WHO Prequalification of In Vitro Diagnostics
PUBLIC REPORT

Product: Abbott RealTi*me* HCV
WHO reference number: PQDx 0450-027-00

Abbott RealTi*me* HCV with product codes **4J86-90, 4J86-80 and 4J86-70**, manufactured by **Abbott Molecular Inc**, **CE-Marked regulatory version**, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 10 December 2019.

Summary of WHO prequalification assessment for Abbott RealTi*me* HCV

	Date	Outcome
Prequalification listing	10 December 2019	listed
Dossier review	N/A	N/A
Site inspection(s) of quality management system	21 November 2019	MR
Product performance evaluation	Quarter (Q) 3 and Q4 of 2019	MR

MR: Meet Requirements
N/A: Not Applicable

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	The manufacturer has submitted a change request to extend the claim of the Abbott RealTime HCV assay (4J86) to include quantification of hepatitis C viral RNA (HCV RNA) in whole blood spotted on cards as dried blood spots (DBS) (i.e. obtained via venipuncture or capillary blood) from HCV-infected individuals.	16 September 2020
3.0	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 November 2021

Intended use:
According to the claim of intended use from Abbott Molecular Inc*, "Abbott RealTime HCV assay is an in vitro reverse transcription polymerase chain reaction (RT- PCR) assay for the quantitation of hepatitis C viral ribonucleic acid (HCV RNA) in human serum, plasma and whole blood spotted on cards as dried blood spots (DBS) (ie, obtained via venipuncture or capillary blood) from HCV-infected individuals. The Abbott RealTime HCV assay is intended for use as an aid in the management of HCV-infected patients undergoing antiviral therapy. The Abbott RealTime HCV assay is not for screening blood, plasma, serum or tissue donors for HCV, or to be used as a diagnostic test to confirm the presence of HCV infection.*

The intended users for the Abbott RealTime HCV assay are laboratory and healthcare professionals."

Assay description:
According to the claim of assay description from Abbott Molecular Inc, "*Abbott RealTime HCV assay consists of 3 reagent kits:*
• *Abbott RealTime HCV Amplification Reagent Kit*
• *Abbott RealTime HCV Control Kit*
• *Abbott RealTime HCV Calibrator Kit*
The Abbott RealTime HCV assay uses RT-PCR to generate amplified product from the RNA genome of HCV in clinical specimens. An RNA sequence that is unrelated to the HCV target sequence is introduced into each specimen at the beginning of sample preparation. This unrelated RNA sequence is simultaneously amplified by RT-PCR and serves as an internal control (IC) to demonstrate that the process has proceeded correctly for each sample. The amount of HCV target sequence that is present at each amplification cycle is measured through the use of fluorescent-labeled oligonucleotide probes on the Abbott m2000rt instrument. The probes do not generate signal unless they are specifically bound to the amplified product. The amplification cycle at which fluorescent signal is detected by the Abbott m2000rt is proportional to the log of the HCV RNA concentration present in the original sample".

Test kit contents:

Component	**Number of tests and product code**
Abbott RealTi*me* HCV Amplification Reagent Kit	**96 tests (product code 4J86-90)**
Abbott RealTi*me* HCV Internal Control	4 vials x 1.2mL (4J86Y)
Abbott RealTi*me* HCV Amplification Reagent Pack	4 packs X 24 tests/pack (4J86)

Abbott RealTi*me* HCV Control Kit	**product code 4J86-80**
Abbott RealTi*me* HCV Negative Control	8 vials x 1.8mL/ vial (4J86Z)
Abbott RealTi*me* HCV Low Positive Control	8 vials x 1.3ml/vial (4J86W)
Abbott RealTi*me* HCV High Positive Control	8 vials x 1.3mL/vial (4J86X)
Abbott RealTi*me* HCV Calibrator Kit	**Product code 4J86-70**
Abbott RealTi*me* HCV Calibrator A	12 vials x 1.3 mL/vial (4J86A)
Abbott RealTi*me* HCV Calibrator B	12 vials x 1.3 mL/vial (4J86B)

Items required but not provided:

Component	**Product code and description**
Sample preparation area	
Abbott *m*2000sp instrument	9K14-02
Abbott m1000sp instrument	4J72-01
Abbott *m*24sp instrument	3N06-01
Abbott RealTi*me* HCV m2000 ROW System Combined Application CD-ROM	1L69-07 or higher
Abbott m2000sp software with version 6.0 or higher	50-148400 or higher
5 mL Reaction Vessels	General Lab material
Abbott *m*Sample Preparation System	04J70-24
Calibrated precision pipettes	20 µL to 1 000 µL
Aerosol barrier pipette tips	20 µL to 1 000µL
13 to 16 mm sample tubes	13 to 16 mm sample tubes
200 µL disposable tips	04J71-17
1000 µL disposable tips	04J71-17
Vortex mixer	General lab equipment
Abbott Optical Adhesive Covers	04J71-75
Abbott Adhesive Cover Applicator	9K32-01
Abbott Splash-Free Support Base	09K31-01
200 mL Reagent Vessels	4J71-60
Master Mix Tube	04J71-80
Abbott 96-Deep-Well Plate	04J71-30
Abbott 96-Well Optical Reaction Plate	04J71-70
Centrifuge capable of 2000g	General lab equipment
1.4 mL Micro Vial 15 mm Caps (optional)	3N20-01
Amplification area	

Abbott m2000rt	9K15-01
Abbott RealTi*me* HCV m2000 ROW System Combined Application CD-ROM	1L69-07 or higher
Abbott m2000rt Optical Calibration Kit	List No. 4J71-93
Other Materials	
Biological safety cabinet approved for working with infectious materials	General lab equipment
Sealable plastic bags	General lab material
RNase-free water (Eppendorf or equivalent) †	General lab material
1.7 mL molecular biology grade microcentrifuge tubes (Dot Scientific, Inc. or equivalent) †	
Cotton Tip Applicators (Puritan or equivalent) †	General lab material

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

Storage:

- The Abbott RealTi*me* HCV Amplification Reagent Pack and Internal Control vials(4J86-90) must be stored at – 10°C or colder when not in use. Care must be taken to separate the Abbott RealTi*me* HCV Amplification Reagent Pack that is in use from direct contact with samples, calibrators, and controls.
- The Abbott RealTi*me* HCV Negative and Positive Controls (4J86-80) must be stored at – 10°C or colder.
- The Abbott RealTi*me* HCV Calibrator A and Calibrator B (4J86-70) must be stored at – 10°C or colder.

Shipping:

• Abbott RealTi*me* HCV Amplification Reagent Kit: Ship on dry ice.
• Abbott RealTi*me* HCV Control Kit: Ship on dry ice.
• Abbott RealTi*me* HCV Calibrator Kit: Ship on dry ice.

Shelf-life upon manufacture:

18 months.

Warnings/limitations:

Refer to the current instructions for use.

Prioritization for prequalification
Based on the established eligibility criteria, Abbott RealTi*me* HCV was given priority for WHO prequalification assessment.

Product dossier assessment

In accordance with the WHO procedure for abridged prequalification assessment, Abbott Molecular Inc. was not required to submit a product dossier for Abbott RealTi*me* HCV as per the *"Instructions for compilation of a product dossier"* (PQDx_018 version 3). Notwithstanding, certain aspects of the product dossier previously submitted for stringent regulatory review were reviewed by an assessor during the site inspection.

Commitments for prequalification:

Abbott Molecular Inc to include a table of numbers of specimens per analyte tested for potentially interfering substances in the IFU by May 31, 2024.

Manufacturing site inspection

In accordance with the WHO procedure for abridged prequalification assessment, a desk assessment was performed in lieu of an onsite inspection. The desk assessment covered the site(s) of manufacture of Abbott RealTi*me* HCV, as per the "*Information for manufacturers on prequalification inspection procedures for the sites of manufacture of diagnostics*" (PQDx_014 version 4).

The desk assessment found that the manufacturer had an acceptable quality management system and good manufacturing practices in place that ensured the consistent manufacture of a product of good quality.

The desk assessment was found to be acceptable on 21 November 2019 and that the quality management system for Abbott RealTi*me* HCV, meets WHO prequalification requirements.

Product performance evaluation

The Abbott RealTi*m*e HCV assay is an in vitro reverse transcription polymerase chain reaction (RT-PCR) assay for the quantitation of hepatitis C viral ribonucleic acid (HCV RNA) in human serum and EDTA/ACD plasma from HCV-infected individuals. The Abbott RealTi*me* HCV assay is intended for use as an aid in the management of HCV-infected patients undergoing antiviral therapy. A volume of 0.2 mL or 0.5 mL of specimen is used to perform the assay (however, at least 0.7 – 1.3 mL of specimen should be available for use with the *m*1000 and *m*2000*sp* systems). This type of assay requires laboratory equipment and cannot be performed in laboratories with limited facilities.

The Abbott RealTi*m*e HCV assay was evaluated at the National Serology Reference Laboratory (NRL), Melbourne, Australia, on behalf of WHO in Q3 and Q4 2019. From this evaluation, we drew the following conclusions.

Analytical evaluation

The precision (intra- and inter-assay) of measurement was found to be acceptable. At 10^2 IU/mL, CV% were <8% and at 10^4 IU/mL, CV% were < 3%.

The limit of detection of the assay was estimated at 31 IU/mL (95% CI: 11-52 IU/mL), which is consistent with the claim (30 IU/mL with the 0.2 mL sample preparation procedure).

There was no cross-contamination when high positive and negative specimens were ran together.

All 6 HCV genotypes included in the 4th HCV RNA Genotype Panel for Nucleic Acid Amplification (NIBSC reference: 14/209) and in the NRL HCV Mixed Genotype Panel were detected by the assay.

Clinical evaluation

In this limited performance evaluation on a panel of 197 ACD plasma specimens, using a sample volume of 0.2 mL, we found a bias of -0.05 $\log_{10}$ IU/ mL (limits of agreement: -0.42 to 0.32 $\log_{10}$ IU/mL) compared to the reference results using the cobas HCV quantitative nucleic acid test with cobas 6800 System (Roche Diagnostics GmbH). The sensitivity (N=99) was 100% (95% CI: 96.3% – 100%) and specificity (N=98) was 100% (95% CI: 96.2% - 100%) compared to the reference results.

In this study, the invalid rate was 0%.

Labelling

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

1. Labels

1.1 Abbott RealTi*me* HCV Amplification Reagent Kit box label (4J86-90)

Abbott RealTi*me* HCV

Amplification Reagent Kit

(4x24 Tests)

(en) For *In Vitro* Diagnostic Use. The Abbott RealTi*me* HCV assay is an *in vitro* reverse transcription-polymerase chain reaction (RT-PCR) assay for the quantitation of hepatitis C viral ribonucleic acid (HCV RNA) in human serum and plasma from HCV-infected individuals. The Abbott RealTi*me* HCV assay is intended for use as an aid in the management of HCV-infected patients undergoing antiviral therapy. The Abbott RealTi*me* HCV assay is not for screening blood, plasma, serum or tissue donors for HCV, or to be used as a diagnostic test to confirm the presence of HCV infection.

Contents:

1. **INTERNAL CONTROL** Abbott RealTi*me* HCV Internal Control (4 vials, 1.2 mL per vial). <0.01% noninfectious Armored RNA® with internal control sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HBV DNA, HCV RNA, HIV RNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin® 300 and 0.15% ProClin 950.
2. **AMPLIFICATION REAGENT PACK** Abbott RealTi*me* HCV 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) HCV Oligonucleotide Reagent. <0.1% synthetic oligonucleotides (4 primers and 2 probes), 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 RealTi*me* is a trademark of Abbott Laboratories.

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

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

REF 4J86-90

IVD

CE 1434

−10°C

51-602125/R5

GTIN

LOT

REF

(de) In-vitro-Diagnostikum. Der Abbott RealTi*me* HCV Assay ist ein in-vitro-Reverse-Transkription-Polymerasekettenreaktions-Assay (RT-PCR) zur quantitativen Bestimmung von Hepatitis-C-viraler Ribonukleinsäure (HCV RNA) in Humanserum und -plasma von HCV-infizierten Personen. Der Abbott RealTi*me* HCV Assay ist als Hilfsmittel bei der Behandlung von HCV-infizierten Patienten unter antiviraler Therapie. Der Abbott RealTi*me* HCV Assay darf nicht als Mittel zum Screening von Blut-, Plasma-, Serum- oder Gewebespendern des HCV oder zur Verwendung als diagnostischer Test zur Bestätigung einer HCV-Infektion vorgesehen.

Inhalt:

1. **INTERNAL CONTROL** Abbott RealTi*me* HCV Interne Kontrolle (4 Fläschchen, 1,2 ml pro Fläschchen). <0,01 % nicht infektiöse quantitativen de l'acide ribonucléique en virus de l'hépatite C (ARN VHC) dans le sérum ou le plasma d'individus infectés par le VHC. Le dosage Abbott RealTi*me* HCV a été conçu pour détecter et quantifier les génotypes 1 à 6 du VHC. Ce dosage Abbott RealTi*me* HCV n'est pas destiné au dépistage du VHC dans des échantillons sanguins, plasmatiques, sériques ou tissulaires de donneurs, ni à la confirmation du diagnostic d'une infection par le VHC.

Contenu :

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

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(fr) Pour usage de diagnostic *in vitro*. Le dosage Abbott RealTi*me* HCV est un dosage *in vitro* de réaction en chaîne de la polymérase avec transcription inverse (RT-PCR) pour la quantification des acides ribonucléiques viraux de l'hépatite C (ARN du VHC) en sérum et plasma humains de sujets infectés par le VHC. Le dosage Abbott RealTi*me* HCV est destiné à être utilisé dans le cadre de la thérapie antivirale des patients infectés par le VHC. Il ne doit pas être utilisé pour le dépistage du VHC dans des échantillons sanguins, plasmatiques, sériques ou tissulaires de donneurs, ni à la confirmation du diagnostic d'une infection par le VHC.

Contenu :

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

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(es) Para uso en diagnóstico *in vitro*. Abbott RealTi*me* HCV es un ensayo *in vitro* de reacción en cadena de la polimerasa previa transcripción inversa (RT-PCR) para la cuantificación del ácido ribonucleico viral de la hepatitis C (RNA del VHC) en suero y plasma humanos de individuos infectados por el VHC. El ensayo Abbott RealTi*me* HCV se ha ideado para ayuda en la gestión de la terapia antiviral en pacientes infectados con el VHC. El ensayo Abbott RealTi*me* HCV no debe utilizarse como análisis de cribado de VHC en donaciones de sangre, plasma, suero o tejidos ni como análisis de diagnóstico para la confirmación de la infección por VHC.

Contenido:

1. **INTERNAL CONTROL** Control interno Abbott RealTi*me* HCV (4 tubos de 1,2 ml cada uno). <0,01% Armored RNA™ no infeccioso con secuencias de control interno en plasma humano negativo. El plasma humano negativo se ha analizado y no se ha encontrado reactividad para el HBsAg, el ADN del VHB, el ARN del VHC, el ARN del VIH, 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** Envase de reactivo de amplificación Abbott RealTi*me* HCV (4 envases de 24 análisis cada uno). Cada envase de reactivo contiene:
   - 1 frasco (0,141 ml) de Thermostable rTth Polymerase Enzyme (enzima polimerasa termoestable rTth) (2,9 a 3,5 unidades/µl) en solución tamponada.
   - 1 frasco (1,1 ml) de HCV Oligonucleotide Reagent (reactivo de oligonucleótidos del VHC). <0,1% síntesis oligonucleótidos (oligonucleótidos sintéticos) (4 cebadores y 2 sondas) y <0,3% dNTPs en una 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 Activation Reagent (reactivo de activación). 30 mM de solución de cloruro de manganeso. 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 RealTi*me* es una marca comercial de Abbott Laboratories.

(it) Per uso diagnostico *in vitro*. Il dosaggio Abbott RealTi*me* HCV è un dosaggio *in vitro* basato sul principio della RT-PCR (trascrizione inversa-reazione a catena della polimerasi) per la determinazione quantitativa dell'acido ribonucleico del virus dell'epatite C (HCV RNA) in campioni di siero e plasma umani prelevati da soggetti con infezione da HCV. Il dosaggio Abbott RealTi*me* HCV è utilizzato come analisi nella gestione da parente di una terapia antivirale di pazienti con infezione da HCV. Il dosaggio Abbott RealTi*me* HCV non deve essere utilizzato come test di screening del sangue, del plasma, del siero o dei tessuti donati per il virus dell'epatite C o come test diagnostico per confermare la presenza dell'infezione da HCV.

Contenuto:

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

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Armored RNA è un marchio commerciale registrato di Ambion.
Abbott RealTi*me* è un marchio commerciale di Abbott Laboratories.

Abbott RealTi*me* HCV

Amplification Reagent Kit

(4x24 Tests)

(pt) Para utilização *in vitro*. O ensaio Abbott RealTi*me* HCV é um ensaio, para utilização *in vitro*, de transcriptase reversa associada à reacção em cadeia da polimerase (RT-PCR) para a quantificação do ácido ribonucleico viral da hepatite C (ARN do HCV) em soro e plasma humanos de indivíduos infectados pelo HCV. O ensaio Abbott RealTi*me* HCV destina-se a ser utilizado como meio auxiliar no tratamento de doentes infectados pelo HCV a receber terapêutica anti-viral. O ensaio Abbott RealTi*me* HCV não se destina a ser utilizado para o rastreio do HCV em amostras de sangue, plasma, soro ou tecido de dadores, nem como teste de diagnóstico confirmatório da presença de infecção pelo HCV.

Conteúdo:

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

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Armored RNA é uma marca comercial registada de Ambion.
Abbott RealTi*me* é uma marca comercial de Abbott Laboratories.

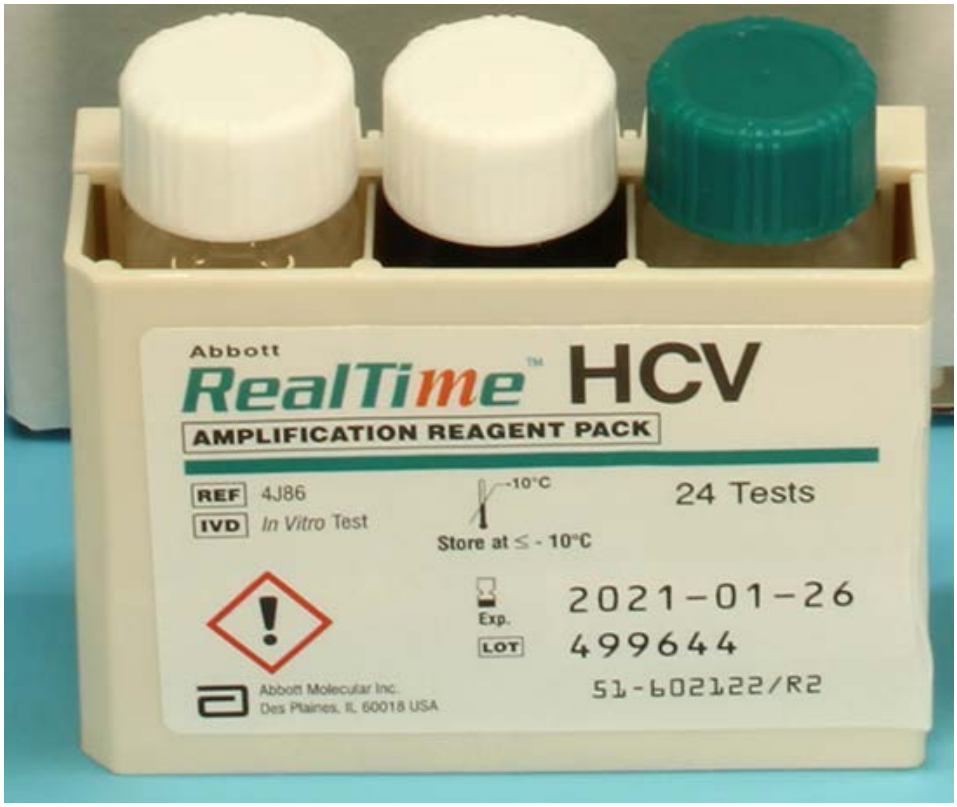
Product of USA / Produkt aus USA / Produit des Etats-Unis / Producto de EE.UU. / Prodotto negli USA / Fabricado nos EUA

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

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

Abbott

1.1.1 Amplification reagent label



1.2 Abbott RealT*ime* HCV Control Kit box label (4J86-80)

Abbott RealTi*me* HCV

Control Kit

(en) For *In Vitro* Diagnostic Use. The Abbott RealTi*me* HCV Controls are used to establish run validity of the Abbott RealTi*me* HCV assay when used for the quantitation of hepatitis C virus (HCV) RNA in human serum and plasma from HCV infected individuals.

Contents:

1. **CONTROL –** Abbott RealTi*me* HCV Negative Control (8 vials, 1.8 mL per vial). Negative human plasma tested and found to be nonreactive for HBsAg, HBV DNA, HCV RNA, HIV RNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin® 300 and 0.15% ProClin 950.
2. **CONTROL L** Abbott RealTi*me* HCV Low Positive Control (8 vials, 1.3 mL per vial). Noninfectious Armored RNA® with HCV sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HBV DNA, HCV RNA, HIV RNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.
3. **CONTROL H** Abbott RealTi*me* HCV High Positive Control (8 vials, 1.3 mL per vial). Noninfectious Armored RNA with HCV sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HBV DNA, HCV RNA, HIV RNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

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Armored RNA is a trademark of Ambion.

Abbott RealTi*me* is a trademark of Abbott Laboratories.

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

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

REF 4J86-80

IVD

1434

-10°C

51-602120/R5

GTIN

LOT

REF

(de) In-vitro-Diagnostikum. Die Abbott RealTime HCV Kontrollen dienen zur Herstellung der Testgültigkeit des Abbott RealTime HCV Assays bei der quantitativen Bestimmung von Hepatitis-C-Virus Ribonukleinsäure (HCV RNA) in Humanserum und -plasma von HCV-infizierten Personen.

Inhalt:

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

ProClin ist ein eingetragenes Warenzeichen von Rohm und Haas.

Armored RNA ist ein Warenzeichen von Ambion.

Abbott RealTime ist ein Warenzeichen von Abbott Laboratories.

(fr) Pour diagnostic *in vitro*. Les contrôles Abbott RealTime HCV sont utilisés pour établir la viabilité des analyses effectuées avec le dosage Abbott RealTime HCV lors de la mesure quantitative de l'ARN du virus de l'hépatite C (VHC) dans le sérum ou le plasma d'individus infectés par le VHC.

Contenu :

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

ProClin est une marque déposée de Rohm and Haas.

Armored RNA est une marque commerciale d'Ambion.

Abbott RealTime est une marque commerciale de Abbott Laboratories.

(es) Para uso en diagnóstico *in vitro*. Los controles Abbott RealTime HCV se usan para establecer la validez del procesamiento del ensayo Abbott RealTime HCV en la determinación cuantitativa del RNA del virus de la hepatitis C (VHC) en plasma y suero humano de individuos infectados con el VHC.

Contenido:

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

Abbott RealTime es una marca comercial de Abbott Laboratories.

(pt) Para utilização *in vitro*. Os Controlos Abbott RealTime HCV destinam-se a estabelecer a validade do processamento do ensaio Abbott RealTime HCV, quando utilizado para a quantificação do ARN de vírus da hepatite C (HCV) em soro e plasma humanos de indivíduos infectados com HCV.

Conteúdo:

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

ProClin é uma marca comercial registada de Rohm and Haas.

Armored RNA é uma marca comercial de Ambion.

Abbott RealTime é uma marca comercial de Abbott Laboratories.

Abbott RealTi*me* HCV

Control Kit

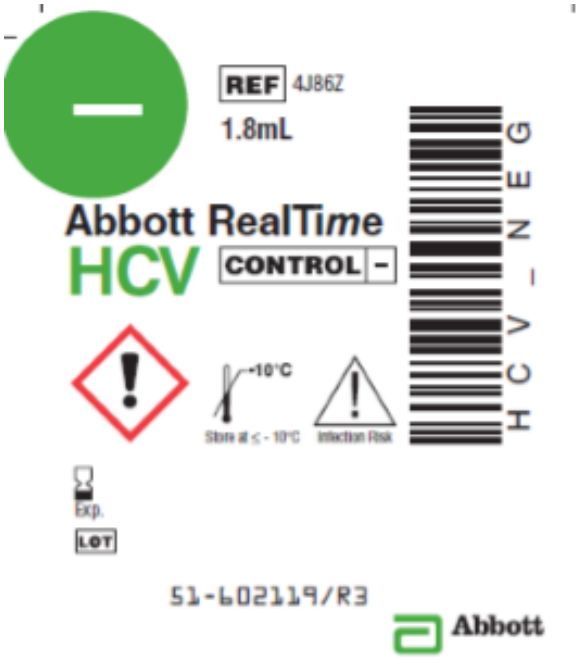
Product of USA / Produkt aus USA / Produit des Etats-Unis / Producto de EE.UU. / Product of USA / Prodotto in USA / Fabricado nos EUA

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

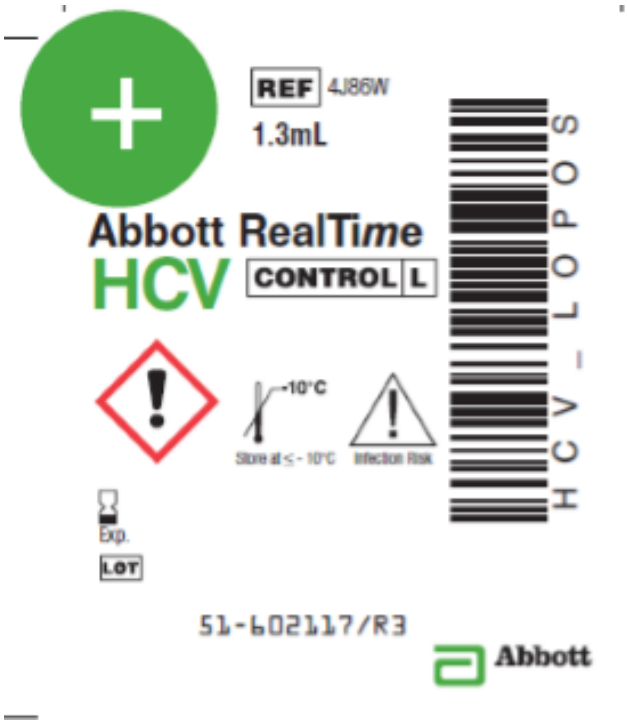
EC REP Abbott GmbH
Max-Planck-Ring 2
65205 Wiesbaden, Germany

Abbott

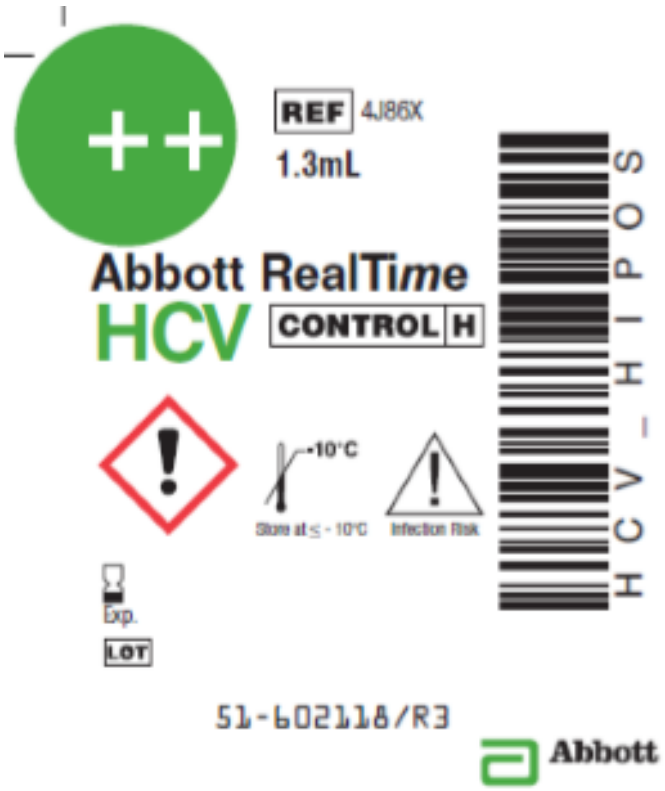
1.2.1 Abbott RealTi*me* HCV Negative Control vial Label (4J86Z)



1.2.2 Abbott RealTi*me* HCV Low Positive Control vial Label (4J86W)



1.2.3 Abbott RealTi*me* HCV High Positive Control vial Label (4J86X)



1.3 Abbott RealTi*me* HCV Calibrator Kit box label (4J86-70)

(de) In-vitro-Diagnostikum. Die Abbott RealTime HCV Kalibratoren dienen zur Kalibrierung des Abbott RealTime HCV Assays bei der quantitativen Bestimmung von Hepatitis-C-viraler Ribonukleinsäure (HCV RNA) in Humanserum und -plasma von HCV-infizierten Personen.
Inhalt:
1. CAL A Abbott RealTime HCV Kalibrator A (12 Fläschchen, 1,3 ml pro Fläschchen). Nicht infektiöses Armored RNA® mit HCV-Sequenzen in negativem humanplasma. Negatives humanplasma wurde getestet und war nicht reaktiv für HBsAg, HBV DNA, HCV RNA, HIV RNA, anti-HIV-1/HIV-2 und anti-HCV. Konservierungsmittel: 0,1 % ProClin® 300 und 0,15 % ProClin 950.
2. CAL B Abbott RealTime HCV Kalibrator B (12 Fläschchen, 1,3 ml pro Fläschchen). Nicht infektiöse Armored RNA mit HCV-sequenzen in negativem Humanplasma. Negatives Humanplasma wurde getestet und war nicht reaktiv für HBsAg, HBV DNA, HCV RNA, HIV RNA, anti-HIV-1/HIV-2 und anti-HCV. Konservierungsmittel: 0,1 % ProClin 300 und 0,15 % ProClin 950.

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Armored RNA ist ein eingetragenes Warenzeichen von Ambion.
Abbott RealTime ist ein Warenzeichen von Abbott Laboratories.

(fr) Pour diagnostic *in vitro*. Les calibrateurs Abbott RealTime HCV sont utilisés pour la calibration du dosage Abbott RealTime HCV lors de la mesure quantitative de l'ARN du virus de l'hépatite C (HCV) dans du sérum ou du plasma d'individus infectés par le VHC.
Contenu :
1. CAL A Calibrateur A Abbott RealTime HCV (12 flacons de 1,3 ml chacun). Armored RNA® non infectieux comprenant des séquences de VHC dans du plasma humain négatif. Le plasma humain négatif a été testé et trouvé non réactif pour l'AgHBs, l'ADN VHB, l'ARN VHC, l'ARN VIH et pour les anticorps anti-VIH-1/VIH-2 et anti-VHC. Conservateurs : ProClin® 300 à 0,1 % et ProClin 950 à 0,15 %.
2. CAL B Calibrateur B Abbott RealTime HCV (12 flacons de 1,3 ml chacun). Armored RNA non infectieux comprenant des séquences de VHC dans du plasma humain négatif. Le plasma humain négatif a été testé et trouvé non réactif pour l'AgHBs, l'ADN VHB, l'ARN VHC, l'ARN VIH et pour les anticorps anti-VIH-1/VIH-2 et anti-VHC. Conservateurs : ProClin 300 à 0,1 % et ProClin 950 à 0,15 %.

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

(es) Para uso en diagnóstico *in vitro*. Los calibradores Abbott RealTime HCV se usan para la calibración del ensayo Abbott RealTime HCV en la determinación cuantitativa del RNA del virus de la hepatitis C (VHC) en suero y plasma humano de individuos infectados con el VHC.
Contenido:
1. CAL A Calibrador A Abbott RealTime HCV (12 tubos de 1,3 ml cada uno). Armored RNA® no infeccioso con secuencias de VHC en plasma humano negativo. El plasma humano negativo se ha analizado y no se ha reconocido reactividad para el HBsAg, ni para el DNA del VHB, ni el RNA del VHC, ni el RNA del VIH, 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. CAL B Calibrador B Abbott RealTime HCV (12 tubos de 1,3 ml cada uno). Armored RNA no infeccioso con secuencias de VHC en plasma humano negativo. El plasma humano negativo se ha analizado y no se ha reconocido reactividad para el HBsAg, ni para el DNA del VHB, ni el RNA del VHC ni el RNA del VIH, ni reactividad de anticuerpos anti-VIH-1/VIH-2 ni anti-VHC. 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) Per uso diagnostico *in vitro*. I calibratori Abbott RealTime HCV vengono utilizzati per la calibrazione del dosaggio Abbott RealTime HCV per la determinazione quantitativa dell'RNA del virus dell'epatite C (HCV) in campioni di siero e plasma umani prelevati da soggetti con infezione da HCV.
Contenuto:
1. CAL A Calibratore A Abbott RealTime HCV (12 provette, 1,3 ml per provetta). Armored RNA® non infettivo con sequenze di HCV in plasma umano negativo. Plasma umano negativo analizzato e risultato non reattivo per l'HBsAg, l'HBV DNA, l'HCV RNA, l'HIV RNA e per gli anticorpi anti-HIV-1/HIV-2 e anti-HCV. Conservanti: ProClin® 300 allo 0,1% e ProClin 950 allo 0,15% .
2. CAL B Calibratore B Abbott RealTime HCV (12 provette, 1,3 ml per provetta). Armored RNA non infettivo con sequenze di HCV in plasma umano negativo. Plasma umano negativo analizzato e risultato non reattivo per l'HBsAg, l'HBV DNA, l'HCV RNA, l'HIV RNA e per gli anticorpi anti-HIV-1/HIV-2 e anti-HCV. Conservanti: ProClin 300 allo 0,1% e ProClin 950 allo 0,15%.

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

Abbott RealTime HCV

Calibrator Kit

(en) For *In Vitro* Diagnostic Use. The Abbott RealTime HCV Calibrators are for calibration of the Abbott RealTime HCV assay when used for the quantitative determination of hepatitis C virus (HCV) RNA in human serum and plasma from HCV infected individuals.
Contents:
1. CAL A Abbott RealTime HCV Calibrator A (12 vials, 1.3 mL per vial). Noninfectious Armored RNA® with HCV sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HBV DNA, HCV RNA, HIV RNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin® 300 and 0.15% ProClin 950.
2. CAL B Abbott RealTime HCV Calibrator B (12 vials, 1.3 mL per vial). Noninfectious Armored RNA with HCV sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HBV DNA, HCV RNA, HIV RNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

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

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

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

REF 4J86-70

IVD

CE 1434

−10°C

51-602115/R5

GTIN

LOT

REF

(pt) Para utilização *in vitro*. Os Abbott RealTime HCV Calibrators destinam-se à calibração do ensaio Abbott RealTime HCV, quando utilizado para a determinação quantitativa do ARN do vírus da hepatite C (HCV) em soro e plasma humanos de indivíduos infectados pelo HCV.
Conteúdo:
1. CAL A Abbott RealTime HCV Calibrator A (12 frascos, 1,3 ml por frasco). Armored RNA® (ARN protegido) não-infeccioso com sequências de HCV em plasma humano negativo. Plasma humano negativo testado e considerado não-reactivo para HBsAg, ADN do HBV, ARN do HCV, ARN do HIV, anticorpos anti-HIV-1/HIV-2 e anti-HCV. Conservantes: 0,1% de ProClin® 300 e 0,15% de ProClin 950.
2. CAL B Abbott RealTime HCV Calibrator B (12 frascos, 1,3 ml por frasco). ARN protegido não-infeccioso com sequências de HCV em plasma humano negativo. Plasma humano negativo testado e considerado não-reactivo para HBsAg, ADN do HBV, ARN do HCV, ARN do HIV, anticorpos anti-HIV-1/HIV-2 e anti-HCV. Conservantes: 0,1% de ProClin 300 e 0,15% de ProClin 950.

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

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

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1300 East Touhy Avenue
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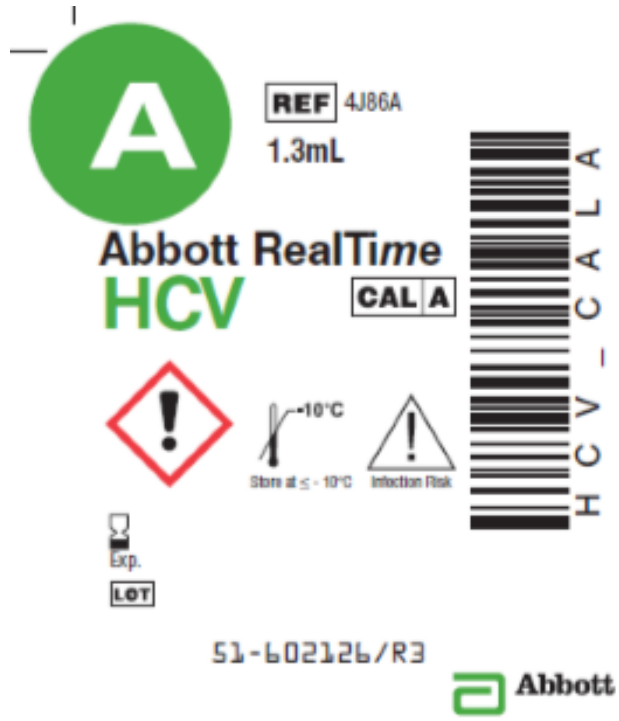
EC REP Abbott GmbH
Max-Planck-Ring 2
65205 Wiesbaden, Germany

Abbott

1.3.1 Abbott RealT*ime* HCV CAL A vial label (4J86A)



1.3.2 Abbott RealT*ime* HCV CAL B vial label (4J86B)

2. Instructions for use[1]

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

Abbott RealTi*me*

en

IN VITRO TEST

REF 4J86-70

51-602116/R6

HCV Calibrators

NOTE: Changes Highlighted

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

Notice to User

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

Intended Use

The Abbott RealTi*me* HCV Calibrators are for calibration of the Abbott RealTi*me* HCV assay when used for the quantitative determination of Hepatitis C Virus (HCV) RNA in human serum and plasma from HCV infected individuals.

Intended User

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

Contents

1. CAL A **Abbott RealTi*me* HCV Calibrator A (List No. 4J86A) (12 vials, 1.3 mL per vial)**. Noninfectious Armored RNA® with HCV sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HBV DNA, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin® 300 and 0.15% ProClin 950.
2. CAL B **Abbott RealTi*me* HCV Calibrator B (List No. 4J86B) (12 vials, 1.3 mL per vial).** Noninfectious Armored RNA with HCV sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HBV DNA, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

- Calibrator concentrations are specified in each Abbott RealTi*me* HCV Calibrator Kit Card.
- The Abbott RealTi*me* HCV Calibrator Kit must only be used with the Abbott RealTi*me* HCV assay (List No. 4J86-90).

Standardization

Abbott manufactures internal reference standards for the Abbott RealTi*me* HCV assay. These internal standards are referenced to the Second WHO International Standard for Hepatitis C Virus RNA (NIBSC Code 96/798)[1] at each concentration level. The Abbott RealTi*me* HCV calibrators are manufactured against these internal standards.

Precautions

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

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

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

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

Components of the Abbott RealTi*me* HCV Calibrator Kit (List No. 4J86-70) contain the following components:

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

The following warnings apply:

Warning

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


Consult instructions for use

Store at ≤ -10°C

Shipping Conditions

Ship on dry ice.

BIBLIOGRAPHY

1. Saldanha J, Lelie N, Heath A. Establishment of the first international standard for nucleic acid amplification technology (NAT) assays for HCV RNA. *Vox Sang.* 1999;76:149-58.
2. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; December 2009. [Also available online. *Type*> www.cdc.gov, *search*>BMBL5>*look up* sections III and IV.]
3. US Department of Labor, Occupational Safety and Health Administration. 29 CFR Part 1910.1030. *Bloodborne Pathogens*.
4. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline—Third Edition*. CLSI Document M29-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
5. World Health Organization. *Laboratory Biosafety Manual*. 3rd ed. Geneva, Switzerland: World Health Organization; 2004.

Technical Assistance

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

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


Abbott Molecular Inc.
Des Plaines, IL 60018 USA

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

www.abbottmolecular.com

June 2020

Abbott

Abbott RealTi*me*

en

IN VITRO TEST

REF 4J86-80

51-602121/R6

HCV Controls

NOTE: Changes Highlighted

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

Notice to User

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

Intended Use

The Abbott RealTi*me* HCV Controls are used to establish run validity of the Abbott RealTi*me* HCV assay when used for the quantitation of Hepatitis C Virus (HCV) RNA in human serum and plasma from HCV infected individuals.

Intended User

The intended users for the Abbott RealTi*me* HCV Controls are laboratory and healthcare professionals.

Contents

1. CONTROL – **Abbott RealTi*me* HCV Negative Control (List No. 4J86Z) (8 vials, 1.8 mL per vial).** Negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HBV DNA, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin® 300 and 0.15% ProClin 950.
2. CONTROL L **Abbott RealTi*me* HCV Low Positive Control (List No. 4J86W) (8 vials, 1.3 mL per vial)**. Noninfectious Armored RNA® with HCV sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HBV DNA, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.
3. CONTROL H **Abbott RealTi*me* HCV High Positive Control (List No. 4J86X) (8 vials, 1.3 mL per vial)**. Noninfectious Armored RNA with HCV sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HBV DNA, HIV RNA, HCV RNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

- Control concentrations are specified in each Abbott RealTi*me* HCV Control Kit Card.
- The Abbott RealTi*me* HCV Control Kit must only be used with the Abbott RealTi*me* HCV assay (List No. 4J86-90).

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* HCV Control Kit (List No. 4J86-80) contain the following components:

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

The following warnings apply:

Warning

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


Consult instructions for use

Store at ≤ -10°C

Shipping Conditions

Ship on dry ice.

BIBLIOGRAPHY

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

Technical Assistance

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

The Abbott RealTi*m*e HCV Controls Kit is imported into the European Union by Abbott Diagnostics GmbH, located at Max-Planck-Ring 2, 65205 Wiesbaden, Germany.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Des Plaines, IL 60018 USA

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

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June 2020

Abbott

Abbott RealTi*me* HCV

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REF 4J86

51-602124/R12

REF 4J86

51-602124/R12

NOTE: Changes Highlighted

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

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

NOTICE TO USER

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

CUSTOMER SERVICE

INTERNATIONAL: CALL YOUR ABBOTT REPRESENTATIVE

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

NAME

Abbott RealTi*me* HCV

INTENDED USE

The Abbott RealTi*me* HCV assay is an in vitro reverse transcription-polymerase chain reaction (RT-PCR) assay for the quantitation of hepatitis C viral ribonucleic acid (HCV RNA) in human serum and plasma from HCV-infected individuals. The Abbott RealTi*me* HCV assay is intended for use as an aid in the management of HCV-infected patients undergoing antiviral therapy. The Abbott RealTi*me* HCV assay is not for screening blood, plasma, serum or tissue donors for HCV, or to be used as a diagnostic test to confirm the presence of HCV infection.

INTENDED USER

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

SUMMARY AND EXPLANATION OF THE TEST

HCV is a single-stranded RNA virus, with a genome of 9,500 nucleotides.[1] HCV has been identified as the major etiological agent for post-transfusion non-A, non-B hepatitis worldwide.[2,3] HCV has been transmitted primarily through intravenous drug use and through blood products. Sensitive serological tests for HCV antibodies have greatly reduced the incidence of new infections from donated blood. About 85% of HCV-infected individuals develop chronic hepatitis, with up to 20% of chronically infected individuals developing cirrhosis. In patients with cirrhosis, the incidence of hepatocellular carcinoma is 1-4% per year.[4,5]

Quantitation of HCV RNA has been instrumental in understanding the effectiveness of antiviral response to interferon monotherapy, interferon plus ribavirin combination therapy, and peginterferon plus ribavirin combination therapy.[6-10] Current guidelines for the management and treatment of HCV recommend quantitative testing for HCV RNA before the start of antiviral therapy, during therapy, and after the conclusion of treatment. The objective of treatment is a sustained virological response (SVR), defined as the absence of HCV RNA detectable by a sensitive test after the end of treatment. SVR is almost always preceded by an early virological response (EVR), defined as a 2-log or greater decrease in HCV viral load after 12 weeks of therapy.[4,5,11]

HCV RNA in serum or plasma can be quantitated using nucleic acid amplification or signal amplification technologies.[12-14] The Abbott RealTi*me* HCV assay uses RT-PCR technology combined with homogeneous real time fluorescent detection for the quantitation of HCV RNA. The selection of a conserved region of the HCV genome provides for the detection of genotypes 1 through 6. The assay is standardized against the Second WHO International Standard for Hepatitis C Virus RNA (NIBSC Code 96/798)[15] and results are reported in international units/mL (IU/mL).

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The Abbott RealTi*me* HCV assay consists of 3 reagent kits:

- Abbott RealTi*me* HCV Amplification Reagent Kit
- Abbott RealTi*me* HCV Control Kit
- Abbott RealTi*me* HCV Calibrator Kit

The Abbott RealTi*me* HCV assay uses RT-PCR[16] to generate amplified product from the RNA genome of HCV in clinical specimens. An RNA sequence that is unrelated to the HCV target sequence is introduced into each specimen at the beginning of sample preparation. This unrelated RNA sequence is simultaneously amplified by RT-PCR and

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

Sample Preparation

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

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

Two automated instrument systems, the Abbott *m*2000*sp* or the Abbott *m*1000 System can be used to prepare samples for the Abbott RealTi*m*e HCV assay. The Abbott *m*2000*sp* provides automated sample eluate transfer and reaction assembly in the Abbott 96-Well Optical Reaction Plate, whereas the Abbott *m*1000 System requires manual sample eluate transfer and reaction assembly. Alternatively, samples can be prepared manually using the Abbott *m*Sample Preparation System followed by manual reaction assembly.

Reagent Preparation and Reaction Plate Assembly

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

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

Amplification

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

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

The target sequence for the Abbott RealTi*m*e HCV assay is in the 5´UTR region of the HCV genome. This region is specific for HCV and is highly conserved.[17] The primers are designed to hybridize to the 5´UTR region with the fewest possible mismatches among HCV genotypes 1 through 6.

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

Detection

During the read cycles of amplification on the Abbott *m*2000*rt*, the temperature is lowered further to allow fluorescent detection of amplification products as the HCV and IC probes anneal to their targets (real-time fluorescence detection). The HCV and IC probes are single-stranded DNA oligonucleotides consisting of a probe sequence with a fluorescent moiety that is covalently linked to the 5´ end of the probe and a quenching moiety that is covalently linked to the 3´ end of the probe.

In the absence of the HCV or IC target sequences, probe fluorescence is quenched. In the presence of HCV or IC target sequences, probe hybridization to complementary sequences separates the fluorophore and the quencher and allows fluorescent emission and detection. The HCV and IC probes are each labeled with a different fluorophore, thus allowing for simultaneous detection of both amplified products at each cycle. The amplification cycle at which fluorescent signal is detected by the Abbott *m*2000*rt* is proportional to the log of the HCV RNA concentration present in the original sample.

PREVENTION OF NUCLEIC ACID CONTAMINATION

The possibility of nucleic acid contamination is minimized because:

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

REAGENTS

Abbott RealTi*m*e HCV Amplification Reagent Kit (List No. 4J86-90)

1. **INTERNAL CONTROL** Abbott RealTi*m*e HCV Internal Control (List No. 4J86Y) (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, HBV DNA, HCV RNA, HIV RNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin® 300 and 0.15% ProClin 950.
2. **AMPLIFICATION REAGENT PACK** Abbott RealTi*m*e HCV Amplification Reagent Pack (List No. 4J86) (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). HCV Oligonucleotide Reagent. < 0.1% synthetic oligonucleotides (4 primers and 2 probes) 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 HCV Control Kit (List No. 4J86-80)

1. **CONTROL −** Abbott RealTi*m*e HCV Negative Control (List No. 4J86Z)(8 vials, 1.8 mL per vial)
   Negative human plasma tested and found to be nonreactive for HBsAg, HBV DNA, HCV RNA, HIV RNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.
2. **CONTROL L** Abbott RealTi*m*e HCV Low Positive Control (List No. 4J86W) (8 vials, 1.3 mL per vial)
   Noninfectious armored RNA with HCV sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HBV DNA, HCV RNA, HIV RNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.
3. **CONTROL H** Abbott RealTi*m*e HCV High Positive Control (List No. 4J86X) (8 vials, 1.3 mL per vial)
   Noninfectious armored RNA with HCV sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HBV DNA, HCV RNA, HIV RNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

Abbott RealTi*m*e HCV Calibrator Kit (List No. 4J86-70)

1. **CAL A** Abbott RealTi*m*e HCV Calibrator A (List No. 4J86A) (12 vials, 1.3 mL per vial)
   Noninfectious armored RNA with HCV sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HBV DNA, HCV RNA, HIV RNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.
2. **CAL B** Abbott RealTi*m*e HCV Calibrator B (List No. 4J86B)(12 vials, 1.3 mL per vial)
   Noninfectious armored RNA with HCV sequences in negative human plasma. Negative human plasma tested and found to be nonreactive for HBsAg, HBV DNA, HCV RNA, HIV RNA, anti-HIV-1/HIV-2, and anti-HCV. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

WARNINGS AND PRECAUTIONS

IVD

For In Vitro Diagnostic Use

The Abbott RealTi*m*e HCV assay is not for screening blood, plasma, serum or tissue donors for HCV, or to be used as a diagnostic test to confirm the presence of HCV infection.

Safety Precautions

Refer to the Abbott *m*1000 Operating Manual, Safety Section, the Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals, Hazard Section, or the Manual Sample Preparation for Abbott RealTi*m*e RNA Assays, Handling Precaution 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,[18] OSHA Standards on Bloodborne Pathogens,[19] CLSI Document M29-A3,[20] and other appropriate biosafety practices.[21] 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.[18]
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and federal regulations.[21]

Components of the Abbott RealTi*m*e HCV Calibrator Kit (List No. 4J86-70), the Abbott RealTi*m*e HCV Control Kit (List No. 4J86-80), and the Abbott RealTi*m*e HCV Amplification Reagent Kit (List No. 4J86-90) 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.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves/protective clothing/ eye protection.
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
P362+P364	Take off contaminated clothing and wash before reuse.
P501	Dispose of contents / container in accordance with local regulations.

SPECIAL PRECAUTIONS

Handling Precautions

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

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

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

Work Areas

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

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

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

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

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

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

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

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

Decontaminate and dispose of all potentially biohazardous materials in accordance with local, state, and federal regulations.[21] All materials should be handled in a manner that minimizes the chance of potential contamination of the work area.

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

Aerosol Containment

To reduce the risk of nucleic acid contamination due to aerosols formed during manual pipetting, aerosol barrier pipette tips must be used for

all manual pipetting. The pipette tips must be used only 1 time. Clean and disinfect spills of specimens and reagents as stated in the Abbott *m*1000 Operating Manual or the Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals.

Contamination and Inhibition

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

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

STORAGE INSTRUCTIONS

Abbott RealTi*m*e HCV Amplification Reagent Kit (List No. 4J86-90).

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

Abbott RealTi*m*e HCV Control Kit (List No. 4J86-80).

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

Abbott RealTi*m*e HCV Calibrator Kit (List No. 4J86-70).

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

SHIPPING CONDITIONS

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

INDICATION OF INSTABILITY OR DETERIORATION OF REAGENTS

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

INSTRUMENT PROCEDURE

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

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

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE

Specimen Collection and Storage

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

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

Separate serum or plasma from cells by centrifugation.

After centrifugation, serum or plasma may be removed from cells. Serum or plasma specimens may be stored:

- at 15 to 30°C for up to 24 hours
- at 2 to 8°C for up to 3 days
- at −70°C or colder for longer term[22-25]

Multiple freeze/thaw cycles should be avoided. If frozen, thaw specimens at 15 to 30°C or at 2 to 8°C. Once thawed, if specimens are not being processed immediately, they can be stored at 2 to 8°C for up to 6 hours.

NOTE: Serum and plasma specimens should not be frozen in non-gel blood collection tubes.

Specimen Transport

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

covering the transport of clinical, diagnostic, or biological specimens.

ABBOTT REALTI*M*E HCV ASSAY PROCEDURE

This Abbott RealTi*m*e HCV package insert contains 2 assay protocols:

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

The Abbott RealTi*m*e HCV assay provides 2 sample volume options (0.2 mL and 0.5 mL).

(See assay protocol step 6 and **INTERPRETATION OF RESULTS** section.)

Materials Provided

- Abbott RealTi*m*e HCV Amplification Reagent Kit (List No. 4J86-90)

Materials Required But Not Provided

- Abbott RealTi*m*e HCV Calibrator Kit (List No. 4J86-70)
- Abbott RealTi*m*e HCV Control Kit (List No. 4J86-80)

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

For Abbott *m*1000 System
Sample Preparation Area

- Abbott *m*1000 System
- Reaction Vessels
- Abbott *m*Sample Preparation System (4 × 24 Preps) (List No. 04J70-24)
- Calibrated precision pipettes capable of delivering 20 to 1000 µL
- Aerosol barrier pipette tips for 20 to 1000 µL pipettes
- 11.6 to 16 mm sample tubes
- 200 µL and 1000 µL disposable tips
- Abbott 96 Deep-Well Plate (List No. 04J71-30)

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

- Abbott *m*2000*sp* instrument
- 5 mL Reaction Vessels
- Abbott *m*Sample Preparation System (4 × 24 Preps) (List No. 04J70-24)
- Calibrated precision pipettes capable of delivering 20 to 1000 µL
- Aerosol barrier pipette tips for 20 to 1000 µL pipettes
- 13 to 16 mm sample tubes
- 200 µL and 1000 µL disposable tips
- Vortex mixer

- Vortex mixer
- Abbott Optical Adhesive Covers (List No. 04J71-75)
- Abbott Adhesive Cover Applicators
- Abbott Splash-Free Support Base (List No. 09K31-01)
- Reagent troughs
- 1.5 mL output tubes
- Centrifuge capable of 5,000*g*
- Abbott Optical Adhesive Covers (List No. 04J71-75)
- Abbott Adhesive Cover Applicators
- Abbott Splash-Free Support Base (List No. 09K31-01)
- Master Mix Vial
- 200 mL reagent vessels
- Abbott 96-Deep-Well Plate (List No. 04J71-30)
- Abbott RealTi*m*e HCV *m*2000 ROW System Combined Application CD-ROM (List No. 1L69)
- Abbott 96-Well Optical Reaction Plate (List No. 04J71-70)
- Centrifuge capable of 2,000*g*

For Abbott *m*1000 System Reagent Preparation Area

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

For Abbott *m*2000*rt* Instrument Amplification Area

- Abbott *m*2000*rt* instrument
- Abbott RealTi*m*e HCV *m*2000 ROW System Combined Application CD-ROM (List No. 1L69)
- Abbott *m*2000*rt* Optical Calibration Kit (List No. 04J71-93)

Other Materials

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

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

Procedural Precautions

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

The Abbott RealTi*m*e HCV Calibrators, Internal Control, Negative Control, and Low and High Positive Control vials are intended for single-use only and should be discarded after use.

Use aerosol barrier pipette tips or disposable pipettes only 1 time when pipetting specimens, IC, or amplification reagents. To prevent contamination to the pipette barrel while pipetting, care should be taken to avoid touching the pipette barrel to the inside of the sample tube or container. The use of extended aerosol barrier pipette tips is recommended.

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

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

The Abbott RealTi*m*e HCV Calibrators and Controls must be prepared in conjunction with the specimens to be tested. The use of the Abbott RealTi*m*e HCV Controls and Calibrators is integral to the performance of the Abbott RealTi*m*e HCV assay. Refer to the **QUALITY CONTROL PROCEDURES** section of this package insert for details.

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

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

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

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

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

Sample Preparation Area

For sample preparation using the Abbott *m*1000 System, follow steps 3 through 10. For the manual sample preparation method refer to Extraction Protocol Section of the Manual Sample Preparation for Abbott RealTi*m*e RNA Assays procedure.

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

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

[a] Refers to sample tube outer diameter

   - If frozen, thaw specimens at 15 to 30°C or at 2 to 8°C. Once thawed, specimens can be stored at 2 to 8°C for up to 6 hours if not processed immediately.
   - Vortex each specimen 3 times for 2 to 3 seconds before loading on the Abbott *m*1000 System worktable. **Specimens showing particulate matter or turbidity should be clarified by centrifugation at 2,000*g* for 5 minutes prior to testing**. Aliquot each specimen into clean tubes or vials if necessary. Refer to the Abbott *m*1000 Operating Manual for tube sizes. Avoid touching the inside of the cap when opening tubes.
7. Place the calibrators (if applicable), the low and high positive controls, the negative control, and the patient specimens into the Abbott *m*1000 sample rack. Follow directions for performing a user-defined protocol, as described in the Abbott *m*1000 Operating Manual, Operation section.

8. Place the Reaction Vessels into the Abbott *m*1000 1 mL subsystem carrier.
9. Load the Abbott *m*Sample Preparation System reagents and the 1.5 mL Output Tubes on the Abbott *m*1000 System worktable as described in the Abbott *m*1000 Operating Manual, Operation section.
10. Initiate the Abbott *m*1000 protocol as described in the Abbott *m*1000 Operating Manual, Operation Section. From the Protocol screen, select the appropriate application file corresponding to the sample volume being tested.
    - **The assembly of the amplification master mix and sample eluates into the Abbott 96-Well Optical Reaction Plate (step 17) must be initiated within 1 hour after completion of Sample Preparation.**

Amplification Area

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

    NOTE: The Abbott *m*2000*rt* instrument requires 15 minutes to warm up.
12. Create the Abbott *m*2000*rt* test order. Refer to the Operating Instructions section of the Abbott *m*2000*rt* Operations Manual. From the Protocol screen, select the appropriate application file corresponding to the sample volume being tested.
    - Enter calibrator (needed if a calibration curve has not been stored on the Abbott *m*2000*rt*) and control lot specific values in the test order for accurate calibration and control evaluation. Lot-specific values are specified in each Abbott RealTi*m*e HCV Calibrator and Control Kit Card.

Reagent Preparation Area

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

NOTE: Change gloves before handling the amplification reagents.

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

Sample Preparation Area

17. In the Sample Preparation Area, transfer 50 µL of sample eluate to the Abbott 96-Well Optical Reaction Plate on the PCR cooler. **Use a separate pipette tip for each sample eluate transfer**. During the transfer of each sample, mix the reaction by pipetting up and down 3 to 5 times. Visually verify that 100 µL has been dispensed into each well.
18. Seal the Abbott 96-Well Optical Reaction Plate according to the instructions in the Abbott *m*2000*rt* Operations Manual.
19. Remove the Abbott 96-Well Optical Reaction Plate from the PCR cooler to the Abbott Splash-Free Support Base. Centrifuge the Abbott 96-Well Optical Reaction Plate in the Abbott Splash-Free Support Base at 5000*g* for 5 minutes. Transfer to the Amplification Area.

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

Amplification Area

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

POST PROCESSING PROCEDURES

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

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

For a detailed description of how to perform an Abbott *m*2000*sp* instrument and Abbott *m*2000*rt* instrument protocol refer to the Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals, Operating Instructions sections. The 96-sample capability requires Abbott *m*2000*sp* Software Version 4.0 or higher. Please follow Abbott *m*2000*sp* Operations Manual (List 09K20-04 or higher) and addendum or addenda.

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

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

    NOTE: Use 1 bottle of *m*Lysis Buffer, 1 vial of IC, and 1 Abbott RealTi*m*e HCV Amplification Reagent Kit to support up to 24 reactions. Use a second set of reagents to support 25 to 48 reactions, a third set of reagents to support 49 to 72 reactions and a fourth set of reagents to support 73 to 96 reactions WITH THE EXCEPTION OF *m*MICROPARTICLES. USE ONLY TWO BOTTLES OF *m*MICROPARTICLES WHEN PROCESSING 25 TO 96 REACTIONS.
3. Gently invert the Abbott *m*Sample Preparation bottles to ensure a homogeneous solution. If crystals are observed in any of the reagent bottles upon opening, allow the reagent to equilibrate at room temperature until the crystals disappear. Do not use the reagents until the crystals have dissolved.
4. Vortex each IC 3 times for 2 to 3 seconds before use.
5. Use a calibrated precision **PIPETTE DEDICATED FOR INTERNAL CONTROL USE ONLY** to add 500 µL of IC to each bottle of *m*Lysis Buffer. Mix by gently inverting the container 5 to 10 times to minimize foaming.

6. **A total of 96 samples can be processed in each run**. A negative control, a low positive control, and a high positive control are included in each run, therefore allowing a maximum of 93 specimens to be processed per run.
   - The Abbott RealTi*m*e HCV assay minimum sample volume and associated rack requirements on the Abbott *m*2000*sp* are:

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

[a] Refers to sample tube outer diameter

   - If frozen, thaw specimens at 15 to 30°C or at 2 to 8°C. Once thawed, specimens can be stored at 2 to 8°C for up to 6 hours, if not processed immediately.
   - Vortex each specimen 3 times for 2 to 3 seconds before loading on the Abbott *m*2000*sp* worktable. **Specimens showing particulate matter or turbidity should be clarified by centrifugation at 2,000*g* for 5 minutes prior to testing.** Aliquot each specimen into clean tubes or vials if necessary. Refer to the Abbott *m*2000*sp* Operations Manual for tube sizes. Avoid touching the inside of the cap when opening tubes.
7. Place the low and high positive controls, the negative control, the calibrators, if applicable, and the patient specimens into the Abbott *m*2000*sp* sample rack.
8. Place the 5 mL Reaction Vessels into the Abbott *m*2000*sp* 1 mL subsystem carrier.
9. Load the Abbott *m*Sample Preparation System reagents and the Abbott 96 Deep-Well Plate on the Abbott *m*2000*sp* worktable as described in the Abbott *m*2000*sp* Operations Manual, Operating Instructions.
10. From the Protocol screen, select the appropriate application file corresponding to the sample volume being tested. Initiate the sample extraction protocol as described in the Abbott *m*2000*sp* Operations Manual, Operating Instruction.
    - Enter calibrator (needed if a calibration curve has not been stored on the Abbott *m*2000*rt*) and control lot specific values in the **Sample Extraction: Worktable Setup, Calibrator and Control** fields. Lot-specific values are specified in each Abbott RealTi*m*e HCV Calibrator and Control Kit Card.
    - **The Abbott *m*2000*sp* Master Mix Addition protocol (step 12) must be initiated within 1 hour after completion of Sample Preparation.**

NOTE: Change gloves before handling the amplification reagents.

11. Load the amplification reagents and the master mix vial on the Abbott *m*2000*sp* worktable after sample preparation is completed.
    - Each amplification reagent pack supports up to 24 reactions.
    - Prior to opening the amplification reagents, ensure that the contents are at the bottom of the vials by tapping the vials in an upright position on the bench.
    - Remove and discard the amplification vial caps.
    - A second amplification reagent pack is required if performing 25 to 48 reactions.
    - A third amplification reagent pack is required if performing 49 to 72 reactions.
    - A fourth amplification reagent pack is required if performing 73 to 96 reactions.
12. Select the appropriate deep well plate that matches the corresponding sample preparation extraction. Initiate the Abbott *m*2000*sp* Master Mix Addition protocol. Follow the instructions as described in the Abbott *m*2000*sp* Operations Manual, Operating Instructions section.

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

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

    NOTE: System instructions for use of the automated plate-filling feature are found in the Abbott *m*2000*sp* Operations Manual (List No. 9K20-04 or higher), section 5, Operating Instructions, Sample Extraction—Closed Mode.

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

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

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

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

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

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

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

POST PROCESSING PROCEDURES

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

QUALITY CONTROL PROCEDURES

Abbott *m*2000*rt* Optical Calibration

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

Optical calibration of the Abbott *m*2000*rt* instrument is required for the accurate measurement and discrimination of dye fluorescence during the Abbott RealTi*m*e HCV 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 HCV assay:

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

Assay Calibration

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

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

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

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

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

Detection of Inhibition

An IC threshold cycle (C_t) assay validity parameter is established during a calibration run.

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

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

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

Negative and Positive Controls

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

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

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

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

Monitoring the Laboratory for the Presence of Contamination

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

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

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

RESULTS

Calculation

The concentration of viral HCV RNA in a sample or control is calculated from the stored calibration curve. The Abbott *m*2000*rt* instrument automatically reports the results on the Abbott *m*2000*rt* workstation. Assay results can be reported in IU/mL or Log IU/mL.

INTERPRETATION OF RESULTS

Sample Volume	Result	Interpretation
0.5 mL	Not Detected	Target not detected
	< 1.08 Log IU/mL[a]	Detected
	1.08 to 8.00 Log IU/mL	
	> 8.00 Log IU/mL	> ULQ[c]
0.2 mL	Not Detected	Target not detected
	< 1.48 Log IU/mL[b]	Detected
	1.48 to 8.00 Log IU/mL	
	> 8.00 Log IU/mL	> ULQ

[a] 12 IU/mL

[b] 30 IU/mL

[c] ULQ = upper limit of quantitation

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

SPECIFIC PERFORMANCE CHARACTERISTICS

The following performance characteristics were determined using the Abbott RealTi*m*e HCV assay with the Abbott *m*2000*sp* and the 0.5 mL sample preparation procedure, unless otherwise specified.

Limit of Detection (LOD)

The LOD is defined as the HCV RNA concentration detected with a probability of 95% or greater.

Limit of Detection, 0.5 mL Sample Volume

The LOD of the Abbott RealTi*m*e HCV assay is 12 IU/mL with the 0.5 mL sample preparation procedure.

The LOD was determined by testing dilutions of the Second WHO International Standard for Hepatitis C Virus RNA (NIBSC 96/798) prepared in HCV negative human plasma. Testing was performed with 3 lots of amplification reagents on 3 instrument systems. The results, representative of the analytical sensitivity of the Abbott RealTi*m*e HCV assay, are summarized in **Table 1**.

Table 1.

(IU/mL)	Number Tested	Number Detected	Percent Detected
25.0	57	57	100
20.0	57	57	100
15.0	57	55	96
12.5	57	53	93
10.0	57	56	98
7.5	57	51	89
5.0	57	46	81
2.5	57	33	58

Probit analysis of the data determined that the concentration of HCV RNA detected with 95% probability was 10.5 IU/mL (95% CI 8.6-14.0 IU/mL).

Limit of Detection, 0.2 mL Sample Volume

The LOD of the Abbott RealTi*m*e HCV assay is 30 IU/mL with the 0.2 mL sample preparation procedure and was determined as described for the 0.5 mL sample preparation procedure. The results, representative of the analytical sensitivity performance of the Abbott RealTi*m*e HCV assay, are summarized in **Table 2**.

Table 2.

(IU/mL)	Number Tested	Number Detected	Percent Detected
50.0	60	59	98
35.0	60	60	100
25.0	60	59	98
20.0	59[a]	48	81
15.0	60	53	88
12.5	60	54	90
10.0	60	39	65
7.5	60	27	45

[a] One sample was inhibited and was deleted from the data analysis.

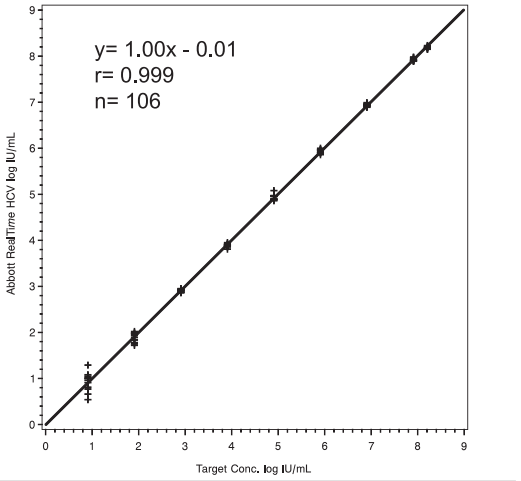
Probit analysis of the data determined that the concentration of HCV RNA detected with 95% probability was 23.8 IU/mL (95% CI 17.4-59.7 IU/mL).

Linear Range

The upper limit of quantitation (ULQ) for the Abbott RealTi*m*e HCV assay is 100 million IU/mL and the lower limit of quantitation is equivalent to LOD (12 IU/mL for the 0.5 mL sample preparation procedure, 30 IU/mL for the 0.2 mL sample preparation procedure).

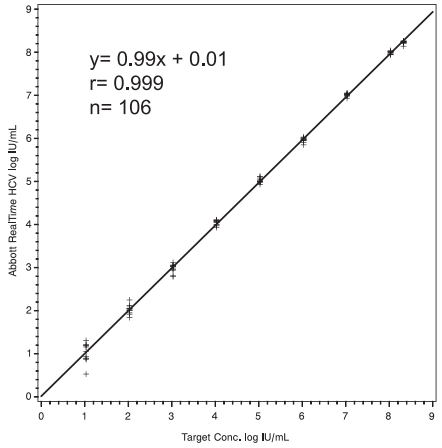
A 9-member panel prepared by diluting HCV armored RNA from 8.21 log IU/mL to 0.91 log IU/mL in HCV negative human plasma was tested. The results, representative of the Abbott RealTi*m*e HCV assay linearity, are shown in **Figure 1**.

Figure 1. Plasma



A 9-member panel prepared by diluting HCV armored RNA from 8.33 log IU/mL to 1.03 log IU/mL in HCV negative human serum was tested. The results, representative of the Abbott RealTi*m*e HCV assay linearity, are shown in **Figure 2**.

Figure 2. Serum



Linearity analysis was performed following the NCCLS EP6-A[26] guideline. The Abbott RealTi*m*e HCV assay was shown to be linear in serum and plasma across the range of HCV RNA concentrations tested.

Precision

The precision of the Abbott RealTi*m*e HCV assay was evaluated for the 0.5 mL sample preparation procedure using the Abbott *m*1000 and the Abbott *m*2000*sp* instruments and the manual sample preparation method. An 8-member HCV RNA panel was prepared. Panel members 1, 3, and 5 were dilutions of an HCV viral stock in negative human plasma. Panel members 2 and 4 were dilutions of an HCV viral stock in negative human serum. Panel members 6 through 8 were prepared by diluting HCV armored RNA in negative human plasma. One lot of amplification reagents was assigned to each of 3 Abbott *m*2000 instruments. For the precision studies with the Abbott *m*1000 and the Abbott *m*2000*sp* the panel members were tested in replicates of 4 in the first run on each instrument pair and replicates of 5 in each subsequent run, for a total of 15 runs. For the precision study using the manual sample preparation method, new dilutions of panel members 1, 2, 3, 5, 7, and 8 were prepared. These panel members were tested in replicates of 2 or 3 in 5 runs on each Abbott *m*2000*rt* instrument. Analysis was performed according to NCCLS EP10-A2.[27] Within-run, between-run, and inter-assay (within-run and between-run) standard deviations were determined. The Abbott RealTi*m*e HCV assay was designed to achieve an inter-assay standard deviation (SD) of less than or equal to 0.25 log IU/mL of HCV RNA for samples containing HCV concentrations from 100 to 100 million IU/mL. The results, representative of the precision of the Abbott RealTi*m*e HCV assay, are summarized in **Tables 3**, **4**, and **5**.

Table 3. Precision with the Abbott *m*1000 and Abbott *m*2000*rt* Instruments

Panel Member	n	Conc. Mean (IU/mL)	Conc. Mean (Log IU/mL)	Within-Run Component SD[a]	Between-Run Component SD[a]	Inter-Assay SD[a,b]
1	66[c,d]	11	1.03	0.26	0.00	0.26
2	72	92	1.96	0.08	0.07	0.11
3	72	664	2.82	0.04	0.07	0.09
4	72	9,182	3.96	0.05	0.05	0.07
5	70[d,e]	62,682	4.80	0.05	0.07	0.08
6	71[e]	917,350	5.96	0.05	0.07	0.08
7	72	10,599,314	7.03	0.07	0.08	0.11
8	71[d]	113,005,242	8.05	0.03	0.06	0.07

[a] Standard deviations (SD) are in log IU/mL.

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

[c] HCV RNA was not detected in 5 replicates.

[d] One replicate was deleted from the data analysis due to an instrument error.

[e] One replicate was aborted during sample preparation due to an instrument error.

Table 4. Precision with the Abbott *m*2000*sp* and Abbott *m*2000*rt* Instruments

Panel Member	n	Conc. Mean (IU/mL)	Conc. Mean (Log IU/mL)	Within-Run Component SD[a]	Between-Run Component SD[a]	Inter-Assay SD[a,b]
1	65[c]	8	0.88	0.25	0.00	0.25
2	72	91	1.96	0.08	0.04	0.09
3	72	564	2.75	0.05	0.03	0.06
4	72	8,853	3.95	0.03	0.02	0.04
5	71[d]	61,320	4.79	0.04	0.02	0.04
6	71[d]	925,118	5.97	0.04	0.02	0.04
7	71[d]	10,045,506	7.00	0.07	0.01	0.07
8	71[d]	109,257,360	8.04	0.04	0.01	0.04

[a] Standard deviations (SD) are in Log IU/mL.

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

[c] HCV RNA was not detected in 7 replicates.

[d] One replicate was deleted from the data analysis due to an instrument error.

Table 5. Precision with Manual Sample Preparation Method

Panel Member	n	Conc. Mean (IU/mL)	Conc. Mean (Log IU/mL)	Within-Run Component SD[a]	Between-Run Component SD[a]	Inter-Assay SD[a,b]
1	41[c]	8	0.93	0.20	0.00	0.20
2	42	79	1.90	0.09	0.03	0.10
3	42	651	2.81	0.06	0.08	0.10
5	41[d]	51,114	4.71	0.09	0.08	0.12
7	42	9,628,784	6.98	0.07	0.01	0.07
8	42	95,761,398	7.98	0.05	0.04	0.06

[a] Standard deviations (SD) are in log IU/mL.

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

[c] HCV RNA was not detected in 1 replicate.

[d] One replicate was deleted from the data analysis due to an instrument error.

Potentially Interfering Substance

The susceptibility of the Abbott RealTi*m*e HCV assay to interference by elevated levels of endogenous substances was evaluated. HCV negative samples and samples containing 1,000 IU/mL of HCV RNA were tested.

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

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

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

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

The assay was also evaluated by testing 70 specimens that had been either obtained from individuals diagnosed or screened for an autoimmune disorder (systemic lupus erythematosus [SLE], anti-nuclear antibodies [ANA], and rheumatoid factor [RF]) or serologically characterized as positive for the following viral markers: HBsAg, anti-HTLV-I/II, anti-HIV-1, anti-HIV-2. An additional 10 specimens that were PCR positive for a flavivirus (West Nile virus [n=4] and GB virus C [n=6]) were tested.

HCV RNA was not detected in 77 of the 80 specimens tested. HCV RNA was detected, but less than LOD, in 3 specimens (1 RF, 1 SLE and 1 anti-HIV-1). Insufficient volume did not allow for confirmation.

Specificity

The target specificity of the Abbott RealTi*m*e HCV assay is greater than or equal to 99.5% after resolution. In a representative study, specificity was evaluated by testing 56 HCV seronegative serum and 56 HCV seronegative plasma specimens. The specimens were tested on 3 Abbott *m*2000 instrument systems with 3 lots of amplification reagents. HCV RNA was not detected, resulting in 100% (112/112) specificity (95% CI 96.76-100.00%).

Cross-Reactivity

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

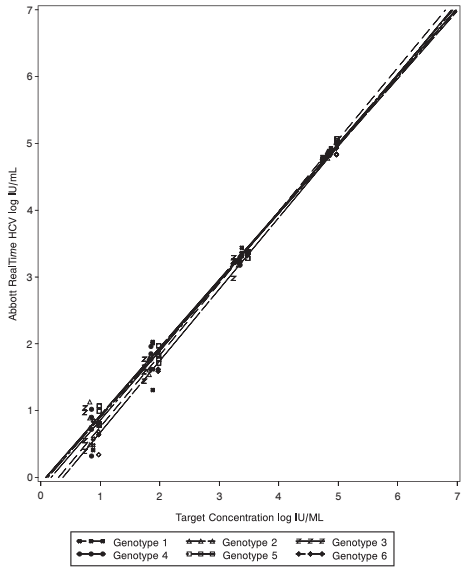
Human immunodeficiency virus 1	Vaccinia virus
Human immunodeficiency virus 2	BK human polyomavirus
Human T-lymphotropic virus 1	Human papilloma virus 16
Hepatitis B virus	Human papilloma virus 18
Epstein-Barr virus	*Neisseria gonorrhoeae*
Herpes simplex virus 1	*Chlamydia trachomatis*
Herpes simplex virus 2	*Candida albicans*
Cytomegalovirus	*Staphylococcus aureus*
Human herpesvirus 6B	*Staphylococcus epidermidis*
Human herpesvirus 8	*Mycobacterium gordonae*
Varicella-zoster virus	*Mycobacterium smegmatis*
Dengue virus 4	

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

Detection and Quantitation of HCV Genotypes

The ability of the Abbott RealTi*m*e HCV assay to detect and quantitate HCV genotypes was evaluated in 3 studies. In the first study, dilution linearity was demonstrated by diluting 6 specimens, one of each genotype 1 through 6, to target concentrations of 5.0 log IU/mL, 3.5 log IU/mL, 2.0 log IU/mL, and 1.0 log IU/mL. Four replicates were tested at each concentration of each genotype. These representative data are shown in **Figure 3**. The correlation coefficients ranged from 0.994 to 0.998.

Figure 3

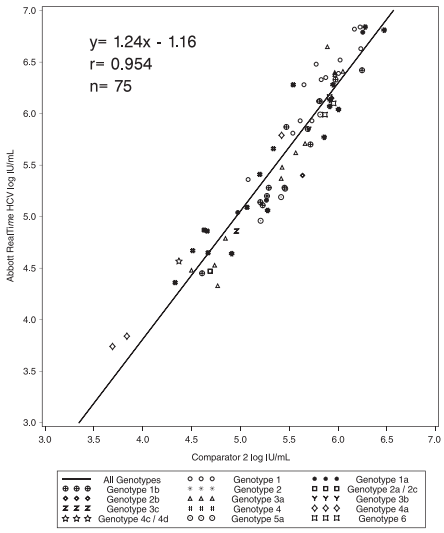


In the second study, a total of 75 specimens representative of each genotype were tested with the Abbott RealTi*m*e HCV assay. The results are summarized in **Table 6**. The correlation between the Abbott RealTi*m*e HCV assay results and those of a comparator assay is shown in **Figure 4**. Method comparison analysis was performed according to NCCLS Document EP9-A2.[28]

Table 6.

HCV Genotypes	Number of Specimens Tested	Number of Specimens Detected	Percent Detected
1	14	14	100
1a	3	3	100
1b	12	12	100
2	9	9	100
2a/2c	1	1	100
2b	2	2	100
3a	12	12	100
3b	1	1	100
3c	1	1	100
4	9	9	100
4a	3	3	100
4c/4d	1	1	100
5a	4	4	100
6	3	3	100

Figure 4.



In the third study, purified RNA transcripts from genotypes 1 through 6 were diluted to a target concentration of 1,000 IU/mL and tested. The results are summarized in **Table 7**. The results demonstrated equivalent quantitation of the genotypes tested.

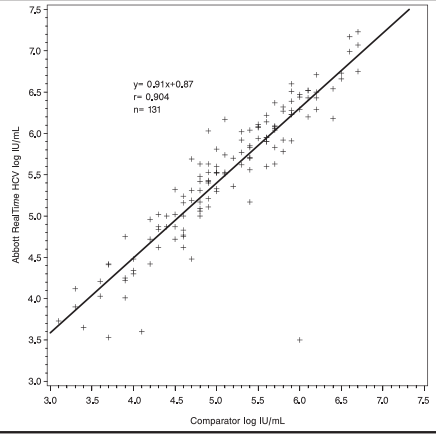
Table 7.

HCV Genotype	Mean Concentration (log IU/mL)
1	3.00
2	3.04
3	2.98
4	2.80
5	2.82
6	3.14

Correlation

Specimens from 131 HCV infected patients were tested with the Abbott RealTi*m*e HCV assay and a comparator assay. Method comparison analysis was performed following NCCLS EP9-A2.[28] The correlation plot is shown in **Figure 5**. One specimen was identified as an outlier and upon re-analysis excluding the outlier, the correlation coefficient was 0.950.

Figure 5.



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