caps.
    - Prepare the master mix by using a **PRECISION PIPETTE DEDICATED FOR REAGENT USE ONLY** to add 271 µL of the HIV-1 Activation Reagent (Reagent 1) and 949 µL of the HIV-1 Oligonucleotide Reagent (Reagent 2) to the Thermostable rTth DNA Polymerase Enzyme bottle (Reagent 3). Mix the enzyme vial containing the reaction mixture (master mix) by gently pipetting up and down 5 to 7 times. Avoid creating foam.
    - If performing 25 to 48 reactions, prepare the amplification master mix from 2 amplification reagent packs.
    - If performing 49 to 72 reactions, prepare the amplification master mix from 3 amplification reagent packs.
    - If performing 73 to 96 reactions, prepare the amplification master mix from 4 amplification reagent packs.

    NOTE: The Abbott *m*2000*rt* protocol (step 50) must be started within 50 minutes of the addition of the activation reagent into the first enzyme bottle (step 43).

44. Pipette the contents of the master mix from the enzyme bottle(s) into a single-use RNase/DNase-free tube or container. Mix by gently pipetting up and down 5 to 7 times. Avoid creating foam.
45. Using a **PRECISION PIPETTE DEDICATED FOR REAGENT USE ONLY**, dispense 50 µL aliquots of the amplification master mix into each well of the Abbott 96-Well Optical Reaction Plate that will be used to run the samples and controls. A calibrated repeat pipettor may be used.
    - Add the master mix in an order starting with column 1 (from top to bottom), and moving to each consecutive column from left to right.
    - Visually verify that 50 µL has been dispensed into each well.
    - Transfer the Abbott 96-Well Optical Reaction Plate on the StrataCooler 96 Benchtop Cooler or Eppendorf PCR Cooler to the Sample Preparation Area.

Sample Preparation Area

46. Using a precision pipettor and a fresh, sterile 200 µL aerosol barrier pipette tip for each sample, transfer 50 µL of each eluted sample to the Abbott 96-Well Optical Reaction Plate on the StrataCooler 96 Benchtop Cooler or Eppendorf PCR Cooler. During the transfer of each sample, mix the reaction by pipetting up and down 3 to 5 times. Visually verify that a total of 100 µL has been dispensed into each well.
47. Seal the Abbott 96-Well Optical Reaction Plate according to the instructions in the Abbott *m*2000*rt* Operations Manual, Operating Instructions section.

48. Remove the Abbott 96-Well Optical Reaction Plate from the StrataCooler 96 Benchtop Cooler or Eppendorf PCR Cooler and place in the Abbott Splash-Free Support Base. **Centrifuge the Abbott 96-Well Optical Reaction Plate in the Abbott Splash-Free Support Base at 5000*g* for 5 minutes.**
49. Transfer the Abbott 96-Well Optical Reaction Plate in the Abbott Splash-Free Support Base to the Amplification Area.

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

Amplification Area

50. Place the Abbott 96-Well Optical Reaction Plate in the Abbott *m*2000*rt* instrument, select the test order created (step 41), and initiate the Abbott RealTi*m*e HIV-1 Qualitative assay protocol, as described in the Abbott *m*2000*rt* Operations Manual, Operating Instructions section. At the completion of the run, assay results are reported on the Abbott *m*2000*rt*. Refer to the **RESULTS** section of the package insert for further details.

POST PROCESSING PROCEDURES

1. At the end of each run, clear and clean all work areas:
   - For Abbott *m*2000*sp* Automated Sample Preparation System users (Protocols I and II), clean the Abbott *m*2000*sp* worktable as stated in the Abbott *m*2000*sp* Operations Manual.
   - For Abbott Manual Sample Preparation Method users (Protocols III and IV), clean sample racks, temperature blocks, and magnetic racks by soaking in a 0.5% sodium hypochlorite solution for approximately 10 minutes and then rinsing completely with water and thoroughly air drying. Decontaminate and wipe work area according to laboratory guidelines. Clean the StrataCooler 96 Benchtop Cooler or Eppendorf PCR Cooler as described in the StrataCooler 96 Benchtop Cooler instruction manual or Eppendorf PCR Cooler Instructions for Use and return to the Reagent Preparation Area.
2. Clean the Abbott *m*2000*rt* and the Abbott Splash-Free Support Base according to the Abbott *m*2000*rt* Operations Manual.
3. Decontaminate and dispose of all specimens, controls, reagents, and other potentially contaminated materials in accordance with local, state, and federal regulations.
4. Abbott *m*Sample Preparation System$_{DNA}$ reagents are single use only. The remaining reagents and liquid waste must be discarded in accordance with local, state, and federal regulations.

   WARNING: Do not mix any oxidizing agents, such as sodium hypochlorite, with the *m*Lysis$_{DNA}$, *m*Microparticles$_{DNA}$, and *m*Wash 1$_{DNA}$ in the *m*Sample Preparation System$_{DNA}$ reagents. Do not mix any oxidizing agents, such as sodium hypochlorite, with the liquid waste. Toxic gases may be generated from these mixtures. Pressure may build up in the closed container of the mixtures. It is the responsibility of each facility to characterize its waste and ensure that it is handled and disposed of in accordance with local, state and federal regulations.
5. Remove and discard all disposables and solid waste in accordance with local, state, and federal regulations.
6. Place the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose of according to the Abbott *m*2000*rt* Operations Manual along with the gloves used to handle the plate.

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 Qualitative 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 Qualitative assay:

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

Detection of Inhibition

A defined, consistent quantity of IC is introduced into each specimen and control at the beginning of sample preparation and detected on the Abbott *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. A flag or an error code is displayed when IC cycle number (CN) value of a specimen or control exceeds the validity range. Refer to the Abbott *m*2000*rt* Operations Manual for an explanation of the corrective actions for errors.

Negative and Positive Controls

A negative control and a positive control are required for every run to verify that the sample processing, the amplification, and the detection steps are performed correctly. The Abbott RealTi*m*e HIV-1 Qualitative Controls need to be processed together with the specimens prior to running the amplification portion of the assay.

A flag is displayed when a control result is out of range. If negative control or positive control is out of range, all of the specimens and controls from that run must be reprocessed, beginning with sample preparation. HIV-1 must not be detected in the negative control. HIV-1 detected in the negative control is indicative of contamination from other specimens or amplified product introduced during sample preparation or during preparation of the Abbott 96-Well Optical Reaction Plate.

To remove contamination, clean the Abbott *m*2000*sp* and *m*2000*rt* and all work areas according to the Abbott *m*2000*sp* and *m*2000*rt* Operations Manuals and the instructions in the **POST PROCESSING PROCEDURES** section of this package insert. Following cleaning, repeat sample processing for controls and specimens. If negative controls are persistently reactive, contact your Abbott representative.

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

Monitoring the Laboratory for the Presence of Contamination

It is recommended that the following procedure be done at least once a month to monitor laboratory surfaces and equipment for contamination. It is very important to test all areas that may have been exposed to processed specimens and controls and/or amplification product. These include routinely handled objects such as pipettes, the Abbott *m*2000*sp* and *m*2000*rt* function keys, bench surfaces and other equipment that may be present in the work areas.

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

   NOTE: Chlorine solutions may pit equipment and metal. Use sufficient amounts or repeated applications of 70% ethanol until chlorine residue is no longer visible.
9. Repeat testing of the contaminated area by following steps 1 through 6.
10. If the presence of contamination is detected again, repeat steps 8 and 9 until no HIV-1 amplification is detected.

RESULTS

A specimen tested by the Abbott RealTi*m*e HIV-1 Qualitative assay will have a result of "HIV-1 Detected" or "Not Detected." When using the dried blood spot application, "HIV-1 Detected" specimen results will also display the Cycle Number (CN) value. The Cycle Number value is the PCR cycle at which the fluorescence has risen above the predefined fluorescence threshold established for determining HIV-1 detected specimens with the Abbott Realti*m*e HIV-1 Qualitative assay. No Cycle Number will be reported for HIV-1 "Not Detected" dried blood spot specimens. The Cycle Number will not be reported for any plasma specimens.

Caution: the Cycle Number value displayed with HIV-1 Detected samples using dried blood spots should not be used for quantitative determination of HIV-1 viral load. Refer to World Health Organization Guideline WHO/CDS/HIV/18.51 UPDATED RECOMMENDATIONS ON FIRST-LINE AND SECOND-LINE ANTIRETROVIRAL REGIMENS AND POST-EXPOSURE PROPHYLAXIS AND RECOMMENDATIONS ON EARLY INFANT DIAGNOSIS OF HIV, December, 2018.

A minimum of 1 negative control and 1 positive control are required with each run. The negative control serves to verify that HIV-1 contamination of the negative control did not occur during the sample preparation and set-up of the amplification reaction. If HIV-1 signal is detected for the negative control, the -QC flag is displayed next to all specimen results for the run. Specimens with the -QC flag may have been similarly contaminated with analyte during processing. If the negative control is not processed, the -QC flag is also indicated next to all specimen results for that run.

The IC signal in specimens serves to confirm that each specimen was processed correctly and to indicate whether inhibitors of amplification are present. If the IC is out of range (ie, IC CN not generated or greater than or equal to a fixed cutoff cycle) and HIV-1 is detected, the specimen will have a result of "HIV-1 Detected." An IC flag will be reported next to the result. If the IC is out of range and HIV-1 is not detected, no result will be reported and an error code will be generated. The specimen with an error code must be retested starting with sample preparation. For more information about error codes and flags, refer to the Abbott *m*2000*rt* Operations Manual Version 3.0, Operations Manual Addendum Version 3.0, or higher versions.

LIMITATIONS OF THE PROCEDURE

- FOR IN VITRO DIAGNOSTIC USE
- Optimal performance of this test requires appropriate specimen collection, handling, preparation, and storage (refer to the **SPECIMEN COLLECTION AND HANDLING INSTRUCTIONS** 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 Qualitative assay. The use of other anticoagulants has not been validated with the Abbott RealTi*m*e HIV-1 Qualitative assay.
- Use of the Abbott RealTi*m*e HIV-1 Qualitative assay is limited to personnel who have been trained in the procedures of a molecular diagnostic assay and the Abbott *m*2000*sp* and the Abbott *m*2000*rt* instruments, and/or the manual sample preparation method for Abbott RealTi*m*e HIV-1 Qualitative.
- The instruments and assay procedures reduce the risk of contamination by amplification product. However, nucleic acid contamination from the positive controls or specimens must be controlled by good laboratory practice and careful adherence to the procedures specified in this package insert.
- A specimen with a result of "Not Detected" cannot be presumed to be negative for HIV-1.
- As with any diagnostic test, results from the Abbott RealTi*m*e HIV-1 Qualitative assay should be interpreted in conjunction with other clinical and laboratory findings.
- The Abbott RealTi*m*e HIV-1 Qualitative is not intended to be used as a screening test for HIV-1.

SPECIFIC PERFORMANCE CHARACTERISTICS

The performance characteristics were determined using the Abbott RealTi*m*e HIV-1 Qualitative assay with the Abbott *m*2000*sp* sample preparation system unless otherwise specified.

Limit of Detection (LOD)

LOD is defined by the HIV-1 concentration detected with a probability of 95%. LOD was determined for both the plasma and DBS procedures 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 or in HIV-1 negative human whole blood that was spotted on filter paper cards to create dried blood spots. Testing was performed with 3 lots of amplification reagents on 3 Abbott *m*2000 Systems. The results, representative of the performance of the Abbott RealTi*m*e HIV-1 Qualitative assay, are summarized in **Tables 1** and **2** for the plasma and DBS procedures, respectively.

Table 1. Plasma Procedure, VQA Viral Standard

Concentration (copies/mL)	Number Tested	Number Detected	Percent Detected
30	45	32	71
50	45	36	80
75	45	42	93
100	45	44	98
150	45	45	100
200	45	45	100

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

Table 2. DBS Procedure, VQA Viral Standard

Concentration (copies/mL)	Number Tested	Number Detected	Percent Detected
800	45	31	69
1000	45	32	71
1500	45	41	91
2000	45	41	91
2500	45	42	93
3000	45	44	98

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

Detection of WHO 2nd International Standard (NIBSC 97/650)

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

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

Concentration (IU/mL)	Number Tested	Number Detected	Percent Detected
50	45	27	60
80	45	39	87
120	45	39	87
160	45	44	98
240	45	44	98
320	45	45	100

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

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

Concentration (IU/mL)	Number Tested	Number Detected	Percent Detected
1600	45	28	62
2400	45	39	87
3000	45	42	93
5000	45	45	100
7000	45	45	100
9000	45	45	100

Probit analysis of the data determined that the concentration of HIV-1 RNA in whole blood detected with 95% probability was 3,085 IU/mL (95% CI 2,644 - 4,199 IU/mL) using the DBS procedure.

Seroconversion Sensitivity

The diagnostic sensitivity of the Abbott RealTi*m*e HIV-1 Qualitative assay was evaluated by testing sequential plasma specimens from 13 HIV seroconversion panels. These panels are commercially available and pre-characterized for HIV infection.

The Abbott RealTi*m*e HIV-1 Qualitative assay detected HIV-1 in 57 out of 82 total number of bleeds compared with 16 out of 82 that were detected by an HIV-1 antibody test (Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA List No. 3A77). The first reactive bleed (i.e., panel member) for Abbott RealTi*m*e HIV-1 Qualitative assay occurred earlier in all 13 panels (median 10.0 days) compared to the Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA. The seroconversion sensitivity is presented in **Table 5**.

Table 5. Seroconversion Sensitivity

Panel ID	Number of Panel Members Tested	Number of Reactive Panel Members: Abbott RealTi*me* HIV-1 Qualitative	Number of Reactive Panel Members: Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA[a]	Days to First Reactive Result: Abbott RealTi*me* HIV-1 Qualitative	Days to First Reactive Result: Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA[a]	Difference in Days to First Reactive Result (Based on Bleed Date)[b]
PRB943	7	6	3	5	14	9
PRB950	4	3	1	18	28	10
PRB951	6	4	1	8	19	11
PRB952	6	5	2	7	17	10
PRB954	7	4	0	10	21[d]	11
PRB955	5	5	2	0[c]	12	12
PRB956	5	4	0	40	50[d]	10
PRB957	7	4	2	14	23	9
PRB962	6	4	0	7	17[d]	10
PRB963	7	4	0	9	21[d]	12
PRB964	6	3	0	15	22[d]	7
PRB965	6	6	3	0[c]	12	12
PRB966	10	5	2	35	48	13
Total	**82**	**57**	**16**			**Median = 10.0** **Mean = 10.5**

[a] Based on data from the vendor.
[b] The dates of the first reactive test results were compared in the Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA and the Abbott RealTi*me* HIV-1 Qualitative.
[c] All bleeds in these panels were detected with the Abbott RealTi*me* HIV-1 Qualitative. Zero was used as the "Days to First Reactive Result."
[d] All bleeds in these panels were nonreactive with the Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA. The last bleed day was used as the "Days to First Reactive Result."

Performance for Detection of HIV-1 Infection in Infants

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

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

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

		Abbott RealTi*me* HIV-1 Qualitative DBS Procedure: Detected	Abbott RealTi*me* HIV-1 Qualitative DBS Procedure: Not Detected
Roche Amplicor HIV-1 DNA Test Version 1.5	**Detected**	54	1
	Not Detected	12	221

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

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

		Abbott RealTi*me* HIV-1 Qualitative Plasma Procedure: Detected	Abbott RealTi*me* HIV-1 Qualitative Plasma Procedure: Not Detected
Roche Amplicor HIV-1 DNA Test Version 1.5	**Detected**	72	0
	Not Detected	8	287

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

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

Detection of HIV-1 Subtypes and Groups

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

Table 8: Subtype Detection

Group/Subtypes	n[a]	Detected by Abbott RealTi*me* HIV-1 Qualitative assay
Group M/Subtype A	10	10
Group M/Subtype B	10	10
Group M/Subtype C	10	10
Group M/Subtype D	10	10
Group M/Subtype AE	10	10
Group M/Subtype F	10	10
Group M/Subtype AG	10	10
Group M/Subtype G	10	10
Group M/Subtype H	10	10
Group O	10	10
Group N	10	10

[a] "n" represents the number of clinical specimens tested. For Group M/Subtype H and Group N, "n" represents the number of replicates of transcript samples tested.

The Abbott RealTi*me* HIV-1 Qualitative assay detected all groups and subtypes tested.

Specificity

The specificity of the Abbott RealTi*me* HIV-1 Qualitative assay was evaluated for both the plasma and DBS procedures by testing 550 HIV-1 seronegative plasma specimens and 550 HIV-1 seronegative whole blood specimens. Both plasma and whole blood specimens were collected from each of the 550 subjects. For each assay procedure, the specimens were tested on 2 Abbott *m*2000 Systems with 4 lots of amplification reagents. HIV-1 was not detected for 550 out of 550 samples in both specimen types, resulting in 100.0% specificity (95% CI 99.33 – 100.00%) for both the plasma and DBS assay procedures in this representative study.

The specificity of the Abbott RealTi*me* HIV-1 Qualitative assay was further evaluated by testing plasma specimens that had been either obtained from individuals diagnosed or screened for an autoimmune disorder or serologically characterized as positive for the following markers: systemic lupus erythematosus (n=10), anti-nuclear antibodies (n=10), rheumatoid factor (n=10), multiple sclerosis (n=7), multiple myeloma (n=10), HBsAg (n=10), anti-HTLV-I (n=10), anti-HCV (n=10). Also tested were flu vaccinees (n=10) and HBV vaccinees (n=10). HIV-1 was not detected in any of the specimens tested. In addition, HIV-1 was detected in all the specimens that had been spiked with HIV-1 RNA. The results demonstrated that the presence of an autoimmune disorder or serologic markers for autoimmune disease or viral pathogens other than HIV-1 did not affect the Abbott RealTi*me* HIV-1 Qualitative assay.

Reproducibility

The reproducibility of the Abbott RealTi*me* HIV-1 Qualitative assay was evaluated using the Abbott *m*2000*sp*. A four-member HIV-1 RNA panel was prepared consisting of 1 negative member and 3 positive members at 1,000,000, 100,000 and 10,000 copies/mL. The panel was tested by 3 operators. Each operator, using a unique combination of reagent

lot and instrument pair, tested 10 replicates of each panel member once per day for 5 days, for a total of 50 replicates per operator (150 total replicates per panel member). The Abbott RealTi*m*e HIV-1 Qualitative assay detected HIV-1 in all 450 HIV-1 positive samples (150 samples at each high, medium and low concentration). The assay results in all negative samples were "Not Detected." The overall agreement for 600 results compared with expected results was 100.0% (95% CI 99.39 – 100.00%).

Reproducibility Between Manual and Automated *m*2000*sp* Sample Preparation Methods

In addition to the automated Abbott *m*2000*sp* sample preparation method, the Abbott RealTi*m*e HIV-1 Qualitative assay also provides a manual sample preparation option. The reproducibility between the manual and the automated sample preparation methods was determined by testing the same HIV-1 RNA panel as evaluated in the Reproducibility study. The panel was tested by a single operator using both sample preparation methods and using 1 reagent lot and 1 Abbott *m*2000*rt* instrument. For each method, the operator tested 10 replicates of each panel member once per day for 5 days, for a total of 50 replicates (200 total replicates for all panel members). The overall agreement between the manual and Abbott *m*2000*sp* sample preparation methods was 99.0% (95% CI 96.43 – 99.88%).

Cross-Reactivity

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

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

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

Potentially Interfering Substances

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

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

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

Carryover

Potential sample carryover in the automated Abbott *m*2000 systems when used with the Abbott RealTi*m*e HIV-1 Qualitative assay was evaluated by testing high titer HIV-1 positive samples (with a target concentration of 1,000,000 copies/mL) interspersed with negative samples. The Abbott RealTi*m*e HIV-1 Qualitative assay did not exhibit detectable carryover from high positive samples to negative samples.

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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 http://www.abbottmolecular.com.

THE PURCHASE OF THIS PRODUCT ALLOWS THE PURCHASER TO

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

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

The Abbott RealTi*m*e HIV-1 Qualitative 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

Abbott RealTi*me*

en

REF 4N66-80

51-608401/R4

HIV-1 Qualitative Control Kit

NOTE: Changes Highlighted

Key to Symbols used	
REF	List Number
IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
	Expiration Date
CONTROL –	Negative Control
CONTROL H	High Positive Control
-10°C	Store at ≤ -10°C
	Consult instructions for use
	Warning
	Caution: 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 Qualitative Controls are used to establish run validity of the Abbott RealTi*me* HIV-1 Qualitative assay when used for the qualitative detection of Human Immunodeficiency Virus Type 1 (HIV-1) nucleic acids from human plasma and dried blood spots (DBS).

Intended User

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

Contents

1. CONTROL – **Abbott RealTi*me* HIV-1 Negative Control (2G31Z) (12 vials, 1.8 mL per vial).** Negative human plasma tested and found to be nonreactive for HBsAg, anti-HIV-1/HIV-2, anti-HCV, HIV RNA, HCV RNA, and HBV DNA. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.
2. CONTROL H **Abbott RealTi*me* HIV-1 High Positive Control (2G31X) (12 vials, 1.8 mL per vial).** Noninfectious Armored RNA with HIV-1 sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, anti-HIV-1/HIV-2, anti-HCV, HIV RNA, HCV RNA, and HBV DNA. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.
   The Abbott RealTi*me* Qualitative Reagents are intended for single-use only and unused reagents should be discarded.
- The Abbott RealTi*me* HIV-1 Qualitative Control Kit must only be used with the Abbott RealTi*me* HIV-1 Qualitiative assay (List No. 4N66-90).

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 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.[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 Qualitative Control Kit (List No. 4N66-80) contain the following components:

- 2-Methyl-2H-isothiazol-3-one
- Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one (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

-10°C **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.

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