Abbott RealTi*me* CMV

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REF 09N21-090

51-608290/R1

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51-608290/R1

Rx ONLY

Key to Symbols Used	
REF	List Number
IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
In Vitro Test	In Vitro Test
For In Vitro Diagnostic Use	For In Vitro Diagnostic Use
PRODUCT OF USA	Product of USA
GTIN	Global Trade Item Number
	Use by
CAL A	Calibrator A
CAL B	Calibrator B
CONTROL –	Negative Control
CONTROL LOW +	Low Positive Control
CONTROL HIGH ++	High Positive Control
INTERNAL CONTROL	Internal Control
AMPLIFICATION REAGENT PACK	Amplification Reagent Pack
	Contains sufficient for <n> tests
	Temperature Limit
	Consult instructions for use
	Warning
	Caution
	For Prescription Use Only
	Manufacturer
	Maximum Time Allowed

CUSTOMER SERVICE: 1-800-553-7042

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* CMV

INTENDED USE

The Abbott RealTi*me* CMV test is an in vitro polymerase chain reaction (PCR) assay for the quantitation of cytomegalovirus (CMV) DNA in human EDTA plasma. The Abbott RealTi*me* CMV test is intended for use as an aid in the management of Hematopoietic Stem Cell Transplant patients who are undergoing anti-cytomegalovirus therapy. In this population, serial DNA measurement can be used to assess virological response to anti-cytomegalovirus therapy. The results from the RealTi*me* CMV test must be interpreted within the context of all relevant clinical and laboratory findings. The RealTi*me* CMV test is not intended as a screening test for the presence of CMV DNA in blood or blood products.

SUMMARY AND EXPLANATION OF TEST

Found worldwide, human CMV is a double-stranded DNA virus of over 230 kb and belongs to the ß-herpesvirus subgroup of the human herpesvirus family.[1,2] After primary infection, CMV establishes life-long latency.[3,4] CMV infection is the leading viral cause of hearing loss and mental disability in newborns.[5] Whereas the virus causes mild or subclinical diseases in immunocompetent individuals, it may lead to severe life-threatening complications in immunocompromised individuals, such as transplant recipients and AIDS patients.[4] CMV infection in hematopoietic stem cell transplant recipients remains a significant cause of morbidity and mortality.[6-9] CMV is a direct cause of tissue-invasive infections, and an indirect cause of acute and chronic graft rejection and secondary bacterial, fungal, and viral infections, which ultimately reduces allograft success and patient survival.[8-10] Quantitation of CMV DNA in conjunction with clinical presentation and other laboratory markers provides clinicians with a means to monitor CMV DNA levels for patient management.[9-12]

The Abbott RealTi*me* CMV assay uses PCR technology combined with homogeneous real-time fluorescent detection for the quantitation of CMV DNA. The assay amplifies two select targets of conserved regions of the CMV genome. The assay uses human plasma (EDTA). The assay is standardized against the 1st World Health Organization (WHO) International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques (NIBSC 09/162).[13] Results are reported in International Units per milliliter (IU/mL) or log IU/mL.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

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

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

The Abbott RealTi*me* CMV assay also utilizes 2 accessory kits:

- Abbott Proteinase K
- Abbott *m*Sample Preparation System$_{DNA}$

The Abbott RealTi*me* CMV assay uses PCR[14] to generate amplified product from the CMV DNA genome in clinical specimens. The presence of CMV target sequence is indicated by the fluorescent signal generated through the use of fluorescently-labeled oligonucleotide probes on the Abbott *m*2000*rt* instrument. The probes do not generate a signal unless they are specifically bound to the amplified product. The amplification cycle at which fluorescent signal is detected by the Abbott *m*2000*rt* is inversely proportional to the log of the CMV DNA concentration present in the original sample. A DNA sequence that is unrelated to the CMV target sequence is introduced into each specimen at the beginning of sample preparation. This unrelated DNA sequence is simultaneously amplified by PCR, and serves as an internal control (IC) to demonstrate that the process has proceeded correctly for each sample.

Sample Preparation

The purpose of sample preparation is to extract and concentrate nucleic acid, to make the target accessible for amplification, and to remove potential inhibitors of amplification from the extract. This process is accomplished by the Abbott *m*2000*sp*, an automated sample preparation system designed to use magnetic microparticle processes for the purification of nucleic acids from plasma samples. The Abbott *m*Sample Preparation System$_{DNA}$ (4 x 24 Preps) reagents lyse the virion, capture the nucleic acids, and wash the particles to remove unbound sample components. Proteinase K is included in the lysis step for plasma samples to digest proteins associated with the sample. The bound nucleic acids are eluted and transferred to the Abbott 96-Deep-Well Plate. The nucleic acids are then ready for amplification. The IC is introduced into the sample preparation procedure and is processed along with the calibrators, controls, and specimens.

Reagent Preparation and Reaction Plate Assembly

The Abbott *m*2000*sp* combines the Abbott RealTi*m*e CMV amplification reagent components (CMV Amplification Reagent, DNA Polymerase, and Activation Reagent). The Abbott *m*2000*sp* dispenses the resulting master mix to the Abbott 96-Well Optical Reaction Plate along with aliquots of the nucleic acid samples prepared by the Abbott *m*2000*sp*. After manual application of the optical seal, the plate is ready for transfer to the Abbott *m*2000*rt*, an automated system used for real-time PCR amplification and detection.

Amplification

During the amplification/detection reaction on the Abbott *m*2000*rt* instrument, the target DNA is amplified by the DNA Polymerase in the presence of deoxynucleotide triphosphates (dNTPs) and magnesium. The amplification reagent contains specific sets of amplification primers for CMV and IC. During PCR amplification, high temperature is used to separate the strands of double-stranded DNA. When the reaction is cooled to a temperature where DNA annealing can again occur, the analyte-specific, single-stranded DNA oligonucleotide primers bind to the analyte DNA. The primers are extended by DNA Polymerase, thereby making an exact copy of a short target stretch of the analyte DNA. The DNA Polymerase enzyme is a thermophilic enzyme that has been modified in its active site by a molecule that renders it inactive. When the DNA Polymerase is heated prior to the initiation of PCR, the inhibitory molecule is cleaved from the DNA Polymerase allowing it to regain its activity. In this way, the DNA Polymerase is only active at temperatures where specific DNA-DNA interactions occur. This greatly reduces non-specific PCR artifacts such as primer dimers.

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 target is achieved through repeated cycling between high and lower temperatures. Amplification of the CMV and IC targets takes place simultaneously in the same reaction.

The Abbott RealTi*m*e CMV assay targets 2 short sequences within the UL34 and UL80.5 genes of the CMV genome.[15-17] The regions are specific for CMV and are highly conserved based on analysis of published CMV sequences. The redundancy in target amplification is designed to provide robust, accurate, and sensitive amplification of CMV DNA.

The IC target sequence is derived from the hydroxypyruvate reductase gene from the pumpkin plant *Cucurbita pepo*, and is provided as a linearized DNA plasmid in a buffer solution with carrier DNA.

Detection

During each round of PCR amplification, the fluorescent probes anneal to the amplified target DNA, if present. The probes are labeled with different fluorescent molecules, which allow CMV and IC to be distinguished from each other. The probes are single-stranded, linear DNA oligonucleotides modified with a fluorescent moiety covalently linked to one end of the probe and a quenching moiety to the other end. In the absence of target sequences, the probes adopt a conformation that brings the quencher close enough to the excited fluorophore to absorb its energy before it can be fluorescently emitted. When the probe binds to its complementary sequence in the target, the fluorophore and the quencher are held apart, allowing fluorescent emission and detection. Since this fluorescence occurs during every cycle, the PCR reaction can be read in real-time. The amplification cycle at which fluorescent signal is detected by the Abbott *m*2000*rt* is inversely proportional to the log of the CMV DNA concentration present in the original sample.

Optional Amplification Reagent Extended Use Feature

An overview of this feature is provided on the last page of this package insert.

The optional amplification reagent extended use feature allows amplification reagent packs containing prepared master mix to be stored at – 20°C (± 5°C), capped and protected from light, for up to 14 days before a second use. The internal control (IC) may be used again within 14 days if the vial remains capped at – 20°C (± 5°C) until the second use.

NOTE: All other assay-related reagents remain single use only.

This package insert provides instructions for running the Abbott RealTi*m*e CMV assay (List No. 09N21-090) with and without the optional amplification reagent extended use feature.

Terminology used in this package insert:

Amplification reagent packs and internal control can be used a total of 2 times. Throughout this package insert, amplification reagent packs and IC that have not yet been used will be referred to as **new** amplification reagent packs and IC (i.e., initial use). Amplification reagent packs that have been used once and contain prepared master mix will be referred to as **partial** amplification reagent packs. IC vials that have been used once will be referred to as **partial** vials of IC.

PREVENTION OF NUCLEIC ACID CONTAMINATION

The possibility of nucleic acid contamination is minimized because:

- The Abbott RealTi*m*e CMV assay performs PCR amplification and oligonucleotide hybridization 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 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 CMV. Refer to the **Contamination Precautions** section of this package insert.

REAGENTS

Abbott RealTi*m*e CMV Amplification Reagent Kit (List No. 09N21-090)

1. INTERNAL CONTROL Abbott RealTi*m*e CMV Internal Control
   (Part No. 9N21Y) (4 vials, 0.53 mL per vial)
   Less than 0.01% noninfectious linearized DNA plasmid in a buffer solution with carrier DNA. Preservatives: Sodium azide and 0.15% ProClin® 950.
2. AMPLIFICATION REAGENT PACK Abbott RealTi*m*e CMV Amplification Reagent Pack
   (List No. 9N21) (4 packs, 24 tests per pack)
   Each reagent pack contains:
   - 1 bottle (0.070 mL) DNA Polymerase (5.4 to 5.9 units/µL) in a buffered solution with stabilizers.
   - 1 bottle (0.600 mL) CMV Oligonucleotide Reagent. Less than 0.1% synthetic oligonucleotides (6 primers and 3 probes). Less than 0.8% dNTPs in a buffered solution with a reference dye. Preservatives: Sodium azide and 0.20% ProClin 950.
   - 1 bottle (0.778 mL) Activation Reagent. 38 mM magnesium chloride in a buffered solution. Preservatives: Sodium azide and 0.15% ProClin 950.

Abbott RealTi*m*e CMV Control Kit (List No. 09N21-080)

1. CONTROL – Abbott RealTi*m*e CMV Negative Control
   (List No. 5N23Z) (8 vials, 0.9 mL per vial).
   Buffered solution with carrier DNA. Preservatives: Sodium azide and 0.15% ProClin 950.
2. CONTROL LOW + Abbott RealTi*m*e CMV Low Positive Control
   (List No. 5N23P) (8 vials, 0.9 mL per vial).
   Inactivated CMV in human plasma. Human plasma found to be nonreactive by FDA-licensed tests for antibody to HCV, antibody to HIV-1, antibody to HIV-2, and HBsAg. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.
3. CONTROL HIGH ++ Abbott RealTi*m*e CMV High Positive Control
   (List No. 5N23X) (8 vials, 0.9 mL per vial).
   Less than 0.01% noninfectious linearized CMV DNA plasmid in a buffer solution with carrier DNA. Preservatives: Sodium azide and 0.15% ProClin 950.

Abbott RealTi*m*e CMV Calibrator Kit (List No. 09N21-070)

1. CAL A Abbott RealTi*m*e CMV Calibrator A
   (List No. 5N23A) (12 vials, 0.9 mL per vial).
   Less than 0.01% noninfectious linearized CMV DNA plasmid in a buffer solution with carrier DNA. Preservatives: Sodium azide and 0.15% ProClin 950.
2. CAL B Abbott RealTi*m*e CMV Calibrator B
   (List No. 5N23B) (12 vials, 0.9 mL per vial).
   Less than 0.01% noninfectious linearized CMV DNA plasmid in a buffer solution with carrier DNA. Preservatives: Sodium azide and 0.15% ProClin 950.

WARNINGS AND PRECAUTIONS

IVD In Vitro Diagnostic Medical Device

For In Vitro Diagnostic Use

The Abbott RealTi*m*e CMV assay is not intended to be used as a screening test for CMV, or to be used as a diagnostic test to confirm the presence of CMV infection.

Due to inherent differences among technologies, it is recommended to assess the impact of any potential change in the method for quantification of CMV DNA during the clinical management of a patient.

Use only USP grade 190 to 200 proof ethanol (95 to 100% ethanol) to prepare the *m*Wash2$_{DNA}$ sample preparation reagent. **Do not use ethanol that contains denaturants.**

Safety Precautions

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

CAUTION: The Abbott RealTi*m*e CMV Low Positive Control 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 micro-organisms 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-A4,[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]

The Abbott RealTi*m*e CMV Calibrator Kit (List No. 09N21-070), Abbott RealTi*m*e CMV Negative Control (5N23Z), Abbott RealTi*m*e CMV High Positive Control (5N23X), and the Abbott RealTi*m*e CMV Internal Control (9N21Y), CMV Amplification Reagent, and Activation Reagent contain the following components:

- 2-Methyl-4-isothiazol-3-one
- Sodium azide

The Abbott RealTi*m*e CMV Low Positive Control contains the following components:

- 2-Methyl-4-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.
EUH032	Contact with acids liberates very toxic gas.
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.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P302+P352	IF ON SKIN: Wash with plenty of water.
P362+P364	Take off contaminated clothing and wash it before reuse.
P501	Dispose of contents / container in accordance with local regulations.

Safety Data Sheet Statement: Important information regarding the safe handling, transport, and disposal of this product is contained in the Safety Data Sheet.

Specimen Collection and Handling Precautions

The Abbott RealTi*m*e CMV assay is only for use with EDTA plasma specimens that have been handled and stored in capped tubes as described in the **SPECIMEN COLLECTION AND HANDLING INSTRUCTIONS** section.

Laboratory Precautions

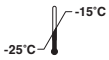
- During preparation of samples, compliance with good laboratory practices is essential to minimize the risk of cross-contamination between samples as well as the inadvertent introduction of nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used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

Contamination Precautions

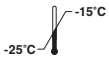
- Amplification reactions such as PCR are sensitive to accidental introduction of product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the reagents used become contaminated by accidental introduction of even a few molecules of amplification product. Measures to reduce the risk of contamination in the laboratory include physically separating the activities involved in performing PCR in compliance with good laboratory practices.
- The use of 2 dedicated areas within the laboratory is recommended for performing the Abbott RealTi*m*e CMV assay with the automated Abbott *m*2000*sp* and *m*2000*rt*:
  - The **Sample Preparation Area** is dedicated to processing samples (specimens, Abbott RealTi*m*e CMV Calibrators and Controls) and to adding processed specimens, calibrators, and controls to the Abbott 96-Well Optical Reaction Plate. All reagents used in the Sample Preparation Area should remain in this dedicated area at all times. Laboratory coats, pipettes, pipette tips, and vortex mixers used in the Sample Preparation Area must remain in this area and not be moved to the Amplification Area. Do not bring amplification product into the Sample Preparation Area.
  - The **Amplification Area** is dedicated to the amplification and the detection of amplified product. Laboratory coats and equipment used in the Amplification Area must remain in this area and not be moved to the Sample Preparation Area.
- If the Abbott *m*2000*sp* run is aborted, dispose of all commodities and reagents according to the Abbott *m*2000*sp* Operations Manual. **NOTE: New amplification reagents may be saved, stored, and used a second time, as described in this package insert.**
- If the Abbott *m*2000*sp* master mix addition protocol is aborted after amplification reagents are added to the Abbott 96-Well Optical Reaction Plate, seal the Abbott 96-Well Optical Reaction Plate and put in a sealable plastic bag and dispose of according to the Abbott *m*2000*sp* Operations Manual, Hazards section, along with the gloves used to handle the plate.
- For all completed, interrupted or aborted Abbott *m*2000*rt* runs, dispose of the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag according to the Abbott *m*2000*rt* Operations Manual along with the gloves used to handle the plate.
- **Autoclaving the sealed Reaction Plate will not degrade the amplified product and may contribute to the release of the amplified product by opening the sealed plate. The laboratory area can become contaminated with amplified product if the waste materials are not carefully handled and contained.**
- Decontaminate and dispose of all specimens, reagents, and other potentially biohazardous or contaminated materials in accordance with local, state, and federal regulations.[21] All materials should be handled in a manner that minimizes the chance of potential contamination of the work area.
- Follow the instructions in this package insert to recap and store amplification reagents that are to be used a second time.

REAGENT STORAGE AND HANDLING INSTRUCTIONS

Abbott RealTi*m*e CMV Amplification Reagent Kit (List No. 09N21-090)



New Abbott RealTi*m*e CMV Amplification Reagent Pack and Internal Control vials must be stored at −20°C (± 5°C) when not in use. Care must be taken to separate the Abbott RealTi*m*e CMV Amplification Reagent Pack that is in use from direct contact with specimens, calibrators, and controls. Reagents are shipped on dry ice.



Partial amplification reagent packs must be stored at −20°C (± 5°C), capped, upright, and protected from light, following initial use. If stored this way, **partial** amplification reagent packs with prepared master mix may be used a second time within 14 days of initial use. IC may also be used a second time within 14 days of being thawed, if stored capped at −20°C (± 5°C). After 2 uses, discard **partial** amplification reagent packs and IC.

Abbott RealTi*m*e CMV Control Kit (List No. 09N21-080)

The Abbott RealTi*m*e CMV Negative, Low Positive, and High Positive Controls must be stored at −20°C (± 5°C). Reagents are shipped on dry ice.

Abbott RealTi*m*e CMV Calibrator Kit (List No. 09N21-070)

The Abbott RealTi*m*e CMV Calibrators A and B must be stored at −20°C (± 5°C). Reagents are shipped on dry ice.

INDICATION OF INSTABILITY OR DETERIORATION OF REAGENTS

When a positive or negative control value is out of the expected range, it may indicate deterioration of the reagents. Associated test results are invalid and samples must be retested. Assay recalibration may be necessary. Refer to the **QUALITY CONTROL PROCEDURES: Assay Calibration** section of this package insert for details.

If you receive reagents, calibrators, or controls that are in a condition contrary to label recommendation, or that are damaged, contact Abbott Molecular Technical Services.

SPECIMEN COLLECTION AND HANDLING INSTRUCTIONS

Human plasma (EDTA) specimens may be used with the Abbott RealTi*m*e CMV assay.

Plasma Specimen Collection and Storage

Follow the manufacturer's instructions for processing plasma collection tubes. Prior to preparing plasma specimens through centrifugation, freshly drawn whole blood specimens may be held at 2 to 30°C for up to 24 hours.

After centrifugation, remove plasma from cells. Plasma specimens may be stored:

- At 15 to 30°C for up to 24 hours
- At 2 to 8°C for up to 5 days
- At −70°C or colder for longer term[22,23]
- Multiple freeze/thaw cycles should be avoided and should not exceed 3 freeze/thaw cycles. Thaw plasma specimens at 15 to 30°C or at 2 to 8°C. Once thawed, if plasma specimens are not being processed immediately, they can be stored at 2 to 8°C for up to 24 hours.

Specimen Transport

NOTE: Ship freshly drawn whole blood specimens with cold packs/boxes. The whole blood specimens should be transported and processed to plasma within 24 hours of draw.

Ship plasma specimens frozen on dry ice. 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.

INSTRUMENT METHODS

The Abbott RealTi*m*e CMV assay is performed using the Abbott *m*2000*sp* for sample processing and the Abbott *m*2000*rt* for amplification and detection. Refer to the **ASSAY PROTOCOL** instructions in this package insert or to the Abbott *m*2000*sp* or *m*2000*rt* Operations Manuals for detailed operating procedures. Prior to performing the assay, the Abbott RealTi*m*e CMV application file must be installed on the Abbott *m*2000*sp* and Abbott *m*2000*rt* systems from the Abbott RealTi*m*e CMV *m*2000 System Combined Application CD-ROM. For detailed information on application file installation, refer to the Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals, Operating Instructions section.

ABBOTT REALTI*M*E CMV ASSAY PROCEDURE

This Abbott RealTi*m*e CMV package insert contains instructions for running the assay with or without the optional amplification reagent extended use feature. The optional amplification reagent extended use feature requires Abbott *m*2000*sp* software version 6.0 or higher and CD-ROM List No. 09N21-001 or higher. The procedures differ in how the amplification reagents are used and stored for extended use. Steps 3, 7, 14, 17, and 18 are affected. An overview of the Abbott RealTi*m*e CMV Amplification Reagent Extended Use feature is provided at the end of this package insert.

Materials Provided

- Abbott RealTi*m*e CMV Amplification Reagent Kit (List No. 09N21-090): **new** and/or **partial** amplification reagent packs and internal control.

Materials Required But Not Provided

- Abbott RealTi*m*e CMV Control Kit (List No. 09N21-080)
- Abbott RealTi*m*e CMV Calibrator Kit (List No. 09N21-070)
- Abbott RealTi*m*e CMV *m*2000 System Combined Application CD-ROM List No. 09N21-001 or higher
- Abbott *m*Sample Preparation System$_{DNA}$ (List No. 06K12-24)
- Abbott Proteinase K (List No. 03L78-061)

SAMPLE PREPARATION AREA

- Abbott *m*2000*sp* instrument with software version 6.0 or higher
- Abbott RealTi*m*e CMV *m*2000 System Combined Application CD-ROM List No. 09N21-001 or higher
- Abbott *m*Sample Preparation System$_{DNA}$ (List No. 06K12-24)

NOTE: One kit is sufficient to complete 96 sample preparations.

- Abbott Proteinase K (List No. 03L78-061)
- 5 mL Reaction Vessels
- Calibrated pipettes capable of delivering 20 to 1000 µL
- Aerosol barrier pipette tips for 20 to 1000 µL Pipettes
- 1000 µL Disposable Tips
- 200 µL Disposable Tips
- Vortex mixer
- USP grade 190 to 200 proof ethanol (95 to 100% ethanol). **Do not use ethanol that contains denaturants.**
- 50 mL polypropylene centrifuge tubes
- Serological pipettes
- Graduated cylinder, 100 mL
- Molecular biology grade water
- Abbott Optical Adhesive Covers
- Abbott Adhesive Cover Applicator
- Abbott Splash-Free Support Base
- Master Mix Tube
- 13 mm Sample Racks or 16 mm Sample Racks
- 200 mL Reagent Vessels
- Abbott 96-Deep-Well Plate
- Abbott 96-Well Optical Reaction Plate
- 1.4 mL Micro Vial 5 mm Caps, optional

AMPLIFICATION AREA

- Abbott *m*2000*rt* instrument with software version 6.0 or higher
- Abbott RealTi*m*e CMV *m*2000 System Combined Application CD-ROM List No. 09N21-001 or higher
- Abbott *m*2000*rt* Optical Calibration Kit (List No. 4J71-93)

OTHER MATERIALS

- Biological safety cabinet approved for working with infectious materials
- Lab coat
- Powder-free disposable gloves
- Protective eyewear
- Solid waste container
- Sealable plastic bags
- 1.7 mL molecular biology grade microcentrifuge tubes (Dot Scientific, Inc. or equivalent)†
- Cotton tip applicators (Puritan or equivalent)†

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

Procedural Precautions

- Read the instructions in this package insert carefully before processing samples.
- The Abbott RealTi*m*e CMV reagents are intended to be used on the Abbott *m*2000*sp* and the Abbott *m*2000*rt* for amplification and detection.
- Do not use kits or reagents after the dates shown on kit or reagent labels.
- The *m*Sample Preparation System$_{DNA}$ reagents are single-use only.
- Amplification reagents and internal control may be used twice when handled according to this package insert.
  IMPORTANT: Amplification reagents that will be used a second time must be stored at −20°C (± 5°C) within 45 minutes of the completion of the master mix addition protocol.
- Controls, calibrators, and *m*Sample Preparation System$_{DNA}$ Reagents are for single-use only and should be discarded after use. Use new reagent vessels and new reaction vessels for every new Abbott RealTi*m*e CMV assay run. At the end of each run, discard all remaining reagents as stated in the Abbott *m*2000*sp* Operations Manual and the **POST PROCESSING PROCEDURES** section of this package insert. Follow instructions in this manual to re-cap and store amplification reagents that are to be used a second time.
- **New** Amplification Reagent Kits, Calibrator Kit, and Control Kit can be thawed and refrozen up to 3 times before use. This does NOT apply to **partial** amplification reagent packs, which should remain at −20°C (± 5°C) until use. Prepared master mix may only be used 2 times.
- A calibration curve must be established before specimens are tested. The use of the Abbott RealTi*m*e CMV Calibrators and Controls is integral to the performance of the Abbott RealTi*m*e CMV assay. Refer to the **QUALITY CONTROL PROCEDURES** section in this package insert for details.
- Use only USP grade 190 to 200 proof ethanol (95 to 100% ethanol) to prepare the *m*Wash2$_{DNA}$ sample preparation reagents. **Do not use ethanol that contains denaturants**.
- Replace any empty or partially used 200 µL and 1000 µL disposable tips on the Abbott *m*2000*sp* with full trays before every run. Refer to the Abbott *m*2000*sp* Operations Manual, Operating Instructions section.
- 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, reagents, and controls by using a detergent solution followed by a tuberculocidal disinfectant such as 1.0% (v/v) sodium hypochlorite or other suitable disinfectant.
- For automated sample preparation on the Abbott *m*2000*sp*, sample tubes should be inspected for air bubbles. If found, remove them with a sterile pipette tip. Reagent bubbles may interfere with proper detection of reagent levels in the reagent vessel, causing insufficient reagent aspiration, which could impact results. **Caution should be taken to avoid cross-contamination between samples by using a new sterile pipette tip for each tube.**
- Use aerosol barrier pipette tips or disposable pipettes only 1 time when pipetting specimens, controls, calibrators, 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.

ASSAY PROTOCOL

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

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

Sample Preparation Area

1. A maximum of 96 samples can be processed in each run. A negative control, a low positive control, and a high positive control must be included in each run, therefore allowing a maximum of 93 specimens to be processed per run.
   - Check sample volume. Do not use flat bottom tubes. The protocol processes 0.5 mL of sample; however, the required minimum sample volume is higher. The Abbott RealTi*m*e CMV assay minimum sample volume and associated rack requirements on the Abbott *m*2000*sp* are:

Rack	Tube Inner Diameter	Abbott RealTi*m*e CMV Minimum Sample Volume (mL)
13 mm	10.0 mm	0.60
	10.5 mm	0.66
	11.0 mm	0.72
	11.5 mm	0.78
	12.0 mm	0.84
	12.5 mm	0.90
16 mm	13.0 mm	0.97
	13.5 mm	1.03
	14.0 mm	1.10
	14.5 mm	1.20

   - Refer to the Abbott *m*2000*sp* Operations Manual for acceptable tube height and appropriate sample rack type.
   - If frozen, thaw specimens at 15 to 30°C or at 2 to 8°C. Once thawed, if specimens are not being processed immediately, store at 2 to 8°C for up to 24 hours.
   - Before use, vortex specimens 3 times for 2 to 3 seconds. **Ensure that bubbles or foam are not created;** if present, remove them with a new sterile pipette tip for each tube. Avoid touching the inside of the cap when opening tubes.

The following table shows the number of sample preparation reagents, controls, calibrators, and Internal Control vials needed based on the number of reactions.

Sample Preparation Reagents and Internal Control Requirements

Reagent	1 to 24 Reactions	25 to 48 Reactions	49 to 72 Reactions	73 to 96 Reactions
Low Positive Control	1 tube	1 tube	1 tube	1 tube
High Positive Control	1 tube	1 tube	1 tube	1 tube
Negative Control	1 tube	1 tube	1 tube	1 tube
Calibrator [a]	3 tubes each	3 tubes each	3 tubes each	3 tubes each
Amplification Reagents [b]	1 if **new;** up to 4 with **partial** packs	2 if **new;** up to 4 with **partial** packs	3 if **new;** up to 4 with **partial** packs	4 **new** or **partial** packs
*m*Sample Prep$_{DNA}$ Reagents	1 set	2 sets	3 sets	4 sets
Internal Control	1 **new** vial or 1 **partial** vial	1 **new** vial or up to 2 **partial** vials	1 **new** vial or up to 3 **partial** vials	1 **new** vial or up to 4 **partial** vials

[a] Calibrators are not required in every run.

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

2. 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, if calibrators, controls, and **new** IC are not being processed immediately, store at 2 to 8°C for up to 24 hours prior to use.
   - Vortex calibrators, controls, and IC 3 times for 2 to 3 seconds before use. Ensure that the contents of each vial are at the bottom after vortexing by tapping the vials on the bench to bring liquid to the bottom of the vial. Ensure bubbles or foam are not generated; if present, remove with a sterile pipette tip, using a new tip for each vial.

3. Select **new** and/or **partial** amplification reagent packs to be used in the run. Refer to the Abbott *m*2000*sp* Operations Manual (List No. 9K20-06 or higher), Operating Instructions section, for instructions pertaining to amplification reagent pack inventory management.

   NOTE: Amplification reagent packs must have the same lot number.

   Thaw **new** amplification reagents at 15 to 30°C or at 2 to 8°C and store at 2 to 8°C until required for the amplification master mix procedure. Once thawed, the **new** amplification reagents can be stored at 2 to 8°C for up to 24 hours if not used immediately.

   NOTE: Partial amplification reagent packs being used a second time should NOT be stored at 2 to 8°C before use. They should be kept at −20°C (± 5°C) until needed for master mix addition. Once removed from the freezer, cumulative room temperature exposure should not exceed 25 minutes, including instances where packs are removed from storage, but not used. If 25 minutes is exceeded, discard the partial amplification reagent packs.

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

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

   NOTE: Do not vortex or invert the Amplification Reagent Pack.

4. Open the Abbott Proteinase K reagent pack. For each bottle of Proteinase K reagent required, pipette 17.15 mL of molecular biology grade water and 2.45 mL of Proteinase K to a 50 mL polypropylene centrifuge tube. Mix by gentle inversion 10 to 15 times.
5. Open the *m*Sample Preparation System$_{DNA}$ reagent pack(s). If crystals are observed in any of the reagent bottles upon opening, allow the reagent to equilibrate at room temperature until the crystals disappear. Do not use the reagents until the crystals have dissolved.
6. Prepare the *m*Wash2$_{DNA}$ by adding 70 mL of USP grade 190 to 200 proof ethanol (95 to 100% ethanol) to each bottle *m*Wash2$_{DNA}$ being used. **Do not use ethanol that contains denaturants.** Invert the bottle(s) to mix contents.
7. Use a calibrated **PRECISION PIPETTE DEDICATED FOR INTERNAL CONTROL USE ONLY** to add 125 µL of IC to each bottle of *m*Lysis$_{DNA}$ being used. Mix by gently inverting the container 5 to 10 times to minimize foaming. **Partial** vials of IC can be re-capped and stored at −20°C (± 5°C) for up to 14 days prior to a second use.
8. Gently invert the *m*Sample Preparation System$_{DNA}$ bottles, except for the *m*Microparticles$_{DNA}$ and *m*Wash1$_{DNA}$ bottles, 5 to 10 times to ensure a homogeneous solution, and pour the contents into the appropriate reagent vessels as indicated in the table below. The *m*Wash1$_{DNA}$ reagent is not needed for this protocol. Ensure bubbles or foam are not generated in the reagent vessels; if present, remove with a sterile pipette tip, using a new tip for each reagent vessel.

   NOTE: Apply labels to the 200 mL reagent vessels according to the layout on the table below.

Sample extraction reagents are distributed as follows:

Samples	1st Vessel	2nd Vessel	3rd Vessel	4th Vessel	5th Vessel	6th Vessel
1–24	1 *m*Lysis$_{DNA}$	empty	1 *m*Microparticles$_{DNA}$	1 Proteinase K	1 *m*Wash2$_{DNA}$	1 *m*Elution Buffer$_{DNA}$
25–48	2 *m*Lysis$_{DNA}$	empty	1 *m*Microparticles$_{DNA}$	2 Proteinase K	1 *m*Wash2$_{DNA}$	1 *m*Elution Buffer$_{DNA}$
49–72	2 *m*Lysis$_{DNA}$	1 *m*Lysis$_{DNA}$	1 *m*Microparticles$_{DNA}$	2 Proteinase K	2 *m*Wash2$_{DNA}$	2 *m*Elution Buffer$_{DNA}$
73–96	2 *m*Lysis$_{DNA}$	2 *m*Lysis$_{DNA}$	1 *m*Microparticles$_{DNA}$	3 Proteinase K	2 *m*Wash2$_{DNA}$	2 *m*Elution Buffer$_{DNA}$

9. Immediately prior to initiation of the sample extraction protocol, vigorously mix or vortex the *m*Microparticles$_{DNA}$ until they are fully resuspended and pour the *m*Microparticles$_{DNA}$ into the appropriate 200 mL reagent vessel as indicated in the table.
10. Place the negative control, the positive controls, the calibrators (if applicable), and the patient specimens into the Abbott *m*2000*sp* sample rack.
    - Remove caps from all specimen, control, and calibrator tubes.
    - Insert specimen, control, and calibrator tubes (uncapped) into sample racks carefully to avoid splashing. Load tubes in consecutive positions, starting with the first position in the first sample rack. Fill all positions in each sample rack without skipping any positions before loading tubes into the next sample rack. If used, bar codes on tube labels must face right for scanning. Ensure that each tube is placed securely in the sample rack so that the bottom of the tube reaches the inside bottom of the rack.
    - Place the 5 mL reaction vessels into the Abbott *m*2000*sp* 1 mL subsystem.
    - Load filled sample racks onto the Abbott *m*2000*sp* in consecutive sample rack positions, with the first rack farthest to the right on the worktable, and any additional rack progressively to the left of the first rack.
11. Initiate the Abbott *m*2000*sp* sample extraction protocol specific for plasma specimens as described in the Abbott *m*2000*sp* Operations Manual, Operating Instructions section.
    - Enter calibrator (needed if a calibration curve has not been stored on the Abbott *m*2000*rt*) and control lot specific values in the **Sample Extraction: Assay Details** screen. Lot specific values are specified in each Abbott RealTi*me* CMV Calibrator and Control Kit card.

      NOTE: Verify the values entered match the values on the kit cards.

Amplification Area

12. Switch on and initialize the Abbott *m*2000*rt* instrument in the amplification area prior to initiation of the master mix protocol. The Abbott *m*2000*rt* requires 15 minutes to warm-up. Refer to the Abbott *m*2000*rt* Operations Manual, Operating Instructions section.

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

Sample Preparation Area

13. **Once sample preparation is completed, the master mix protocol must be initiated within 60 minutes.**

    NOTE: Change gloves before handling the amplification reagents.

14. Load the amplification reagents, the uncapped master mix tube, and the Abbott 96-Well Optical Reaction Plate on the Abbott *m*2000*sp* worktable after sample preparation is completed**.**
    - If only 1 amplification reagent pack is being used, no master mix tube is required.
    - Do not vortex or invert the amplification reagent pack.
    - Each **new** amplification reagent pack supports up to 24 reactions.
    - Ensure the amplification reagents are thoroughly thawed before use.

      IMPORTANT: Partial amplification reagent packs should be stored at −20°C (± 5°C) until immediately before the second use. Confirm that master mix is thawed before placing partial pack(s) on the Abbott *m*2000*sp* worktable. Once removed from −20°C (± 5°C) storage, partial amplification reagent packs being used a second time must be used within 25 minutes or discarded. This applies to cumulative room temperature exposure, including instances where packs are removed from storage, but not used.
    - Prior to opening the **new** amplification reagents, ensure that the contents are at the bottom of the vials by tapping the vials in an upright position on the bench.
    - Do not tap **partial** amplification reagent packs being used a second time. Tapping may result in loss of master mix volume in the cap.
    - Remove caps. If a **new** amplification reagent pack will be stored for a second use, the vials will need to be recapped for storage. If planning to reuse the original caps to recap the reagent vials, save the original caps. If planning to use fresh caps to recap the reagent vials, original caps may be discarded.
    - **Partial amplification reagent packs are loaded to the left of new amplification reagent packs on the Abbott *m*2000*sp* worktable.**
    - **Ensure that amplification reagent packs are firmly seated on the instrument.**
15. Initiate the *m*2000*sp* master mix addition protocol as described in the Abbott *m*2000*sp* Operations Manual, Operating Instructions section.

    NOTE: The Abbott *m*2000*rt* protocol (step 18) must be started within 60 minutes following the completion of the master mix addition protocol.

16. Seal the Abbott 96-Well Optical Reaction Plate after the Abbott *m*2000*sp* instrument has completed addition of eluted samples and master mix according to the Abbott *m*2000*sp* Operations Manual, Operating Instructions section.
17. Place the sealed Abbott 96-Well Optical Reaction Plate into the Abbott Splash-Free Support Base for transfer to the Abbott *m*2000*rt* instrument.
    If a prepared **partial** amplification reagent pack is to be used a second time, cap the 3 reagent vials with the saved or new caps (List No. 3N20-01) and promptly store the reagents at −20°C (± 5°C), protected from light, and in an upright position. Discard any amplification reagent packs that are exhausted or have been used twice.

Amplification Area

18. Place the Abbott 96-Well Optical Reaction Plate in the Abbott *m*2000*rt* instrument and initiate the Abbott RealTi*m*e CMV assay protocol, as described in the Abbott *m*2000*rt* Operations Manual, Operating Instructions section. At the completion of the run, assay results are reported on the Abbott *m*2000*rt*. Refer to the **RESULTS** section of this package insert for further details.
    IMPORTANT: Assembled master mixes that will be used a second time (as a **partial** amplification reagent pack) must be stored at −20°C (± 5°C) within 45 minutes of the completion of the master mix addition protocol.

POST PROCESSING PROCEDURES

1. Remove the Abbott 96-Deep-Well Plate from the worktable and dispose 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. At the end of each run, clear and clean all work areas. Clean the Abbott *m*2000*sp* worktable as stated in the Abbott *m*2000*sp* Operations Manual.
4. The *m*Sample Preparation System$_{DNA}$ reagents are single-use only.
5. Clean the Abbott *m*2000*rt* and the Abbott Splash-Free Support Base according to the Abbott *m*2000*rt* Operations Manual.
6. Decontaminate and dispose of all specimens, controls, reagents, and other potentially contaminated materials in accordance with local, state, and federal regulations.
7. Remove and discard all disposables and solid waste in accordance with local, state, and federal regulations.

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 CMV 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 CMV assay:

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

Assay Calibration

A calibration curve is required to quantitate CMV DNA in the specimens and controls. Two assay calibrators are run in replicates of 3 to generate a calibration curve (CMV concentration [log copies/mL] versus the threshold cycle [Ct] at which a reactive level of fluorescent signal is detected). The lot-specific values for Calibrator A and Calibrator B are specified on each Abbott RealTi*m*e CMV Calibrator Kit Card and must be entered into the assay test order when a run is performed. **It is important that the values for the calibrators be entered exactly as they appear on the calibrator kit card.** The values for the calibrators are entered in log copies/mL. The calibration curve slope and intercept are calculated and stored on the instrument. The concentration of CMV DNA in a sample is calculated from the calibration curve. Results are automatically reported on the Abbott *m*2000*rt* workstation.

The Negative Control, Low Positive Control, and High Positive Control must be included in the calibration run.

Follow the procedure for sample preparation, reagent addition, amplification, and detection protocols as stated in the Abbott *m*2000*sp* and *m*2000*rt* Operations Manuals.

Once an Abbott RealTi*m*e CMV 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 CMV Amplification Reagent Kit with a new lot number is used.
- An *m*Sample Preparation System$_{DNA}$ Kit with a new lot number is used.
- An updated version of the Abbott RealTi*m*e CMV application specification file is installed.
- An optical calibration of the Abbott *m*2000*rt* is performed per the Calibration Procedure section of the Abbott *m*2000*rt* Operations Manual.

Detection of Inhibition

An IC cycle number [CN] assay validity parameter is established during a calibration run.

Prior to sample preparation, a defined, consistent quantity of the IC is introduced into the lysis buffer, which is then used during the processing of each specimen, calibrator, and control, and measured on the Abbott *m*2000*rt* instrument to demonstrate proper sample processing and assay validity. The IC is composed of a DNA sequence unrelated to the CMV DNA sequence.

The median IC cycle number in calibration samples establishes the IC CN validity range to be met by all subsequent processed specimens using that calibration curve.

An error 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 code. Specimens whose IC CN value falls outside of the established range must be retested starting with sample preparation.

Positive and Negative Controls

A Negative Control, a Low Positive Control, and a High Positive Control are included in each run to evaluate run validity.

The lot-specific values for the positive controls are specified on each Abbott RealTi*m*e CMV Control Kit Card and must be entered into the test order when a run is performed. **It is important that the values for the positive controls be entered exactly as they appear on the control kit card.** The values for the positive controls are entered in log copies/mL.

An error 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 code. 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 CMV must not be detected in the negative control. CMV 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*2000*sp*, sample rack, temperature blocks, and Abbott *m*2000*rt*, and repeat the sample processing for controls and specimens following the **Procedural Precautions**. If negative controls are persistently reactive, contact Abbott Molecular Customer Service.

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 and controls, calibrators, and/or amplification product. This includes routinely handled objects such as pipettes, function keys for the Abbott *m*2000*sp*, function keys for the Abbott *m*2000*rt*, sample racks, temperature blocks, laboratory bench surfaces, microcentrifuges, and centrifuge adaptors.

1. Add 0.8 mL molecular biology grade water to a 1.7 mL DNase-free microcentrifuge tube.
2. Saturate the cotton tip of an applicator (Puritan or equivalent) in the molecular biology grade 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 molecular biology grade 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 the *m*Wash1$_{DNA}$ buffer to a clean tube using the pipette dedicated for IC use.
6. Add 20 µL of the *m*Wash1$_{DNA}$ buffer to each microcentrifuge tube.
7. Cap the microcentrifuge tube.
8. Test this sample according to the assay procedure section of this package insert.

9. Transfer liquid from microcentrifuge tube to a 5 mL reaction vessel.
10. The presence of contamination is indicated by the detection of CMV in the swab samples.
11. If CMV is detected on equipment, follow the cleaning and decontaminating guidelines given in that equipment's operations manual. If CMV 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.
12. Repeat testing of the contaminated area by following steps 1 through 9.

RESULTS

Calculation

The concentration of CMV DNA in a sample or control is calculated from either a stored calibration curve or a calibration curve created by calibrators within a sample run. The Abbott *m*2000*rt* instrument automatically reports the results on the *m*2000*rt* workstation. Assay results are reported in IU/mL or log IU/mL.

NOTE: The assay is standardized against the 1st World Health Organization (WHO) International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques (NIBSC 09/162).[24]

Results can be converted to copies/mL using a conversion factor of 1.56. The mathematical relationship between IU/mL and copies/mL is copies/mL = IU/mL / 1.56. Results can also be converted to log copies/mL using a conversion factor of 0.19. The mathematical relationship between log IU/mL and log copies/mL is log copies/mL = log IU/mL – 0.19.

The following table represents the potential Abbott *m*2000*rt* outputs that can be observed by the user.

Interpretation of Results

Result	Interpretation
Not detected	Target not detected
< 1.70 log IU/mL[a]	Detected[b]
1.70 to 8.19 log IU/mL	[c]
> 8.19 log IU/mL	> ULQ[d]

[a] 50 IU/mL

[b] Below LLOQ (lower limit of quantitation); CMV DNA is not quantifiable.

[c] Calcaluated results are within assay quantitation range. If calculated results are obtained, the Interpretation field is left blank.

[d] Above ULOQ (upper limit of quantitation); if log IU/mL results are above the quantitation range of the assay, results are reported as ">8.19 log IU/mL"; if IU/mL results are above the quantitation range of the assay, results are reported as ">156,000,000 IU/mL."

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.

LIMITATIONS OF THE PROCEDURE

- **FOR IN VITRO DIAGNOSTIC USE ONLY.**
- 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.
- Test performance characteristics have been evaluated only for individuals who have undergone Hematopoietic Stem Cell Transplant. No information is available on test performance in patients undergoing other types of transplant procedures, neonates or pediatric patients, AIDS or other immunocompromised patients; nor is information available on test performance in patients who have been diagnosed with CMV disease.
- The detection of viral DNA is dependent upon proper specimen collection, handling, transportation, storage, and preparation (including extraction). Failure to observe proper procedures in any of these steps can lead to incorrect results. There is a risk of false negative results resulting from improperly collected, transported, or handled specimens
- Though rare, mutations within the highly-conserved regions of the viral genome covered by the primers and/or probes in the Abbott RealTi*m*e CMV assay may result in the under-quantitation of virus or failure to detect the presence of virus. The Abbott RealTi*m*e CMV assay mitigates this risk by amplifying two select targets of conserved regions of the CMV genome.
- Optimal performance of this test requires appropriate specimen collection, storage, and transport to the test site (refer to the **SPECIMEN COLLECTION AND HANDLING INSTRUCTIONS** section of this package insert).
- Human plasma (EDTA) may be used with the Abbott RealTi*m*e CMV assay. The use of other anticoagulants has not been validated for use with the Abbott RealTi*m*e CMV assay.
- 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.
- A specimen with a result of "Not Detected" cannot be presumed to be negative for CMV DNA.
- Drug interference was evaluated using drug pools, and effects of individual drugs were not assessed, with the exception of the 13 therapeutic drugs that were tested individually.
- Results from the Abbott RealTi*m*e CMV assay should be interpreted in conjunction with other clinical and laboratory findings.

SPECIFIC PERFORMANCE CHARACTERISTICS

Traceability to the 1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques (NIBSC code: 09/162).

The Abbott RealTi*m*e CMV assay is standardized to the 1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques (NIBSC code: 09/162). Abbott RealTi*m*e CMV Calibrators trace to the WHO International Standard (IS) each time a lot is manufactured by means of value assignment using Abbott CMV Primary Calibrators.

Conversion factors were determined by diluting the CMV WHO IS to approximately 5 log IU/mL in pooled, normal EDTA plasma and testing against the Abbott CMV Primary Calibrators. The conversion factors were confirmed using the CMV WHO IS diluted to approximately 3.5 log IU/mL.

The mathematical relationship between log copies/mL and log IU/mL is log copies/mL + 0.19 = log IU/mL. The mathematical relationship between copies/mL and IU/mL is copies/mL x 1.56 = IU/mL.

LIMIT OF BLANK AND PERFORMANCE WITH NEGATIVE SPECIMENS (SPECIFICITY)

A total of 100 anti–CMV IgG negative specimens were tested with the Abbott RealTi*m*e CMV assay. Two amplification reagent lots were used to test 50 specimens each. Testing was performed on 2 Abbott RealTi*m*e *m*2000 Systems (*m*2000*sp* and *m*2000*rt*) and each lot was used in 2 independent runs testing 25 specimens. All specimens had reported results of "Not detected". The specificity was 100% (100/100) (95% CI of 96.3 to 100.0%). The percent of false positives was 0% (0/100). The limit of blank (LOB) for the Abbott RealTi*m*e CMV assay is confirmed to be 0 IU/mL.

LIMIT OF DETECTION

The limit of detection (LOD) is defined as the CMV DNA concentration detected with a probability of 95%.

Determination of LOD for CMV genotypes gB1 and gB2.

The LOD was determined by testing dilutions of the WHO IS (genotype gB1), and strain AD169 (genotype gB2),[25] in pooled CMV negative human plasma. Testing was performed with 4 lots of amplification reagents on 4 *m*2000 Systems. Testing with each lot was performed over 3 days. Each dilution was tested in replicates of 8 per day. The results obtained with the WHO IS are summarized in **Table 1**. Results for AD169 are summarized in **Table 2**. The results support a claimed LOD of 31.20 IU/mL (1.49 log IU/mL) for the Abbott RealTi*m*e CMV assay.

Table 1. LOD Using the WHO IS, genotype gB1

IU/mL	Number Tested	Number Detected	Percent Detected
46.80	96	96	100
31.20	96	95	99
23.40	96	93	97
15.60	96	91	95
12.48	96	91	95
9.36	96	83	86
7.80	96	86	90
6.24	96	66	69
3.90	96	52	54
1.56	96	24	25

Probit analysis of the data determined that the concentration of CMV DNA detected with 95% probability for genotype gB1 was 16 IU/mL (95% CI of 13 to 19 IU/mL).

Table 2. LOD Using strain AD169, Genotype gB2

IU/mL	Number Tested	Number Detected	Percent Detected
46.80	96	96	100
31.20	95[a]	94	99
23.40	96	88	92
15.60	96	85	89
12.48	96	76	79
9.36	96	79	82
7.80	96	66	69
6.24	96	58	60
3.90	96	40	42
1.56	96	20	21

[a] One replicate was excluded due to instrument error.

Probit analysis of the data determined that the concentration of CMV DNA detected with 95% probability for genotype gB2 was 27 IU/mL (95% CI of 22 to 34 IU/mL).

Confirmation of LOD for CMV genotypes gB3 and gB4.

To confirm the ability of the Abbott RealTi*m*e CMV assay to meet the claimed LOD of 31.20 IU/mL (1.49 log IU/mL), the Toledo strain of CMV (genotype gB3),[26] and a cultured clinical specimen of (genotype gB4)[25] were diluted to 31.20 IU/mL (1.49 log IU/mL) and tested with the Abbott RealTi*m*e CMV assay. For each strain, 2 independent dilutions were made in pooled CMV negative EDTA plasma. Two amplification reagent lots were used to test 30 replicates each for a total of 60 replicates per genotype. Testing used 2 *m*2000 Systems and was performed over 3 days with each lot. Results demonstrated that Abbott RealTi*m*e CMV assay can detect 31.20 IU/mL of CMV DNA testing strains of genotypes gB3 and gB4. Results are summarized in **Table 3**. The results support a claimed LOD of 31.20 IU/mL (1.49 log IU/mL) for the Abbott RealTi*m*e CMV assay.

Table 3. LOD Confirmation, Using Genotype gB3 and gB4

CMV Genotype	IU/mL	Number Tested	Number Detected	Percent Detected
gB3	31.20	60	58	97
gB4	31.20	60	60	100

Confirmation of LOD for anti-viral resistant CMV specimens.

To confirm the ability of Abbott RealTi*m*e CMV assay to meet the claimed LOD of 31.20 IU/mL (1.49 log IU/mL), 2 anti-viral resistant clinical specimens were diluted to 31.20 IU/mL (1.49 log IU/mL) and tested with the Abbott RealTi*m*e CMV assay. For each strain, 2 independent dilutions were made in pooled CMV negative EDTA plasma. Two amplification reagent lots were used to test 30 replicates each for a total of 60 replicates per specimen. Testing used 2 *m*2000 Systems and was performed over 3 days with each lot. Results demonstrated that Abbott RealTi*m*e CMV assay can detect 31.20 IU/mL of CMV DNA testing anti-viral resistant specimens. Results are summarized in **Table 4**. The results support a claimed LOD of 31.20 IU/mL (1.49 log IU/mL) for the Abbott RealTi*m*e CMV assay.

Table 4. LOD Confirmation Using Anti-Viral Resistant Specimens

Anti-Viral Resistant Specimen	IU/mL	Number Tested	Number Detected	Percent Detected
1	31.20	60	58	97
2	31.20	60	60	100

The claimed LOD of Abbott RealTi*m*e CMV assay is 31.20 IU/mL (1.49 log IU/mL) for CMV genotypes gB1 to gB4.

LINEAR RANGE

The upper limit of linearity of the Abbott RealTi*m*e CMV assay for plasma specimens is 8.19 log IU/mL (156 million IU/mL) and the lower limit of linearity is equivalent to the claimed assay LLOQ of 1.70 log IU/mL (50 IU/mL).

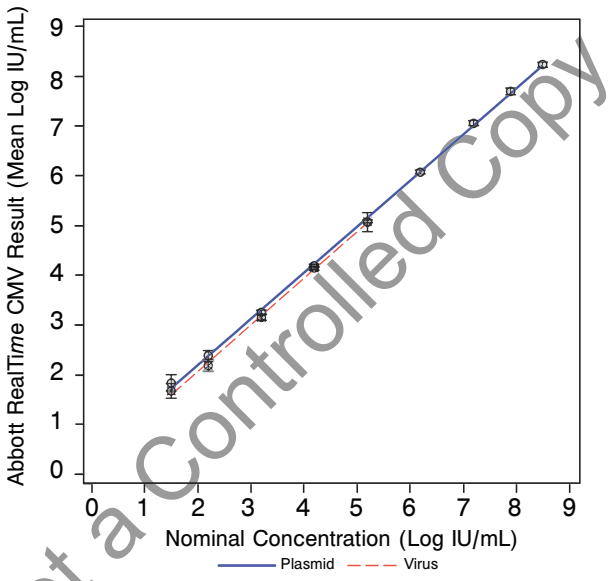
The linear range for genotypes gB1 to gB4 was determined using 2 panels per genotype. The 9-member plasmid panels were prepared by diluting CMV DNA to concentrations ranging from 1.49 to 8.49 log IU/mL in pooled CMV negative human plasma. The 5-member virus panels were made using cultured virus strains of genotypes gB1 to gB4 diluted in pooled CMV negative human plasma.

The concentrations ranged from 1.49 to 5.19 log IU/mL for genotypes gB1, gB2, and gB4 and 1.46 to 5.00 log IU/mL for gB3.

Testing was performed with a single lot of amplification reagents for 3 days with 4 replicates per day for a total of 12 replicates per panel member.

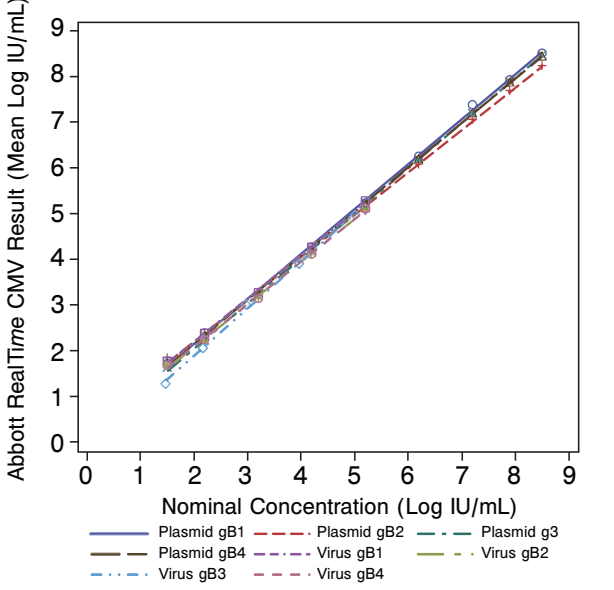
The results are shown in **Figures 1** and **2**. The deviation from linearity, defined as the difference between values predicted using a linear model vs the best fit polynomial model, was ≤0.10 log IU/mL for all panel members of genotypes gB1 to gB4.

Figure 1. Abbott RealTi*m*e CMV Assay Linearity using CMV Genotype gB2 Strain AD169



Note: Error Bars represent ± 1 SD of the mean

Figure 2. Abbott RealTi*m*e CMV Assay Linearity by Genotype



The Abbott RealTi*m*e CMV assay was shown to be linear across the range of CMV concentrations tested. See **Table 5** for linear equations.

Table 5. Linear Equations for CMV Genotypes gB1 to gB4

CMV Genotype	Plasmid Panel	Virus Panel
gB1	y = 0.9821x + 0.1991	y = 0.9485x + 0.3143
gB2	y = 0.9257x + 0.3627	y = 0.9356x + 0.2144
gB3	y = 0.9872x + 0.0833	y = 1.0367x – 0.1767
gB4	y = 0.9689x + 0.2178	y = 0.9305x + 0.2382

PRECISION - WITHIN LABORATORY

Within laboratory precision of the Abbott RealTi*m*e CMV assay was evaluated. Precision panels were made with either plasmid DNA (panel members 8 through 10) or cultured strain AD169 (panel members 1 through 7) spiked in pooled human plasma. Testing was performed with 3 lots of amplification reagents on 3 *m*2000sp and *m*2000rt instrument pairs. A 10-member panel spanning a targeted range from 1.11 to 8.49 Log IU/mL was tested in replicates of 4 in each run for a total of 15 runs per panel.

Results are summarized in **Table 6**.

Table 6. Precision - Within Laboratory

			Within-run Component		Between-run Component		Inter-assay[a]		Between-lot/ instrument Component		Total	
Panel Member	n	Mean Log IU/mL	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	40[b]	1.23[e]	0.232	18.9	0.055	4.5	0.239	19.4	0.000	0.0	0.239	19.4
2	53[c]	1.41[e]	0.269	19.1	0.173	12.3	0.320	22.7	0.000	0.0	0.320	22.7
3	60	1.99	0.134	6.7	0.020	1.0	0.136	6.8	0.024	1.2	0.138	6.9
4	60	2.92	0.058	2.0	0.067	2.3	0.088	3.0	0.024	0.8	0.092	3.1
5	60	3.83	0.053	1.4	0.035	0.9	0.063	1.7	0.011	0.3	0.064	1.7
6	60	4.83	0.046	1.0	0.042	0.9	0.062	1.3	0.065	1.4	0.090	1.9
7	60	6.07	0.036	0.6	0.017	0.3	0.039	0.6	0.052	0.9	0.065	1.1
8	60	7.08	0.050	0.7	0.028	0.4	0.057	0.8	0.054	0.8	0.079	1.1
9	59[d]	7.77	0.036	0.5	0.026	0.3	0.044	0.6	0.079	1.0	0.091	1.2
10	59[d]	8.26	0.042	0.5	0.020	0.2	0.047	0.6	0.079	0.9	0.091	1.1

[a] Between-Run SD (Total Inter-assay SD) contains the Within-run and Between-run components.
[b] CMV DNA was not detected in 20 replicates.
[c] CMV DNA was not detected in 6 replicates. One replicate was identified as an outlier, and was excluded.
[d] One replicate did not generate a result due to instrument error.
[e] The mean concentration is below the claimed assay LLOQ (1.70 log IU/mL).

CLINICAL REPRODUCIBILITY

Clinical reproducibility was evaluated in a multi-center study that included 3 external sites that used the Abbott RealTi*m*e CMV assay to test an 8 member panel spanning a targeted range from 1.19 to 8.49 Log IU/mL. The panel was made with CMV positive clinical specimens, cultured strain AD169 or plasmid DNA diluted in pooled plasma. Each panel member was repeated 4 times within the panel. Testing was performed with 3 lots of Abbott RealTi*m*e CMV Amplification Reagent Kit and Sample Preparation$_{DNA}$ System Kits. Each of the 3 clinical sites tested 2 of the 3 amplification reagent lots and sample preparation reagent lots for 5 non-consecutive days each, resulting in a total of 10 runs at each site.

Results are summarized in **Table 7**.

Table 7. Clinical Reproducibility

				Within-run Component		Between-run Component		Between-lot Component		Between-site Component		Total[g]	
Panel Member	Source	n	Mean Log IU/mL	SD[a]	%CV	SD[a]	%CV	SD[a]	%CV	SD[a]	%CV	SD[a]	%CV
1	Plasmid	120	8.34	0.17	2.0	0.07	0.8	0.16	1.9	0.10	1.1	0.26	3.1
2	Plasmid	120	6.70	0.13	1.9	0.04	0.6	0.08	1.2	0.17	2.5	0.23	3.4
3	Cultured Virus	120	5.29	0.12	2.3	0.04	0.7	0.05	1.0	0.19	3.6	0.23	4.4
4	Cultured Virus	120	3.94	0.14	3.5	0.05	1.4	0.06	1.5	0.19	4.7	0.24	6.2
5	Cultured Virus	119[b]	3.06	0.15	4.9	0.05	1.6	0.04	1.2	0.15	4.9	0.22	7.2
6	Clinical Specimen	119[c]	2.26	0.12	5.4	0.05	2.3	0.03	1.2	0.13	5.8	0.19	8.3
7	Clinical Specimen	119[d]	1.68[f]	0.21	12.8	0.10	6.0	0.04	2.5	0.13	8.0	0.27	16.4
8	Clinical Specimen	101[e]	1.43[f]	0.26	18.5	0.00	0.0	0.06	4.3	0.14	9.5	0.30	21.2

[a] Standard deviations (SD) are in log IU/mL.
[b] A result was not generated for one replicate due to a missing sample.
[c] One replicate was excluded from the analysis due to technician error.
[d] CMV DNA was not detected in one replicate.
[e] CMV DNA was not detected in 19 replicates.
[f] The mean concentration is below the claimed assay LLOQ (1.70 log IU/mL).
[g] Total variability includes within-run, between-run, between-lot, and between-site variability.

PRECISION – OPERATOR TO OPERATOR

In addition, operator-to-operator precision of the Abbott RealTi*m*e CMV assay was evaluated by testing the same 8 member panel. One lot of amplification reagents was run on 1 *m*2000sp and *m*2000rt instrument pair by 3 operators. Each operator completed 1 run per day for 7 days, for a total of 21 runs. Four replicates were tested for each panel member in each run.

Results are summarized in Table 8.

Table 8. Operator-to-Operator Precision

				Within-run Component		Between-run Component		Between-operator Component		Total[f]	
Panel Member	Source	n	Mean Log IU/mL	SD[a]	%CV	SD[a]	%CV	SD[a]	%CV	SD[a]	%CV
1	Plasmid	84	8.24	0.13	1.6	0.06	0.7	0.12	1.4	0.18	2.2
2	Plasmid	84	6.62	0.14	2.1	0.03	0.5	0.07	1.0	0.16	2.4
3	Cultured Virus	83[b]	5.17	0.12	2.4	0.08	1.6	0.10	1.9	0.18	3.5
4	Cultured Virus	84	3.83	0.11	2.9	0.09	2.4	0.10	2.7	0.18	4.6
5	Cultured Virus	83[b]	2.96	0.10	3.4	0.10	3.3	0.11	3.7	0.18	6.0
6	Clinical Specimen	84	2.10	0.11	5.4	0.05	2.5	0.11	5.4	0.17	8.0
7	Clinical Specimen	78[c]	1.43[e]	0.33	23.2	0.00	0.0	0.11	7.7	0.35	24.4
8	Clinical Specimen	70[d]	1.28[e]	0.32	24.7	0.05	3.7	0.04	2.8	0.32	25.1

[a] Standard deviations (SD) are in log IU/mL.
[b] A result was not generated for one replicate due to an instrument error.
[c] CMV DNA was not detected in 6 replicates.
[d] CMV DNA was not detected in 14 replicates.
[e] The mean concentration is below the claimed assay LLOQ (1.70 log IU/mL).
[f] Total variability includes within-run, between-run, and between-operator variability.

LOWER LIMIT OF QUANTITATION

The claimed lower limit of quantitation (LLOQ) for the Abbott RealTi*m*e CMV assay is 50 IU/mL (1.70 log IU/mL).

The total analytical error (TAE) was calculated using estimates determined through analysis of data from limit of detection (LOD) and precision studies. These studies included all four genotypes of CMV including several anti-viral resistant strains.

TAE was estimated by 2 different methods: |Bias| + (2 × SD) and SQRT (2) × 2 × SD . The TAE estimates for panel members that had an observed concentration at or near the claimed assay limit of detection (1.49 log IU/mL) were evaluated.

The TAE analyses demonstrated that the Abbott RealTi*m*e CMV assay can determine CMV DNA concentration of 50 IU/mL (1.70 log IU/mL) in plasma with an acceptable level of accuracy (TAE ≤ 1.00 log IU/mL). The absolute value of the bias plus two SDs (TAE) ≤ 1.00 log IU/mL ensures that, for samples with a true value equal to the LLOQ, there is 95% or greater probability that the measured value will be within 1 log IU/mL of the true value. The square root of two times two SDs ≤ 1.00 log IU/mL ensures that, for samples with a true value equal to the LLOQ, the difference between two measurements of more than 1 log IU/mL is statistically significant.

Table 9 shows the TAE analyses for the lowest concentration that meets both TAE acceptance criteria for genotypes gB1 to gB4, anti-viral resistant, and clinical strains. Across all strains, the lowest concentration that met TAE acceptance criteria across all CMV genotypes and clinical strains was 46.80 IU/mL (1.67 log IU/mL). The results support a claimed LLOQ of 50 IU/mL (1.70 log IU/mL).

Table 9. Lower Limit of Quantitation for CMV Genotypes gB1 to gB4, anti-viral resistant, and clinical strains

CMV Strain	Nominal Concentration IU/mL	Nominal Concentration Log IU/mL	n	Mean (Log IU/mL)	SD (Log IU/mL)	Bias (Log IU/mL)	TAE = |Bias| + (2 x SD) (Log IU/mL)	TAE = SQRT(2) x 2 x SD (Log IU/mL)
gB1	31.20	1.49	95	1.81	0.20	0.32	0.72	0.57
gB2	31.20	1.49	94	1.52	0.28	0.03	0.59	0.79
gB3	31.20	1.49	58	1.60	0.33	0.11	0.77	0.93
gB4	31.20	1.49	60	1.72	0.24	0.23	0.71	0.68
CMV Anti-Viral Resistant Strain 1	46.80	1.67	60	1.79	0.16	0.12	0.44	0.45
CMV Anti-Viral Resistant Strain 2	31.20	1.49	60	1.77	0.24	0.28	0.76	0.68
Clinical Strain	31	1.49	78	1.43	0.33	–0.06	0.72	0.93
Clinical Strain	31	1.49	119	1.68	0.24	0.19	0.67	0.68

CROSS REACTIVITY

The following microorganisms (viruses, bacteria, and fungi) were evaluated for potential cross-reactivity in the Abbott RealTi*m*e CMV assay. Each microorganism was added to CMV DNA negative plasma samples and plasma samples containing approximately 100 IU/mL and 3,120 IU/mL of CMV DNA. Microorganisms were tested at 10^5 – 10^6 copies/mL, IU/mL, viral particles/mL, cells/mL, $TCID_{50}$/mL, IFU/mL or CFU/mL.

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

- Human immunodeficiency virus 1
- Human immunodeficiency virus 2
- Human T lymphotropic virus type I
- Hepatitis A virus
- Human Papillomavirus 18
- Adenovirus
- Parvovirus B19
- JC Polyomavirus

Hepatitis B virus	*Neisseria gonorrhoeae*
Hepatitis C virus	*Chlamydia trachomatis*
Epstein-Barr virus	*Staphylococcus aureus*
Herpes simplex virus type 1	*Staphylococcus epidermidis*
Herpes simplex virus type 2	*Mycobacterium gordonae*
Human herpesvirus 6	*Mycobacterium smegmatis*
Human herpesvirus 7	*Propionibacterium acnes*
Human herpesvirus 8	*Streptococcus pneumoniae*
Varicella-Zoster virus	*Salmonella typhi*
Vaccinia virus	*Aspergillus niger*
BK Polyomavirus	*Candida albicans*
Human Papillomavirus 16	*Cryptococcus neoformans*

POTENTIALLY INTERFERING SUBSTANCES

The susceptibility of the Abbott RealTi*m*e CMV assay to interference by elevated levels of potentially interfering substances was evaluated. Ten Anti-CMV IgG negative plasma samples and plasma samples containing approximately 100 IU/mL and 3,120 IU/mL of CMV DNA were spiked with high levels of hemoglobin, bilirubin, protein, lipids, or genomic DNA and tested. No interference in the performance of the Abbott RealTi*m*e CMV assay was observed in the presence of the following endogenous substances for all CMV positive and negative samples tested:

- Hemoglobin 2 g/L
- Bilirubin 342 µM
- Protein 120 g/L
- Lipid 37 mM
- Genomic DNA 350 µg/dL

Forty-four therapeutic drugs were tested in 9 pools, and individually for 13 drugs. (See table below) CMV DNA negative plasma samples and plasma samples containing approximately 100 IU/mL and 3,120 IU/mL of CMV DNA were spiked with the drugs. No interference in the performance of the Abbott RealTi*m*e CMV assay was observed in the presence of the following drugs and drug pools in excess of peak plasma or serum levels, or in excess of therapeutic dose when peak plasma or serum levels were not available.

Drug Pool	Drugs Tested
1	zidovudine, saquinavir, ritonavir, clarithromycin, interferon 2b
2	abacavir sulfate, amprenavir, peginterferon 2a, peginterferon 2b,[a] ribavirin
3	tenofovir disoproxil fumarate, lamivudine, indinavir sulfate, ganciclovir, valganciclovir hydrochloride, acyclovir
4	stavudine, efavirenz, lopinavir, enfuvirtide, ciprofloxacin
5	nevirapine, nelfinavir, azithromycin, valacyclovir
6	adefovir, didanosine, entecavir, cidofovir, mycophenolate mofetil
7	famotidine, cyclosporine
8	prednisone, sirolimus, tacrolimus, azathioprine
9	atenolol, amlodipine besylate, lisinopril, rabeprazole, valsartan

[a] Peginterferon 2b was not tested with the 100 IU/mL sample.

NOTE: A consideration was made to avoid combining specific drugs within a pool that would not be used together in a clinical setting. For drug interference evaluated using drug pools, effects of individual drugs were not assessed with the exception of the 13 therapeutic drugs that were tested individually.

Drugs Tested Individually
lymphocyte immune globulin
cyclosporine
tacrolimus
mycophenolate mofetil
azathioprine
ganciclovir
valganciclovir
foscarnet
everolimus
adefovir*
didanosine*
entecavir*
cidofovir*

*The drugs were tested individually with CMV positive samples at 100 IU/mL.

The susceptibility of the Abbott RealTi*m*e CMV assay to interference by autoimmune disease states was evaluated. Plasma from 10 patients each with Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis (RA), and Anti-nuclear antibodies (ANA) were tested.

Each sample was tested unspiked and spiked with CMV virus to approximately 100 IU/mL and 3,120 IU/mL. Results showed that these disease states do not interfere with the Abbott RealTi*m*e CMV assay.

ANALYTICAL CARRYOVER

Potential sample carryover in the automated Abbott *m*2000 instrument was determined by testing 218 high concentration CMV positive plasma samples interspersed with 220 negative samples arranged in a checkerboard pattern. The positive samples were spiked with CMV DNA at a target concentration of 15.6 million IU/mL. The carryover rate is defined as the number of CMV negative samples that report a value greater than the assay LOD over the total number of CMV negative samples tested. A total of 5 runs were evaluated. The carryover rate was 0.0% (0/220).

CLINICAL STUDIES

The clinical utility of the Abbott RealTi*m*e CMV assay was evaluated in a prospective, multicenter trial of subjects undergoing allogenic hematopoietic stem cell transplantation (HCT). All HCT recipients were CMV-seropositive. Plasma CMV viral load levels were monitored in subjects according to the following schedule: weekly during days 0 through 100 post-transplant, every other week during days 101 through 180, every 30 days during days 181 through 365. Once a subject commenced CMV-specific antiviral treatment (CMV AVT), plasma viral load testing occurred on a weekly basis until CMV AVT was discontinued; at which point regularly scheduled viral load assessments resumed.

The date and time of initiation and discontinuation of CMV AVT was recorded and CMV viral level in close proximity was used to establish beginning of therapy (BOT) and end of therapy (EOT) viral load levels, respectively. Viral load levels to define baseline were obtained within 0 to + 14 days of transplantation, while BOT viral load levels were collected – 7 to + 2 days of initiation of CMV AVT, EOT was defined as immediately following discontinuation of CMV AVT. If there were multiple viral load measurements within the window, the 1 closest to the baseline, BOT, or EOT date was chosen.

Data from 93 subjects were analyzed. Of the 93 subjects, 64 were treated with CMV AVT. The remaining 29 subjects received AVT that was not specific for CMV infection (non–CMV AVT). The demographics and ages of the CMV AVT and non–CMV AVT subjects included in the analyses are shown in **Table 10** and **Table 11**, respectively. The mean age for the 93 subjects included in the analysis was 52 years old with a range of 21 to 72 years old.

Table 10. Summary of Demographics

		CMV AVT Subjects (n = 64)	Non-CMV AVT Subjects (n = 29)	Total Subjects (n = 93)
	Category	**n (%)**	**n (%)**	**n (%)**
Gender	Female	29 (45.3%)	13 (44.8%)	42 (45.2%)
	Male	35 (54.7%)	16 (55.2%)	51 (54.8%)
Race	Asian	10 (15.6%)	2 (6.9%)	12 (12.9%)
	Black or African American	0 (0.0%)	1 (3.4%)	1 (1.1%)
	White	54 (84.4%)	26 (89.7%)	80 (86.0%)
Ethnicity	Hispanic or Latino	2 (3.1%)	1 (3.4%)	3 (3.2%)
	Not Hispanic or Latino	62 (96.9%)	28 (96.6%)	90 (96.8%)

Table 11. Summary of Age (Years)

		CMV AVT Subjects	Non-CMV AVT Subjects	Total Subjects
	Category	**(n = 64)**	**(n = 29)**	**(n = 93)**
Age	Mean Years (SD)	53 (12)	51 (13)	52 (13)
	Range (min, max)	(21, 72)	(25, 72)	(21, 72)

The mean number and range of CMV viral load measurements for the 93 subjects included in the analyses are shown in **Table 12**.

Table 12. Mean and Range of the Number of Viral Load Measurements

Status	Subjects	Mean	Range
CMV AVT	64	25.0	6, 56
Non-CMV AVT	29	23.6	3, 38
All Subjects	93	24.6	3, 56

The mean and duration of the first course of therapy for 63 of the CMV AVT subjects is shown in **Table 13**.

The first course of CMV AVT is defined as the earliest CMV AVT start date and end date for CMV AVT subjects.

Table 13. Mean and Range of the Duration of First Course of CMV AVT (Days)

Subjects[a]	Mean (Days)	Range
63	28.6	1, 123

[a] One subject was excluded; the CMV AVT end date was not provided.

A summary of the CMV specific drugs used for treating the subjects for their first course of CMV AVT are shown in **Table 14**. Ganciclovir, Valganciclovir Hydrochloride, and Valganciclovir were the most commonly used CMV AVT drugs for the subjects' first CMV AVT.

Table 14. Summary of Drugs Used for First CMV AVT

Drug Name	Total Number of Subjects with CMV AVT	Number of Subjects Receiving the Drug	Percentage of Subjects Receiving the Drug (%)
Ganciclovir	64	31	48.4 (31/64)
Ganciclovir Sodium	64	2	3.1 (2/64)
Valganciclovir Hydrochloride	64	29	45.3 (29/64)
Valganciclovir	64	17	26.6 (17/64)
Foscarnet Sodium	64	3	4.7 (3/64)
Foscarnet	64	2	3.1 (2/64)
Immunoglobulin Cytomegalovirus	64	3	4.7 (3/64)

Comparison of viral loads at BOT versus baseline and peak versus EOT are shown in **Table 15** for CMV AVT subjects. Sixty-two CMV AVT subjects were included in this analysis. Measurements for 58 CMV AVT subjects fell within the time window for baseline and BOT. Measurements for 61 CMV AVT subjects fell within the time window for EOT. The mean difference in viral load between the BOT and baseline for 58 subjects for whom results were available at both time points was 2.62 log IU/mL with a 95% confidence interval of 2.16 to 3.08. The p-value is less than 0.0001 which shows the mean difference is significantly different than zero. The mean difference in viral load between peak and EOT for the 61 subjects for whom results were available at both time points was 2.14 log IU/mL, with a 95% confidence interval of 1.81 to 2.47. The p-value is less than 0.0001 which shows the mean difference is significantly different than zero.

Table 15. Comparison of CMV Viral Load Between Different Time Points (Baseline vs BOT, Peak vs EOT) for the First Course of CMV AVT

	n[a]	Mean Difference (SD)	95% Confidence Interval	p-value
Difference in VL Between BOT and Baseline (log IU/mL)	58[b]	2.62 (1.733)	2.16, 3.08	< 0.0001[d]
Difference in VL Between Peak and EOT (log IU/mL)	61[c]	2.14 (1.293)	1.81, 2.47	< 0.0001[e]

[a] A total of 2 subjects were excluded. One subject was excluded because no CMV AVT end date was provided; 1 subject was excluded because there was no viral load measurement during the first course of CMV AVT.

[b] The viral load measurements of 2 subjects fell outside of the time window of BOT; the viral load measurements of 2 subjects fell outside of the time window of baseline.

[c] The viral load measurement of 1 subject fell outside of the time window of EOT.

[d] p-value comparing VL at BOT vs VL at baseline (using paired t-test).

[e] p-value comparing VL at peak vs VL at EOT (using paired t-test).

Viral loads for CMV AVT subjects during the first course of CMV AVT versus viral loads for non-CMV AVT subjects during 1 year post-transplant were also analyzed and are shown in **Table 16**.

This analysis demonstrates that, during the first course of CMV AVT, the CMV viral loads at BOT and peak are statistically significantly higher than the highest CMV viral loads for subjects with non-CMV AVT.

Table 16. Viral Load Analysis for the First Course of CMV AVT vs non-CMV AVT Populations

	n[a]	Mean (SD)	Range	p-value
VL at BOT (log IU/mL) for CMV AVT Subjects	60[b]	3.48 (1.205)	0, 5.93	0.0001[c]
VL at Peak (log IU/mL) for CMV AVT Subjects	62	3.66 (1.138)	0, 5.93	< 0.001[d]
VL at Peak (log IU/mL) for non-CMV AVT Subjects	29	2.46 (0.976)	0, 4.04	

[a] A total of 2 subjects were excluded. One subject was excluded because no CMV AVT end date was provided; 1 subject was excluded because there was no viral load measurement during the first course of CMV AVT.

[b] The viral load measurements of 2 subjects fell outside of the time window of BOT.

[c] p-value comparing VL at BOT for first CMV AVT subjects vs VL at peak for non-CMV AVT subjects using a t-test.

[d] p-value comparing VL at peak for first CMV AVT subjects vs VL at peak for non-CMV AVT subjects using a t-test.

CONCLUSIONS DRAWN FROM THE STUDIES

The results of the nonclinical and clinical laboratory studies support the use of the Abbott RealTi*m*e CMV assay as an aid in the management of HCT patients who are undergoing anti-cytomegalovirus therapy. In this population, serial DNA measurement can be used to assess virological response to anti-cytomegalovirus therapy.

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Technical Assistance:

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

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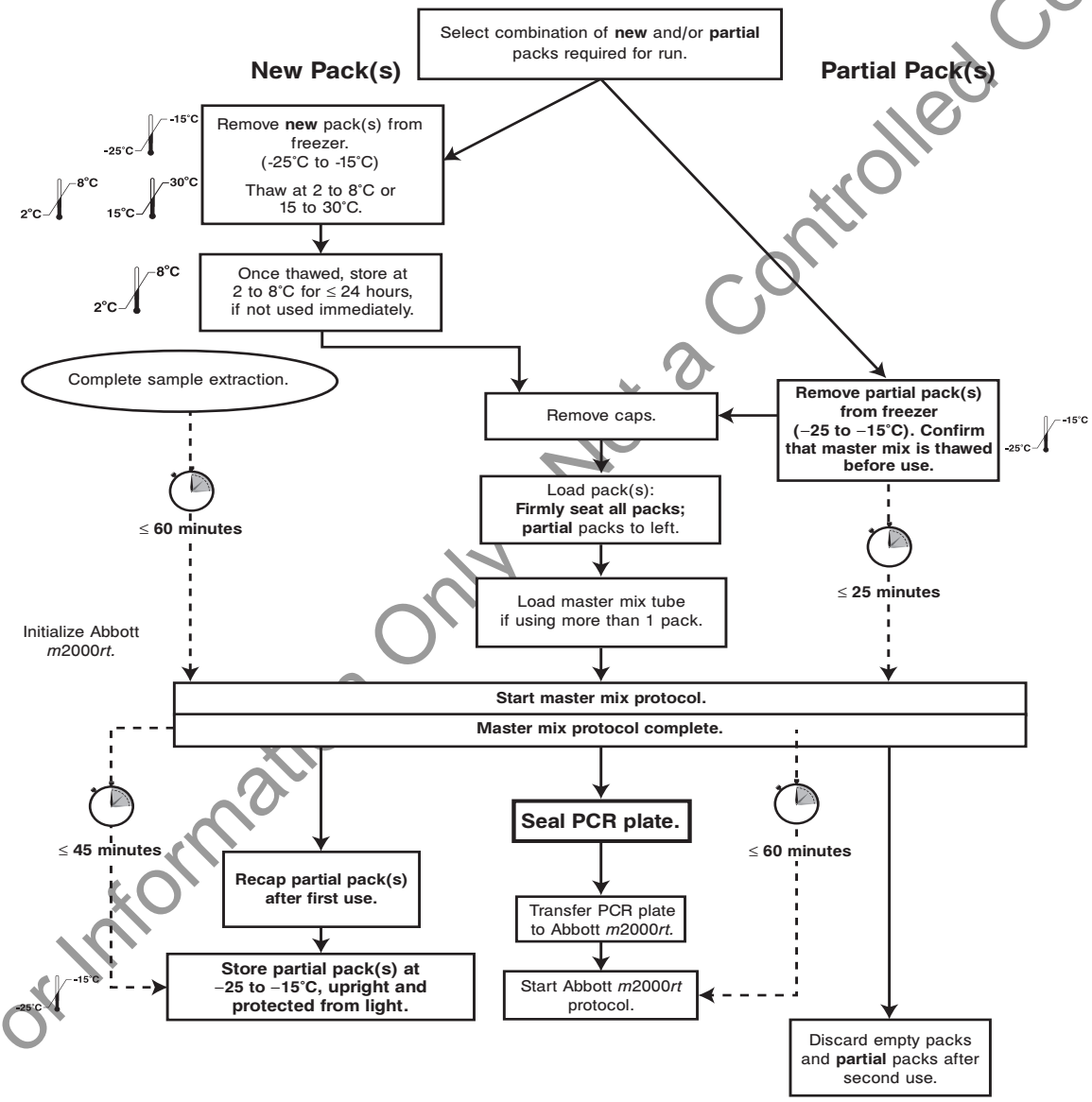
OVERVIEW OF THE ABBOTT REALT*IME* CMV AMPLIFICATION REAGENT EXTENDED USE FEATURE

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

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



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