Aptima® CMV Quant Assay

Instructions for Use
For *in vitro* diagnostic use
For US export only

General Information

Intended Use

The Aptima® CMV Quant assay is an in vitro nucleic acid amplification test for the quantitation of human cytomegalovirus DNA in human EDTA plasma and whole blood on the fully automated Panther® System.

The Aptima CMV Quant assay is intended for use to aid in the diagnosis and in the management of solid-organ and hematopoietic stem cell transplant patients.

The Aptima CMV Quant assay is not intended for use as a screening assay for the presence of CMV in blood or blood products.

Summary and Explanation of the Test

Human CMV is a ubiquitous, linear double-stranded DNA virus of 240 kb that belongs to the herpes family. Depending on the population studied and the geographic region, CMV seroprevalence ranges from 45 to 100% worldwide.[1,2] In immunocompetent hosts, CMV infection is generally asymptomatic and self-limited. However, in immunocompromised individuals, such as transplant recipients and individuals infected with human immunodeficiency virus, CMV is an important cause of morbidity and mortality.

Similar to other herpes viruses, after primary infection CMV establishes a lifelong latent infection that may sporadically reactivate. In transplant recipients, transfer of latent CMV in the graft or reactivation of latent CMV infection in the host may result in wide spread viral replication and dissemination to multiple organs, that is often life-threatening.[3]

Quantitative nucleic acid amplification testing is the preferred method for monitoring of CMV infection and disease in transplant recipients because it is rapid and sensitive.[4] Recent guidelines recommend at least weekly monitoring of CMV viral load to guide decisions to start anti-CMV therapy and to monitor response to therapy.[5,6,7,8] In general, higher viral load values are correlated with increased risk for CMV disease.[4,9] Thus, quantitation of CMV DNA in conjunction with clinical presentation and other laboratory markers is crucial in the management of patients with CMV infection.

Principles of the Procedure

The Aptima CMV Quant assay is an in vitro nucleic acid amplification test that uses real-time transcription-mediated amplification (TMA) technology on the Panther system* to quantify CMV DNA, genotypes 1, 2, 3, and 4. The primer design targets the highly conserved UL56 gene to ensure accurate quantitation of the CMV DNA. The assay is standardized to the 1st WHO International Standard (NIBSC code: 09/162) for human cytomegalovirus.[21]

The Aptima CMV Quant assay involves three main steps, which take place in a single tube on the Panther system: target capture, target amplification by TMA, and detection of the amplification products (amplicon) by the fluorescently labeled probes (torches).

During target capture, viral DNA is isolated from specimens. The specimen is treated with a detergent to solubilize the viral envelope, denature proteins, and release viral genomic DNA. Capture oligonucleotides hybridize to highly conserved regions of CMV DNA, if present, in the test specimen. The hybridized target is then captured onto magnetic microparticles that are separated from the specimen in a magnetic field. Wash steps remove extraneous components from the reaction tube.

* Including variants of the Panther system.

Target amplification occurs via TMA, which is a transcription-mediated nucleic acid amplification method that utilizes two enzymes, Moloney murine leukemia virus (MMLV) reverse transcriptase and T7 RNA polymerase. The reverse transcriptase is used to generate a DNA copy (containing a promoter sequence for T7 RNA polymerase) of the target sequence. T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template.

Detection is achieved using single-stranded nucleic acid torches that are present during the amplification of the target and that hybridize specifically to the amplicon in real time. Each torch has a fluorophore and a quencher. When the torch is not hybridized to the amplicon, the quencher is in close proximity of the fluorophore and suppresses the fluorescence. When the torch binds to the amplicon, the quencher is moved farther away from the fluorophore, which will emit a signal at a specific wavelength when excited by a light source. As more torches hybridize to amplicon, a higher fluorescent signal is generated. The time taken for the fluorescent signal to reach a specified threshold is proportional to the starting CMV concentration. Each reaction has an internal calibrator/internal control (IC) that controls for variations in specimen processing, amplification, and detection. The concentration of a sample is determined by the Panther system software using the CMV and IC signals for each reaction and comparing them to calibration information.

Assay results are converted from copies/mL to IU/mL using a conversion factor equation embedded in the Panther software. The same conversion factor equation is used for both whole blood and plasma specimens. A dilution factor of 4 is applied to CMV viral load results for whole blood specimens when the Whole Blood Conversion Factor is selected on the Panther system.

Summary of Safety and Performance

The SSP (Summary of Safety and Performance) is available in the European database on medical devices (Eudamed), where it is linked to the device identifiers (Basic UDI-DI). To locate the SSP for Aptima CMV Quant assay, refer to the Basic Unique Device Identifier (BUDI): **54200455DIAGAPTCMVAP**.

Warnings and Precautions

A. For *in vitro* diagnostic use.

B. For professional use.

C. To reduce the risk of invalid results, carefully read the entire package insert and the appropriate *Panther/Panther Fusion System Operator's Manual* prior to performing this assay.

Laboratory Related

D. CAUTION: The controls for this assay contain human plasma. The plasma is negative for hepatitis B surface antigen (HBsAg), antibodies to HCV, antibodies to HIV-1 and HIV-2, and HIV antigen when tested with US Food and Drug Administration licensed procedures. In addition, the plasma is nonreactive for CMV DNA, HBV DNA, HCV RNA, and HIV-1 RNA when tested with licensed nucleic acid tests using pooled samples. All human blood sourced materials should be considered potentially infectious and should be handled with Universal Precautions.[10,11,12]

E. Only personnel adequately trained in the use of the Aptima CMV Quant assay and in handling potentially infectious materials should perform this procedure. If a spill occurs, immediately disinfect following appropriate site procedures.

F. Use only supplied or specified disposable laboratory ware.

G. Use routine laboratory precautions. Do not pipet by mouth. Do not eat, drink or smoke in designated work areas. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

H. Work surfaces, pipettes, and other equipment must be regularly decontaminated with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution.

I. Dispose of all materials that have come in contact with specimens and reagents according to regional regulations.[10,11,12,13] Thoroughly clean and disinfect all work surfaces.

J. The controls contain sodium azide as a preservative. Do not use metal tubing for reagent transfer. If solutions containing sodium azide compounds are disposed of in a plumbing system, they should be diluted and flushed with generous amounts of running water. These precautions are recommended to avoid accumulation of deposits in metal piping in which explosive conditions could develop.

K. Good standard practices for molecular laboratories include environmental monitoring. To monitor a laboratory's environment, the following procedure is suggested:

1. Obtain a cotton-tipped swab and pair with the Aptima Specimen Aliquot Tube (SAT).
2. Label each SAT appropriately.
3. Fill each SAT with 1 mL of Aptima Specimen Diluent.
4. To collect the surface samples, lightly moisten a swab with nuclease-free deionized water.
5. Swab the surface of interest using a top to bottom vertical motion. Rotate the swab approximately one-half turn while swabbing the location.
6. Immediately place the swab sample into the tube and gently swirl the swab in the diluent to extract potential swabbed materials. Press the swab on the side of the transport tube to extract as much liquid as possible. Discard the swab and cap the tube.
7. Repeat steps for remaining swab samples.
8. Test swab with molecular assay.

Specimen Related

L. Specimens may be infectious. Use Universal Precautions[10,11,12] when performing this assay. Proper handling and disposal methods should be established according to local regulations.[11] Only personnel adequately trained in the use of the Aptima CMV Quant assay and trained in handling infectious materials should perform this procedure.

M. Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.

N. Avoid cross-contamination during the specimen handling steps. Be especially careful to avoid contamination by the spread of aerosols when loosening or uncapping specimens. Specimens can contain extremely high levels of organisms. Ensure that specimen

containers do not contact one another, and discard used materials without passing over open containers. Change gloves if they come in contact with specimen.

Assay Related

O. In case of an invalid result due to ML2 error, do not retest the neat plasma specimen. Refer to *Panther System Test Procedure*, step E.5, in this package insert for instructions to dilute the plasma specimen.

Note: *For ML2 error, refer to the appropriate Panther/Panther Fusion System Operator's Manual for Mag Wash Clean Instructions.*

P. Do not use the reagent kit, the calibrator, or the controls after the expiration date.

Q. Do not interchange, mix, or combine assay reagents from kits with different master lot numbers. Assay fluids can be from different lot numbers. Controls and the calibrator can be from different lot numbers.

R. Avoid microbial and nuclease contamination of reagents.

S. Cap and store all assay reagents at specified temperatures. The performance of the assay may be affected by use of improperly stored assay reagents. See *Reagent Storage and Handling Requirements* and *Panther System Test Procedure* for more information.

T. Do not combine any assay reagents or fluids without specific instruction. Do not top off reagents or fluids. The Panther system verifies reagent levels.

U. Avoid contact of TER with skin, eyes, and mucous membranes. Wash with water if contact with this reagent occurs. If spills of this reagent occurs, dilute with water and follow appropriate site procedures.

V. Some reagents in this kit are labeled with risk and safety symbols.

Note: *Hazard communication reflects the EU Safety Data Sheets (SDS) classifications. For hazard communication information specific to your region, refer to the region specific SDS on the Safety Data Sheet Library at www.hologicsds.com. For more information on the symbols, refer to the symbol legend on http://www.hologic.com/package-inserts*

EU Hazard Information	
—	**Amplification Reagent** ***Magnesium Chloride 65 - 70%*** — H412 - Harmful to aquatic life with long lasting effects. P273 - Avoid release to the environment. P501 - Dispose of contents/ container to an approved waste disposal plant.
—	**Enzyme Reagent** ***Triton X-100 1 - 5%*** ***HEPES 1 - 5%*** — H402 - Harmful to aquatic life. P273 - Avoid release to the environment. P501 - Dispose of contents/ container to an approved waste disposal plant.

	Enzyme Reconstitution Solution ***Glycerol 20 - 25%*** ***Triton X-100 5 - 10%*** ***HEPES 1 - 5%***
—	— H402 - Harmful to aquatic life. P273 - Avoid release to the environment. P501 - Dispose of contents/ container to an approved waste disposal plant.
	Promoter Reagent ***Magnesium Chloride 55 - 60%***
—	— H412 - Harmful to aquatic life with long lasting effects. P273 - Avoid release to the environment. P501 - Dispose of contents/ container to an approved waste disposal plant.
	Target Capture Reagent ***HEPES 15-20%*** ***Lauryl Sulfate Lithium Salt 5-10%*** ***Succinic Acid 1-5%*** ***Lithium Hydroxide, Monohydrate 1-5%***
—	— H402 - Harmful to aquatic life. P273 - Avoid release to the environment P501 - Dispose of contents/ container to an approved waste disposal plant.
 	**Target Enhancer Reagent (TER)** ***Lithium Hydroxide, Monohydrate 5-10%*** **DANGER** H302 - Harmful if swallowed H314 - Causes severe skin burns and eye damage P264 - Wash face, hands and any exposed skin thoroughly after handling. P270 - Do not eat, drink or smoke when using this product. P301 + P312 - IF SWALLOWED: Call a POISON CENTER or doctor if you feel unwell. P330 - Rinse mouth. P501 - Dispose of contents/ container to an approved waste disposal plant. P260 - Do not breathe dust/fume/gas/mist/vapours/spray. P280 - Wear protective gloves/protective clothing/eye protection/face protection. P301 + P330 + P331 - IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. P303 + P361 + P353 - IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower]. P304 + P340 - IF INHALED: Remove person to fresh air and keep comfortable for breathing. P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P310 - Immediately call a POISON CENTER or doctor. P321 - Specific treatment (see supplemental first aid instructions on this label). P363 - Wash contaminated clothing before reuse. P405 - Store locked up.
	**CMV Kit Controls** ***Human Serum/Human Plasma 95-100%*** ***Sodium Azide < 1%*** — H412 - Harmful to aquatic life with long lasting effects P501 - Dispose of contents/ container to an approved waste disposal plant P273 - Avoid release to the environment
	Kit Calibrator ***Lauryl Sulfate Lithium Salt 0-10%*** ***Succinic Acid 0-10%***
—	— H402 - Harmful to aquatic life P273 - Avoid release to the environment P501 - Dispose of contents/ container to an approved waste disposal plant.

Reagent Storage and Handling Requirements

A. The following table shows the storage conditions and stability for reagents, controls, and calibrator.

Reagent	Unopened Storage	Open Kit (Reconstituted)	
		Storage	Stability
qCMV Amplification Reagent	2°C to 8°C		
qCMV Amplification Reconstitution Solution	2°C to 8°C	2°C to 8°C	30 days[a]
qCMV Enzyme Reagent	2°C to 8°C		
qCMV Enzyme Reconstitution Solution	2°C to 8°C	2°C to 8°C	30 days[a]
qCMV Promoter Reagent	2°C to 8°C		
qCMV Promoter Reconstitution Solution	2°C to 8°C	2°C to 8°C	30 days[a]
qCMV Target Capture Reagent	2°C to 8°C	2°C to 8°C	30 days[a]
qCMV PCAL (Positive Calibrator)	-15°C to -35°C	15°C to 30°C	Single use vial Use within 24 hours
qCMV NC CONTROL – (Negative Control)	-15°C to -35°C	15°C to 30°C	Single use vial Use within 24 hours
qCMV LPC CONTROL + (Low Positive Control)	-15°C to -35°C	15°C to 30°C	Single use vial Use within 24 hours
qCMV HPC CONTROL + (High Positive Control)	-15°C to -35°C	15°C to 30°C	Single use vial Use within 24 hours
qCMV Target Enhancer Reagent	15°C to 30°C	15°C to 30°C	30 days[a]

[a] When reagents are removed from the Panther system, they should be immediately returned to their appropriate storage temperatures.

B. Discard any unused reconstituted reagents, target capture reagent (TCR), and target enhancer reagent (TER) after 30 days or after the Master Lot expiration date, whichever comes first.

C. Reagents stored onboard the Panther system have 96 hours of onboard stability. Reagents can be loaded onto the Panther system up to 8 times. The Panther system logs each time the reagents are loaded.

D. After thawing the calibrator, the solution must be clear, i.e., not cloudy or have precipitates. Ensure that precipitates are dissolved. Do not use the calibrator if gelling, precipitation, or cloudiness is present.

E. The lyophilized promoter reagent and reconstituted promoter reagent are photosensitive. Protect these reagents from light during storage and preparation for use.

F. The qCMV Target Enhancer Reagent must be at 15°C to 30°C before use.

Specimen Collection and Storage

Note: *Handle all specimens as if they contain potentially infectious agents. Use Universal Precautions.*

Note: *Take care to avoid cross-contamination during sample handling steps. For example, discard used material without passing over open tubes.*

Note: *Only plastic secondary tubes are recommended for sample storage.*

Whole blood specimens collected in the following glass or plastic tubes may be used to prepare plasma:

- Tubes containing EDTA anticoagulants
- Plasma preparation tubes (PPTs)

A. Specimen Collection

1. Plasma: Whole blood can be stored at 2°C to 30°C and must be centrifuged within 24 hours of specimen collection. Separate the plasma from the pelleted red blood cells following the manufacturer's instructions for the tube used. Plasma can be tested on the Panther system in a primary tube or transferred to a secondary tube such as an Aptima Specimen Aliquot Tube (SAT). To obtain the 500 µL sample volume, the minimum volume of plasma for primary collection tubes is up to 1200 µL. For secondary tubes, the minimum volume is 700 µL to obtain the 500 µL sample volume. The following table identifies dead volume requirements for each primary and secondary tube type.

Tube (Size and Type)	Dead Volume on Panther
Aptima Sample Aliquot Tube (SAT)	0.2 mL
12x75 mm	0.5 mL
13x100 mm	0.5 mL
13x100 mm with Gel	0.3 mL
16x100 mm with Gel	0.7 mL

If not tested immediately, plasma can be stored in accordance with the specifications below. If transferred to a secondary tube, plasma may be frozen at -20°C or -70°C. Do not exceed 3 freeze–thaw cycles. Do not freeze plasma specimens in EDTA primary collection tubes.

2. Whole blood must be processed using pre-filled Whole Blood Diluent tubes before being tested on the Panther system. Do not exceed 3 freeze–thaw cycles for unprocessed whole blood samples.

B. Specimen Storage Conditions

1. EDTA Plasma Specimens

Whole blood can be stored at 2°C to 30°C and must be centrifuged within 24 hours of specimen collection. Plasma may then be stored under one of the following conditions:

- In the primary collection tube or secondary tube at 2°C to 30°C for up to 24 hours,

- In the primary collection tube or secondary tube at 2°C to 8°C for up to 5 days, or
- In the secondary tube at -20°C or -70°C for up to 60 days.

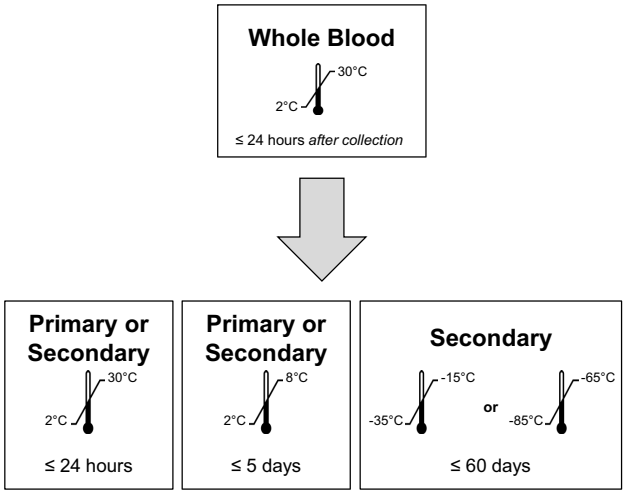

Figure 1. Storage Conditions for EDTA Tubes

2. PPT Specimens

   Whole blood can be stored at 2°C to 30°C and must be centrifuged within 24 hours of specimen collection. Plasma may then be stored under one of the following conditions:

   - In the PPT at 2°C to 30°C for up to 24 hours,
   - In the PPT at 2°C to 8°C for up to 5 days, or
   - In the PPT at -20°C or -70°C for up to 60 days.

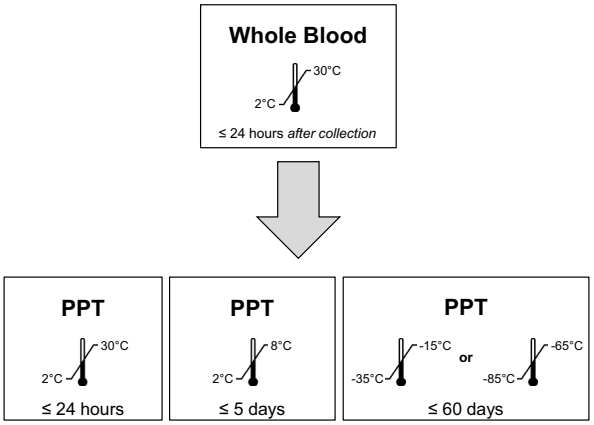

Figure 2. Storage Conditions for PPTs

3. Dilution of Plasma Specimens

   Plasma specimens may be diluted in the SAT or secondary tube for testing on the Panther system. See *Panther System Test Procedure*, step E.5 below for more information.

   Note: *If a specimen is diluted, it should be tested immediately after dilution. Do not freeze a diluted specimen.*

4. Whole Blood Specimens

   Whole blood can be stored at 15°C to 30°C up to 36 hours after specimen collection. Collected whole blood may be stored under one of the following conditions:

   - In the primary collection tube at 2°C to 8°C for up to 5 days or
   - In the primary collection tube at -20°C or -70°C for up to 60 days.

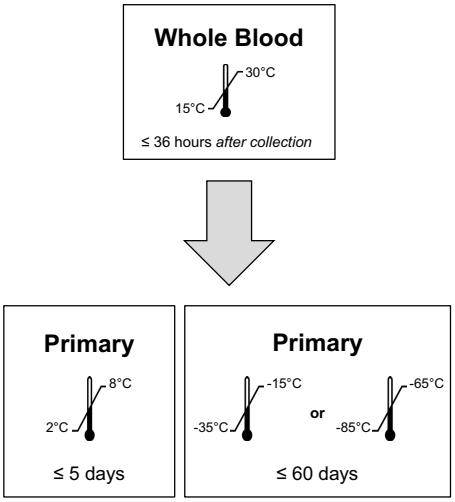

Figure 3. Storage Conditions for Whole Blood Specimens

Samples Onboard the Panther System

Plasma and processed whole blood samples may be left on the Panther system uncapped for up to 8 hours. Samples may be removed from the Panther system and tested as long as the total time onboard does not exceed 8 hours prior to the pipetting of the sample by the Panther system.

Specimen Transport

Maintain sample storage conditions as described in *Specimen Collection and Storage*.

Note: *Specimens must be shipped in accordance with applicable national, international, and regional transportation regulations.*

Panther System

Reagents for the Aptima CMV Quant assay are listed below for the Panther system. Reagent identification symbols are also listed next to the reagent name.

Reagents and Materials Provided

Aptima CMV Quant Assay Kit, 100 tests (Cat. No. PRD-05074)
(1 assay box, 1 target enhancer reagent box, 1 calibrator kit, and 1 controls kit)

Aptima CMV Quant Assay Box
(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity
A	**qCMV Amplification Reagent** *Non-infectious nucleic acids dried in buffered solution.*	1 vial
E	**qCMV Enzyme Reagent** *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution.*	1 vial
PRO	**qCMV Promoter Reagent** *Non-infectious nucleic acids dried in buffered solution.*	1 vial
AR	**qCMV Amplification Reconstitution Solution** *Aqueous solution containing glycerol and preservatives.*	1 x 7.2 mL
ER	**qCMV Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 x 5.8 mL
PROR	**qCMV Promoter Reconstitution Solution** *Aqueous solution containing glycerol and preservatives.*	1 x 4.5 mL
TCR	**qCMV Target Capture Reagent** *Nucleic acids in a buffered salt solution containing solid phase, non-infectious nucleic acids, and Internal Calibrator.*	1 x 72.0 mL
	Reconstitution Collars	3
	Master Lot Barcode Sheet	1 sheet

Aptima CMV Quant Target Enhancer Reagent Box
(store at 15°C to 30°C upon receipt)

Symbol	Component	Quantity
TER	**qCMV Target Enhancer Reagent** *A concentrated solution of lithium hydroxide.*	1 x 46.0 mL

Aptima CMV Quant Calibrator Kit (Cat. No. PRD-05075)
(store at -15°C to -35°C upon receipt)

Symbol	Component	Quantity
PCAL	**qCMV Positive Calibrator** *Plasmid DNA in buffered solution.*	5 x 2.5 mL
	Calibrator Barcode Label	—

Aptima CMV Quant Controls Kit (Cat. No. PRD-05076)
(store at -15°C to -35°C upon receipt)

Symbol	Component	Quantity
NC	**qCMV Negative Control** *CMV negative defibrinated human plasma containing gentamicin and 0.2% sodium azide as preservatives.*	5 x 0.8 mL
LPC	**qCMV Low Positive Control** *Inactivated CMV in defibrinated human plasma containing gentamicin and 0.2% sodium azide as preservatives.*	5 x 0.8 mL
HPC	**qCMV High Positive Control** *Inactivated CMV in defibrinated human plasma containing gentamicin and 0.2% sodium azide as preservatives.*	5 x 0.8 mL
	Control Barcode Label	—

Materials Required but Available Separately

Note: *Materials available from Hologic have catalog numbers listed, unless otherwise specified.*

Material	Cat. No.
Panther® System	303095
Panther Fusion® System	PRD-04172
Panther System, Continuous Fluid and Waste (Panther Plus)	PRD-06067
Panther Run Kit for Real Time Assays (for real-time assays only)	PRD-03455 (5000 tests)
Aptima® Assay Fluids Kit (also known as Universal Fluids Kit) contains Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent	303014 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther Waste Bag Kit	902731
Panther Waste Bin Cover	504405
Or, Panther System Run Kit *(when running non-real-time-TMA assays in parallel with real-time-TMA assays) contains MTUs, waste bags, waste bin covers, auto detect, and assay fluids*	303096 (5000 tests)
Whole Blood Diluent tubes (for processing whole blood specimens only)	PRD-06783 (100 pre-filled tubes per bag)
Tips, 1000 µL filtered, conductive, liquid sensing, and disposable *Not all products are available in all regions. Contact your representative for region-specific information.*	901121 (10612513 Tecan) 903031 (10612513 Tecan) MME-04134 (30180117 Tecan) MME-04128
Bleach, 5% to 8.25% (0.7 M to 1.16 M) sodium hypochlorite solution	—
Disposable, powderless gloves	—
Replacement non-penetrable caps	103036A
Replacement Hologic Solid Caps (single-use tube cap for whole blood processing)	PRD-06720
Reagent replacement caps	
Amplification, Enzyme, and Promoter reagent reconstitution bottles	*CL0041 (100 caps)*
TCR bottle	*CL0040 (100 caps)*
TER bottle	*903302 (100 caps)*
Plastic-backed laboratory bench covers	—
Lint-free wipes	—
Pipettor	—
Tips	—
Primary collection tubes (EDTA and PPT) options: *13 mm x 100 mm* *13 mm x 75 mm* *16 mm x 100 mm*	—
Centrifuge	—
Vortex mixer	—

Optional Materials

Material	Cat. No.
Secondary tube options:	
12 mm x 75 mm	—
13 mm x 100 mm	—
16 mm x 100 mm	—
Aptima Specimen Aliquot Tubes (SATs) (100 pack)	FAB-18184
Transport tube cap (100 pack) *cap for SAT*	504415
Aptima Specimen Diluent	PRD-03003
Aptima Specimen Diluent Kit *contains Aptima Specimen Diluent, 100 SATs and 100 caps*	PRD-03478
Transfer pipets	—
Cotton-tipped swabs	—
Tube rocker	—

Panther System Test Procedure

Note: *See the appropriate Panther/Panther Fusion System Operator's Manual for additional procedural information.*

A. Work Area Preparation

1. Clean work surfaces where reagents will be prepared. Wipe down work surfaces with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and then follow with a deionized (DI) water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface with clean, plastic-backed absorbent laboratory bench covers.
2. Clean a separate work surface where samples will be prepared. Use the procedure described above (Step A.1).
3. Clean any pipettors. Use the cleaning procedure described above (Step A.1).

B. Calibrator and Controls Preparation

Allow the calibrator and controls to reach 15°C to 30°C prior to processing as follows:

1. Remove the calibrator and controls from storage (-15°C to -35°C) and place at 15°C to 30°C. Throughout the thawing process, gently invert each tube to mix thoroughly. Ensure tube contents are fully thawed prior to use.

   Option. Calibrator and control tubes may be placed on a tube rocker to mix thoroughly. Ensure tube contents are fully thawed prior to use.

   Note: *Avoid creating excessive foam when inverting the calibrator and controls. Foam compromises the level-sensing by the Panther system.*
2. When the tube contents have thawed, dry the outside of the tube with a clean, dry disposable wipe.
3. To prevent contamination, do not open the tubes at this time.

C. Reagent Reconstitution/Preparation of a New Kit

Note: *Reconstitution of reagents should be performed prior to beginning any work on the Panther system.*

1. To prepare Target Capture Reagent (TCR), perform the following:
   a. Remove the TCR from storage (2°C to 8°C). Check the lot number on the TCR bottle to make sure that it matches the lot number on the Master Lot Barcode Sheet.
   b. Immediately shake the TCR bottle vigorously 10 times. Allow the TCR bottle to remain at 15°C to 30°C to warm for at least 45 minutes. During this period, swirl and invert the TCR bottle at least every 10 minutes.

      Option. The TCR bottle may be prepared on a tube rocker by following these instructions: Remove the TCR from storage (2°C to 8°C) and immediately shake vigorously 10 times. Place the TCR bottle on a tube rocker and leave the TCR at 15°C to 30°C to warm for at least 45 minutes.
   c. Ensure all precipitate is in solution and the magnetic particles are suspended before use.
2. To reconstitute Amplification, Enzyme, and Promoter Reagents, perform the following:
   a. Remove the lyophilized reagents and corresponding reconstitution solutions from storage (2°C to 8°C). Pair each reconstitution solution with its lyophilized reagent.
   b. Ensure that the reconstitution solution and lyophilized reagent have matching label colors. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired.
      i. Open the lyophilized reagent vial by removing the metallic seal and rubber stopper.
      ii. Firmly insert the notched end of the reconstitution collar (black) onto the vial (Figure 4, Step 1).
      iii. Open the matching reconstitution solution bottle, and set the cap on a clean, covered work surface.
      iv. Place the reconstitution solution bottle on a stable surface (e.g., bench). Then, invert the lyophilized reagent vial over the reconstitution solution bottle and firmly attach the collar to the reconstitution solution bottle (Figure 4, Step 2).
      v. Slowly invert the assembled bottles (vial attached to solution bottle) to allow the solution to drain into the glass vial (Figure 4, Step 3).
      vi. Pick up the assembled bottles, and swirl the assembled bottles for at least 10 seconds (Figure 4, Step 4).
      vii. Wait for at least 30 minutes for the lyophilized reagent to go into solution.
      viii. After the lyophilized reagent has gone into solution, swirl the assembled bottles for at least 10 seconds and then slightly rock the solution within the glass vial back and forth to mix thoroughly.
   c. Slowly tilt the assembled bottles again to allow all of the solution to drain back into the reconstitution solution bottle (Figure 4, Step 5).
   d. Carefully remove the reconstitution collar and glass vial (Figure 4, Step 6).
   e. Recap the bottle. Record operator initials and reconstitution date on the label (Figure 4, Step 7).

f. Discard the reconstitution collar and glass vial (Figure 4, Step 8).

Warning: *Avoid creating excessive foam when reconstituting reagents. Foam compromises the level-sensing by the Panther system.*

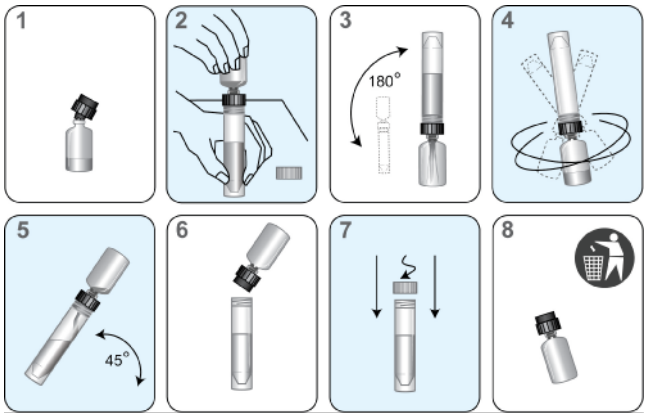

Figure 4. Reagent Reconstitution Process

3. Remove the qCMV Target Enhancer Reagent from storage (15°C to 30°C). Record operator initials and open date on the label. Check the lot number on the TER bottle to make sure it matches the lot number on the Master Lot Barcode Sheet.

D. Reagent Preparation for Previously Prepared Reagents

1. Remove the previously prepared reagents from storage (2°C to 8°C). Previously prepared Amplification, Enzyme and Promoter reagents, and TCR must reach 15°C to 30°C prior to the start of the assay.
2. Remove TER from storage (15°C to 30°C).
3. For previously prepared TCR, perform Step C.1 above prior to loading on the system.
4. Swirl and invert the Amplification, Enzyme, and Promoter reagents to mix thoroughly prior to loading on the system. Avoid creating excessive foam when inverting reagents.

   Option. The previously prepared reagents may be prepared on a tube rocker by following these instructions: Remove the reagents from storage (2°C to 8°C). Place the reagents on a tube rocker and leave at 15°C to 30°C to warm for at least 30 minutes.
5. Do not top off reagent bottles. The Panther system will recognize and reject bottles that have been topped off.

E. Plasma Specimen Handling

1. Ensure that processed specimens in primary tubes or undiluted specimens in secondary tubes are stored properly per *Specimen Collection and Storage*.
2. Ensure frozen specimens are thoroughly thawed. Vortex the thawed specimens for 3 to 5 seconds to mix thoroughly.
3. Allow the specimens to reach 15°C to 30°C prior to processing. See *Samples Onboard the Panther System* for additional onboard information.
4. Ensure each primary collection tube contains up to 1200 µL of specimen or each secondary tube contains at least 700 µL of specimen. Refer to the table provided in *Specimen Collection* to identify dead volume requirements for each primary and

secondary tube type. If plasma specimen dilution is necessary, in cases of low sample volume and/or those that require repeat testing, see step E.5 below for additional information.

5. Dilute Plasma Specimen

   A plasma specimen may be diluted 1:3 in a SAT or a secondary tube for testing on the Panther system.

   a. Thaw Aptima CMV Negative Control

      i. Remove one tube of negative control from storage (-15°C to -35°C) and place at 15°C to 30°C. Throughout the thawing process, gently invert tube to mix thoroughly. Ensure tube content is fully thawed prior to use.

         Option: Control tube may be placed on a tube rocker to mix thoroughly. Ensure tube content is fully thawed prior to use.

      ii. When the tube content has thawed, dry the outside of the tube with a clean, dry disposable wipe.

      iii. To prevent contamination, do not open the tube at this time.

   b. Dilute Plasma Specimen

      Note: *If a specimen is diluted, it must be tested immediately after preparation of dilution.*

      i. Place 240 uL of specimen in the SAT.

      ii. Add 480 uL of negative control.

      iii. Cap the tube.

      iv. Gently invert 5 times to mix.

      Specimens diluted 1:3 can be tested using the 1:3 option on the Panther system (see the *Panther/Panther Fusion system Operator's Manual* for more information). The software will automatically report a neat result by applying the dilution factor. These specimens will be flagged as diluted specimens.

6. Just prior to loading specimens into a Sample Rack, centrifuge each specimen at 1000 to 3000*g* for 10 minutes. Do not remove caps at this step.

   See Step G.2 below, for information about loading the rack and removing the caps.

F. Whole Blood Specimen Handling

1. Ensure that unprocessed specimens in primary tubes are stored properly per *Specimen Collection and Storage*.
2. Ensure frozen specimens are thoroughly thawed. Allow the specimens to reach 15°C to 30°C prior to processing. See *Samples Onboard the Panther System* for additional onboard information.
3. Gently invert whole blood tubes at least 3 times, or mix gently on a rocker, until blood is homogeneous.
4. Before sample processing, perform the following procedure on each specimen.
   a. Blood in the primary tubes should be mixed thoroughly by inversion and the sample should be immediately transferred into the tube containing whole blood diluent.
   b. Add 500 µL whole blood specimen to the pre-filled Whole Blood Diluent tube.
   c. Replace the cap and vortex the sample for at least 5 seconds.

See Step G.2 below, for information about loading the rack and removing the caps.

G. System Preparation

1. Set up the system according to the instructions in the *Panther/Panther Fusion System Operator's Manual* and *Procedural Notes.* Make sure that the appropriately sized reagent racks and TCR adapters are used.
2. Load samples into the Sample Rack. Perform the following steps for each sample tube (specimen, and, when necessary, calibrator and controls):
   a. Loosen one sample tube cap, but do not remove it yet.

      Note: *Be especially careful to avoid contamination by the spread of aerosols. Gently loosen caps on samples.*

   b. Load the sample tube into the Sample Rack.
   c. Repeat Steps 2.a and 2.b for each remaining sample.
   d. After the samples have been loaded into the Sample Rack, remove and discard each sample tube cap in one Sample Rack. To avoid contamination, do not pass a cap over any other Sample Racks or sample tubes.
   e. If necessary, use a new, disposable transfer pipet to remove any bubbles or foam. Bubbles in the tube compromise the level-sensing by the Panther system.
   f. When the last cap has been removed, load the Sample Rack into the Sample Bay.

      Note: *If running other assays and sample types at the same time, secure the Sample Retainer prior to loading the Sample Rack into the Sample Bay.*

   g. Repeat Steps 2.a to 2.f for the next Sample Rack.

H. System Preparation - Applying Whole Blood Specimen Conversion Factor.

1. Set up the system according to the instructions in the *Panther/Panther Fusion System Operator's Manual.*
2. Load specimen rack.
3. Apply Whole Blood Conversion Factor to assay test orders for Whole Blood specimens.

   Note: *Whole Blood Conversion Factor may be applied to an entire rack or a single test order.*

   To apply the Whole Blood Conversion Factor to an entire rack of Whole Blood specimens:

   a. From the *Sample Rack Bay* screen, double-click the loaded rack of interest. The *Sample Rack Loading screen* appears for the selected rack.
   b. Select **Dilute All**.

      The Dilution Factor window appears.

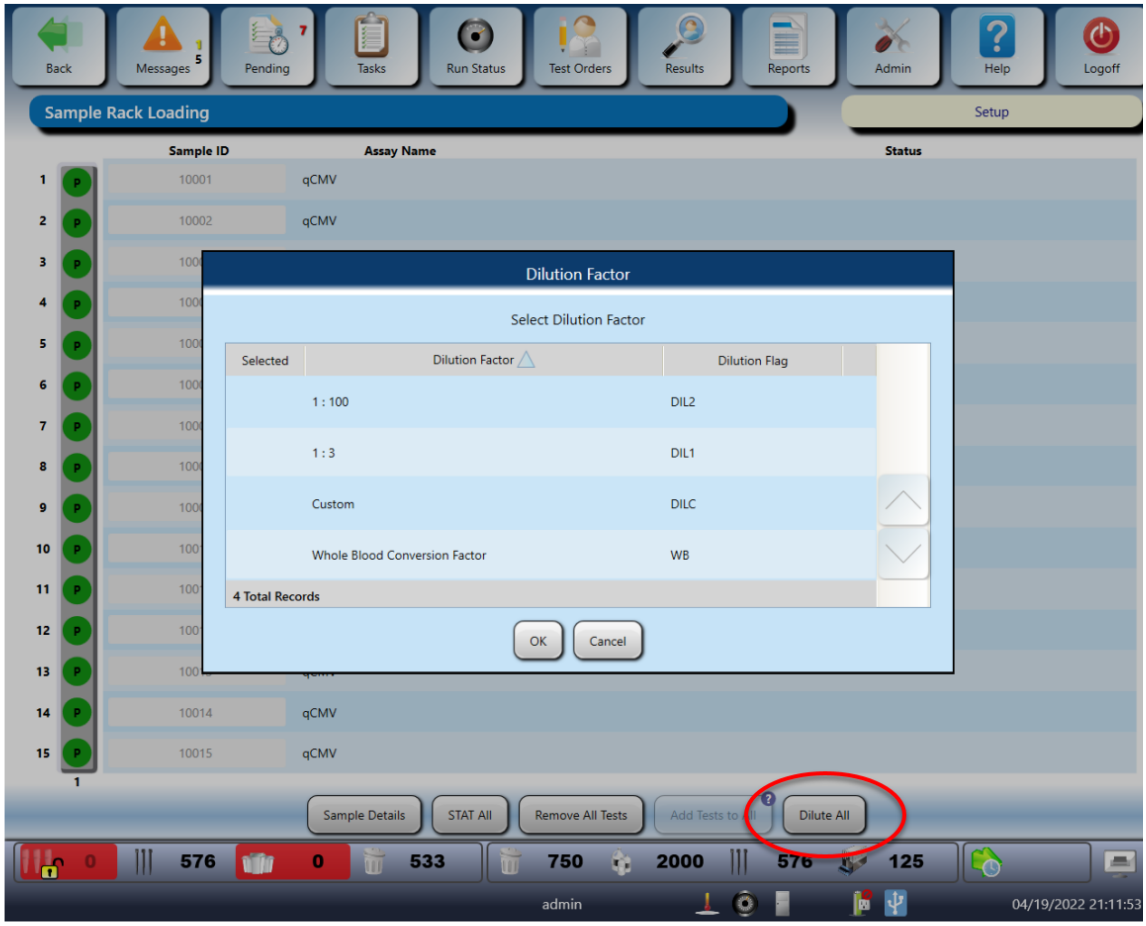

Figure 5. The Dilution Factor Window in the Sample Rack Loading Screen (Example)

c. Select **Whole Blood Conversion Factor**.

d. Select **OK.**

A *Set Dilution Factor for Rack* window appears.

e. Select **Yes** to apply the Whole Blood Conversion Factor flag to the entire rack of Whole Blood specimens.

To apply the Whole Blood Conversion Factor to a single test order (see illustration below):

a. From the *Sample Rack Bay* screen, double-click the loaded rack with the specimen(s) of interest.

The *Sample Rack Loading* screen appears for the selected sample rack.

b. From the *Sample Rack Loading* screen, double-click the specimen of interest.

The *Sample Details* screen appears with the current test orders for the selected specimen.

c. Select the test order of interest from the *Test Orders* panel.

d. Select **Apply Dilution**

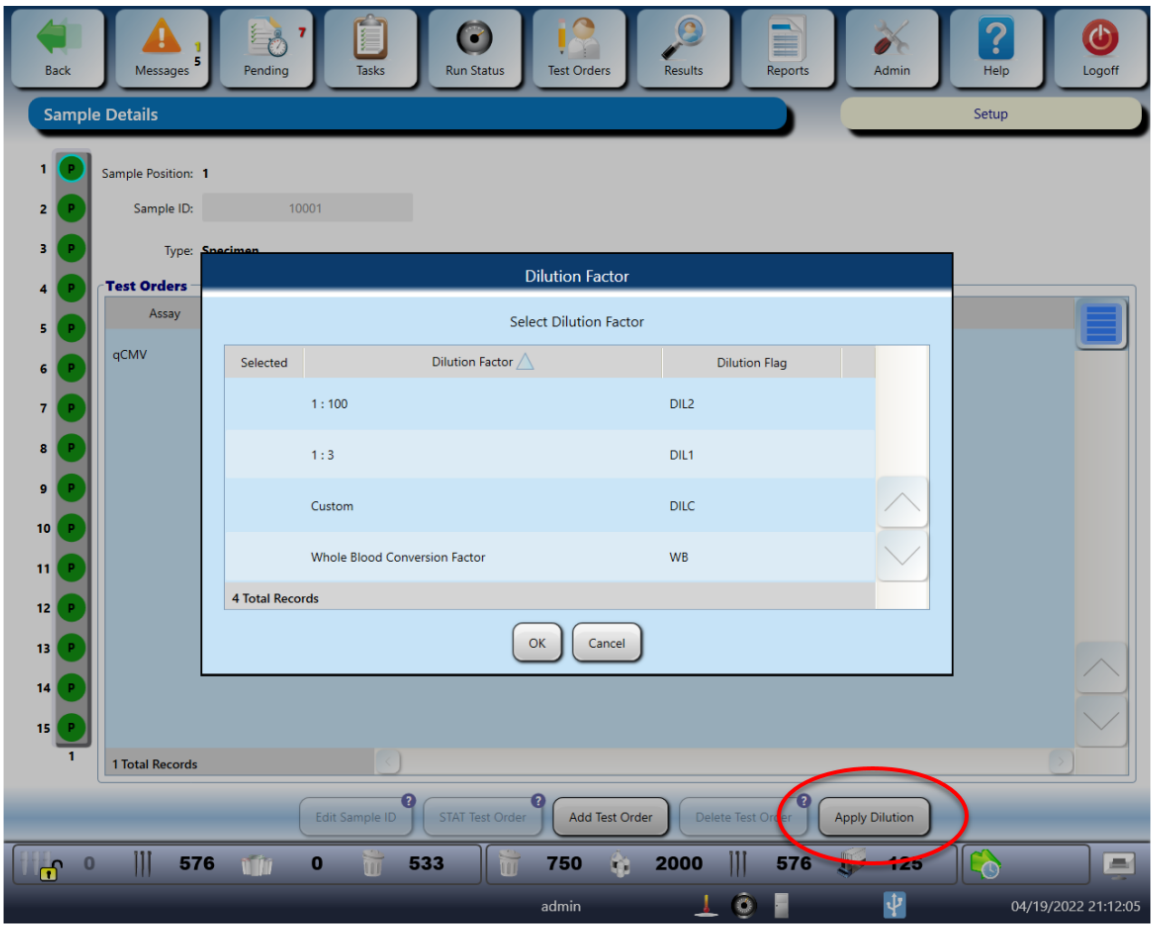

Figure 6. The Dilution Factor Window in the Sample Details Screen (Example)

e. Select **Whole Blood Conversion Factor**.

f. Select **OK** to apply the Whole Blood Conversion Factor flag to all selected test orders.

4. If necessary, the Whole Blood Factor can be removed from test orders prior to the start of processing.

To delete the Whole Blood Conversion Factor from an entire rack:

1. From the *Sample Rack Bay* screen, double-click the loaded rack of interest.

   The *Sample Rack Loading* screen appears for the selected rack.

2. Select **Dilute All**.
3. From the *Dilution Factor* window, de-select **Whole Blood Conversion Factor**.
4. Select **OK.**

   A *Set Dilution Factor for Rack* window appears.

5. Select **Yes** to delete the Whole Blood Conversion Factor from an entire rack.

To delete the Whole Blood Conversion Factor assay test orders:

1. From the *Sample Rack Bay* screen, double-click the loaded rack with the specimen(s) of interest.

The *Sample Rack Loading* screen appears for the selected sample rack.

2. From the *Sample Rack Loading* screen, double-click the specimen of interest.

   The *Sample Details* screen appears with the current test orders for the selected specimen.

3. Select the test order of interest from the *Test Orders* panel.
4. Select **Apply Dilution.**
5. From the *Dilution Factor* window, deselect **Whole Blood Conversion Factor**.
6. Select **OK** to delete the Whole Blood Conversion Factor from the test order.

Procedural Notes

A. Calibrator and Controls

1. The qCMV positive calibrator, qCMV low positive control, qCMV high positive control, and qCMV negative control tubes can be loaded in any position in the Sample Rack and in any Sample Bay Lane on the Panther system. Specimen pipetting will begin when one of the following two conditions has been met:
   a. The calibrator and controls are currently being processed by the system.
   b. Valid results for the calibrator and controls are registered on the system.
2. Once the calibrator and control tubes have been pipetted and are processing for the Aptima CMV Quant assay reagent kit, specimens can be tested with the associated reconstituted kit for up to 24 hours **unless:**
   a. The calibrator or control results are invalid.
   b. The associated assay reagent kit is removed from the system.
   c. The associated assay reagent kit has exceeded stability limits.
3. The calibrator and each control tube can be used once. Attempts to use the tube more than once can lead to processing errors.

B. Glove Powder

As in any reagent system, excess powder on some gloves may cause contamination of opened tubes. Powderless gloves are recommended.

Quality Control

A run or specimen result may be invalidated by an operator if technical, operator, or instrument difficulties are observed while performing the assay and are documented. In this case, specimens must be retested.

Specimens with invalid results must be retested to obtain a valid result.

In case of an invalid result due to ML2 error, do not retest the neat plasma specimen. Refer to *Panther System Test Procedure*, step E.5, in this package insert for instructions to dilute the specimen.

Note: *For ML2 error, refer to the appropriate Panther/Panther Fusion System Operator's Manual for Mag Wash Clean Instructions.*

Assay Calibration

To generate valid results, an assay calibration must be completed. A single positive calibrator is run in triplicate each time a reagent kit is loaded on the Panther system. Once established, the calibration is valid for up to 24 hours. Software on the Panther system alerts the operator when a calibration is required. The operator scans a calibration coefficient found on the Master Lot Barcode Sheet provided with each reagent kit.

During processing, criteria for acceptance of the calibrator are automatically verified by the software on the Panther system. If less than two of the calibrator replicates is valid, the software automatically invalidates the run. Samples in an invalidated run must be retested using a freshly prepared calibrator and freshly prepared controls.

Negative and Positive Controls

To generate valid results, a set of assay controls must be tested. One replicate of the negative control, the low positive control, and the high positive control must be tested each time a reagent kit is loaded on the Panther system. Once established, the controls are valid for up to 24 hours. Software on the Panther system alerts the operator when controls are required.

During processing, criteria for acceptance of controls are automatically verified by software on the Panther system. To generate valid results, the negative control must give a result of “Not Detected” and the positive controls must give results within predefined parameters. If any one of the controls has an invalid result, the software automatically invalidates the run. Samples in an invalidated run must be retested using a freshly prepared calibrator and freshly prepared controls.

Internal Calibrator/Internal Control

Each sample contains an internal calibrator/internal control (IC). During processing, IC acceptance criteria are automatically verified by the Panther system software. If an IC result is invalid, the sample result is invalidated. Every sample with an invalid IC result must be retested to obtain a valid result.

The Panther system software is designed to accurately verify processes when procedures are performed following the instructions provided in this package insert and the *Panther/ Panther Fusion System Operator's Manual.*

Interpretation of Results

The Panther system automatically determines the concentration of CMV DNA for specimens and controls by comparing the results to a calibration curve. CMV DNA concentrations are reported in IU/mL and $\log_{10}$ IU/mL. The interpretation of results is provided in Table 1 and Table 2. If the whole blood or plasma dilution option is used on the Panther system, the software automatically calculates the CMV DNA concentration for the neat specimen by multiplying the diluted concentration by the dilution factor, and the sample results will be flagged.

Note: *For diluted plasma specimens, results listed as "Not Detected" or "<53 detected" may be generated by diluting a specimen with a concentration above, but close to the LoD (limit of detection) or LLoQ (lower limit of quantitation). It is recommended to collect and test another neat specimen if a quantitative result is not obtained.*

Table 1: Plasma Result Interpretation

Reported Aptima CMV Quant Assay Result		Interpretation
IU/mL	**$\log_{10}$ Value**	
Not Detected	Not Detected	CMV DNA not detected.
<53 detected	<1.72	CMV DNA is detected but at a level below the lower limit of quantification (LLoQ).
53 to 10,000,000	1.72 to 7.00	CMV DNA concentration is within the quantitative range between LLoQ to ULoQ IU/mL.
>10,000,000	>7.00	CMV DNA concentration is above the upper limit of quantification (ULoQ).
Invalid[a]	Invalid[a]	There was an error in the generation of the result. Specimen should be retested.

[a]Invalid results are displayed in blue-colored font.

Table 2: Whole Blood Result Interpretation

Reported Aptima CMV Quant Assay Result		Interpretation
IU/mL	**$\log_{10}$ Value**	
Not Detected	Not Detected	CMV DNA not detected.
<176 detected	<2.24	CMV DNA is detected but at a level below the lower limit of quantification (LLoQ).
176 to 10,000,000	2.24 to 7.00	CMV DNA concentration is within the quantitative range between LLoQ to ULoQ IU/mL.
>10,000,000	>7.00	CMV DNA concentration is above the upper limit of quantification (ULoQ).
Invalid[a]	Invalid[a]	There was an error in the generation of the result. Specimen should be retested.

[a]Invalid results are displayed in blue-colored font.

Note: *For diluted plasma specimens, the Panther system will report results greater than ULoQ (upper limit of quantitation) using scientific notation if the diluted specimen's result is within the assay range before applying the dilution factor.*

The acceptance criteria for each of the Aptima CMV Quant assay controls are outlined in Table 3.

Note: *The recovery range listed below shifts based on the assigned value of each specific lot. Refer to the assigned concentration listed on the Control Barcode Sheet insert provided with each Control box.*

Table 3: Acceptance Criteria for Recovery Range for Aptima CMV Quant Assay

Component	Recovery Range for Valid Runs
Negative Control	N/A
Low Positive Control	+/- 0.6 $\log_{10}$ copies/mL
High Positive Control	+/- 0.5 $\log_{10}$ copies/mL

Limitations

A. Use of this assay is limited to personnel who have been trained on the procedure. Failure to follow the instructions given in this package insert may result in erroneous results.

B. Reliable results are dependent on adequate specimen collection, transport, storage, and processing.

C. Though rare, mutations within the highly conserved regions of the viral genome covered by the primers and/or probes in the Aptima CMV Quant assay may result in under quantification of or failure to detect the virus.

Analytical Performance

Limit of Detection Using the 1st WHO International Standard

The limit of detection (LoD) of the assay is defined as the concentration of CMV DNA that is detected at 95% or greater probability according to CLSI EP17-A2.[14]

Limit of Detection using 1st WHO International Standards in Plasma

The LoD was determined by testing panels of the 1st WHO International Standard (NIBSC code 09/162)[21] for CMV diluted in CMV negative human plasma. 60 replicates of each dilution were tested with each of three reagent lots for a total of 180 replicates per dilution. Probit analysis was performed to generate the predicted detection limits. The LoD values shown in Table 4 are the results from the reagent lot with the highest predicted detection limit. The LoD for the Aptima CMV Quant assay using the 1st WHO International Standard is 40.7 IU/mL for plasma.

Table 4: Limit of Detection for Plasma Using the 1st WHO International Standard for CMV

Predicted Detection Limit	Concentration (IU/mL)
10%	1.9
20%	2.9
30%	4.0
40%	5.3
50%	6.9
60%	9.1
70%	12.2
80%	17.1
90%	27.5
95%	40.7

Limit of Detection using 1st WHO International Standards in Whole Blood

The LoD was determined by testing panels of the 1st WHO International Standard for CMV diluted in CMV negative whole blood. 60 replicates of each dilution were tested with each of three reagent lots for a total of 180 replicates per dilution. Probit analysis was performed to generate the predicted detection limits. The LoD values shown in Table 5 are the results from the reagent lot with the highest predicted detection limit. The LoD for the Aptima CMV Quant assay using the 1st WHO International Standard is 131.0 IU/mL for whole blood.

Table 5: Limit of Detection for Whole Blood Using the 1st WHO International Standard for CMV

Predicted Detection Limit	Concentration (IU/mL)
10%	8.8
20%	13.2
30%	17.7
40%	22.7
50%	28.7
60%	36.2
70%	46.5
80%	62.4
90%	93.7
95%	131.0

Limit of Detection of CMV Genotypes and Drug Resistant Mutants

Limit of Detection of CMV Genotypes and Drug Resistant Mutants in Plasma

The LoD was verified for three different genotypes based on Glycoprotein B sequence[7] (gB-2, gB-3, gB-4) and drug resistant mutants by testing various concentrations of CMV around the established LoD for plasma using the 1st WHO International Standard (genotype gB-1). Testing was performed with 30 replicates per panel member per reagent lot using two lots of Aptima CMV Quant reagents. The highest LoD verified for all three genotypes and drug resistant mutants was 40 IU/mL using both reagent lots.

Note: *The performance of the Aptima CMV Quant assay with drug resistant mutations of CMV was only evaluated in plasma specimens.*

Table 6: Limit of Detection of CMV Genotypes and Drug Resistant Mutants in Plasma

Genotypes/Mutants	Concentration (IU/mL)
gB-2	40
gB-3	40
gB-4	35
Drug resistant mutant UL54 and UL97*	35
Drug resistant mutant UL56**	35

*UL54 gene mutations can lead to cross resistance to several antivirals for treatment of CMV infection such as ganciclovir (GCV), cidofovir (CDV), and foscarnet (PFA). UL97 gene mutations also lead to ganciclovir (GCV) resistance.
**UL56 gene mutations lead to letermovir (LET) resistance.

The overall LoD in plasma is 40.7 IU/mL.

Limit of Detection Across CMV Genotypes in Whole Blood

The LoD was verified for three different Glycoprotein B genotypes (gB-2, gB-3, and gB-4) by testing various concentrations of CMV around the established LoD for whole blood using the CMV 1st WHO International Standard (genotype gB-1). Testing was performed with 30 replicates per panel member per reagent lot using two lots of Aptima CMV Quant reagents. The highest LoD verified for all three genotypes was 150 IU/mL using both reagent lots.

Table 7: Limit of Detection Across CMV Genotypes in Whole Blood

Genotype	Concentration (IU/mL)
gB-2	150
gB-3	150
gB-4	130

The overall LoD in whole blood is 150 IU/ml.

Linear Range

Linear Range in Plasma

The linear range was established by testing panels of CMV diluted in CMV negative human plasma according to CLSI EP06-A.[15] Panels ranged in concentration from 1.62 log_{10} IU/mL to 7.30 log_{10} IU/mL. The Aptima CMV Quant assay demonstrated linearity across the range tested. The upper limit of quantitation (ULoQ) of the assay is 7 Log_{10} IU/mL as shown in Figure 7.

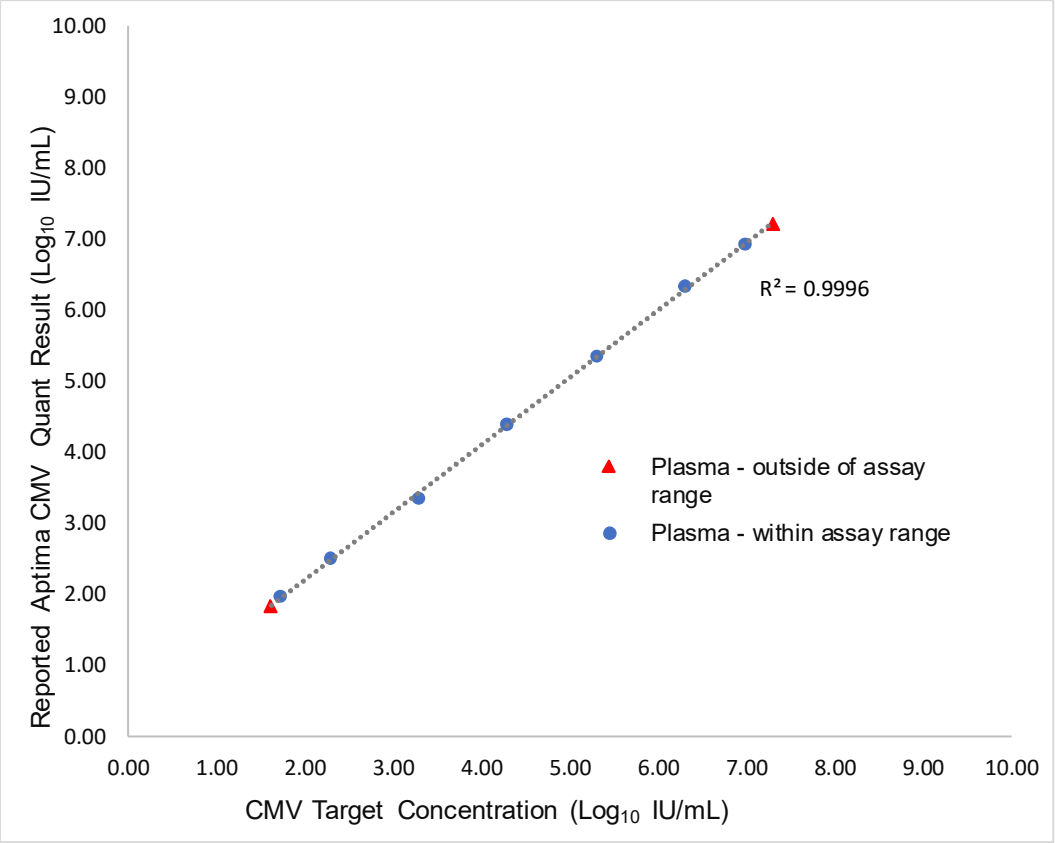

Figure 7. Linearity in Plasma

Linear Range in Whole Blood

The linear range was established by testing panels of CMV diluted in CMV negative human whole blood according to CLSI EP06-A.[15] Panels ranged in concentration from 2.15 log_{10} IU/mL to 7.3 log_{10} IU/mL for whole blood. The Aptima CMV Quant assay demonstrated linearity across the range tested. The upper limit of quantitation (ULoQ) of the assay is 7 log_{10} IU/mL as shown in Figure 8.

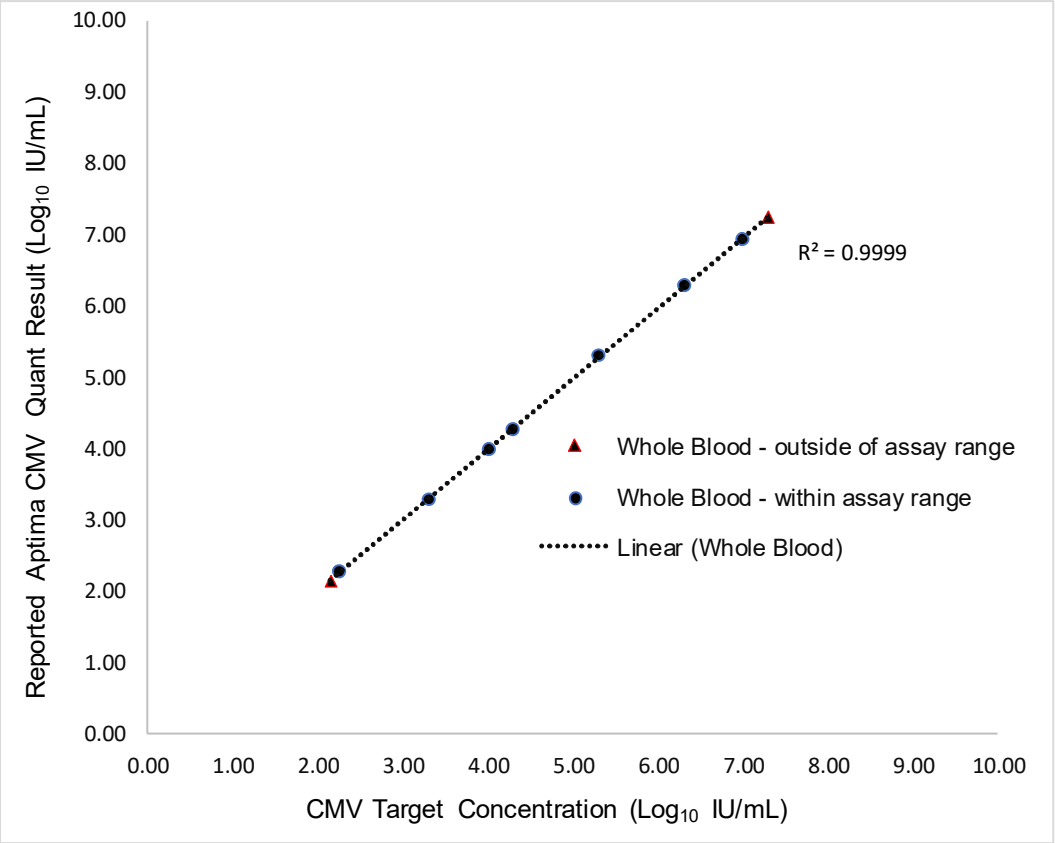

Figure 8. Linearity in Whole Blood

Linearity Across CMV Genotypes

Linearity Across CMV Genotypes in Plasma

The linearity for Glycoprotein genotypes gB-2, gB-3, and gB-4 was verified by testing panels of CMV diluted in CMV negative plasma at concentrations ranging from 1.72 $\log_{10}$ IU/mL to 7.00 $\log_{10}$ IU/mL. Linearity was demonstrated across the range for all genotypes tested as shown in Figure 9.

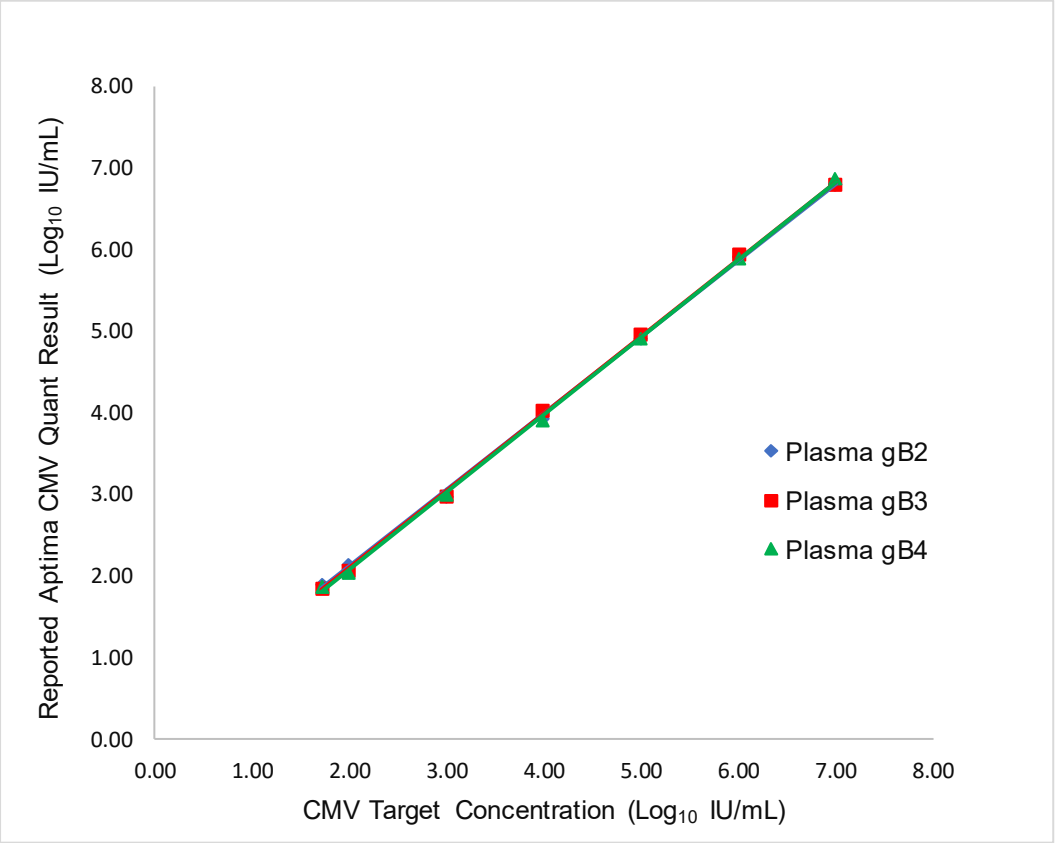

Figure 9. Linearity Across CMV Genotypes gB-2, gB-3, and gB-4 in Plasma

Linearity Across CMV Genotypes in Whole Blood

The linear response for Glycoprotein genotypes gB-2, gB-3, and gB-4 was verified by testing panels of CMV diluted in CMV negative whole blood at concentrations ranging from 2.25 log_{10} IU/mL to 7.00 log_{10} IU/mL. Linearity was demonstrated across the range for all three genotypes tested as shown in Figure 10.

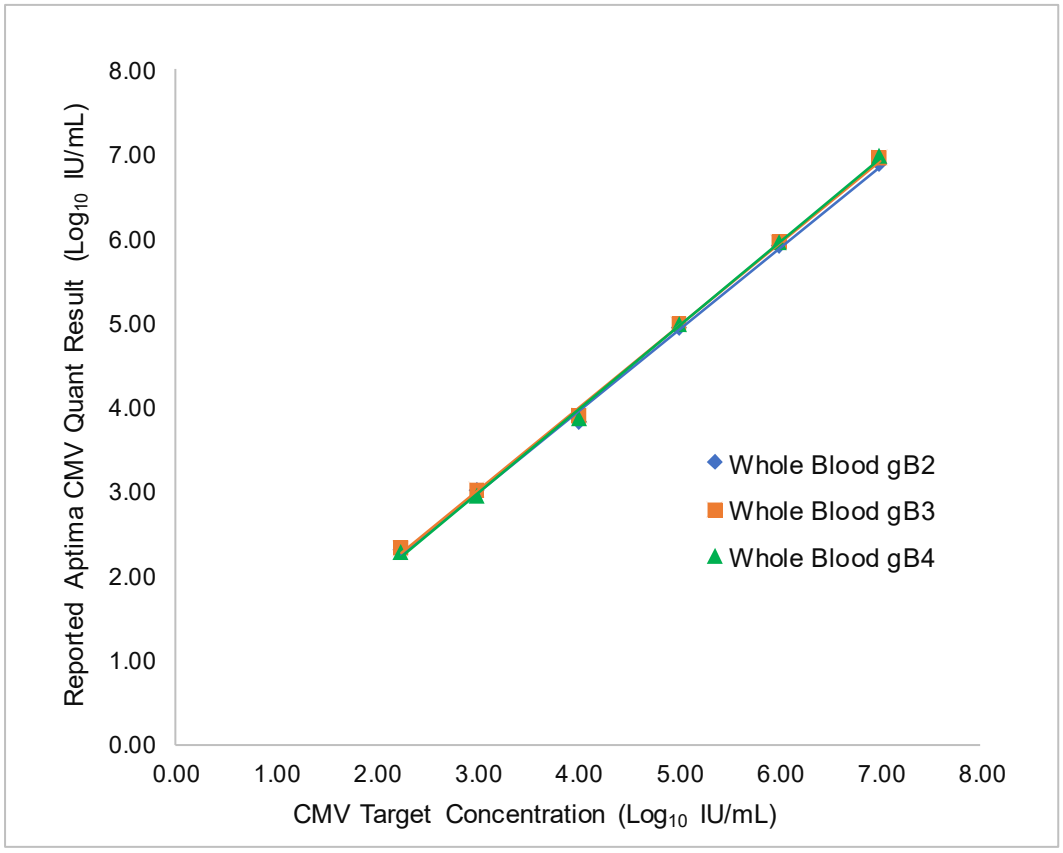

Figure 10. Linearity Across CMV Genotypes gB-2, gB-3, and gB-4 in Whole Blood

Lower Limit of Quantitation Using the 1st WHO International Standard

The lower limit of quantitation (LLoQ) is defined as the lowest concentration at which CMV DNA is reliably quantitated within a total error, according to CLSI EP17-A2.[14] Total error was estimated using the Westgard Model: Total Error (TE) = |bias| + 2SD. To ensure accuracy of measurements, the total error of the Aptima CMV Quant assay was set at 1 $\log_{10}$ IU/mL (i.e., at the LLoQ, a difference of more than 1 $\log_{10}$ IU/mL between two measurements is statistically significant).

Lower Limit of Quantitation using the 1st WHO International Standard in Plasma

The LLoQ was determined by testing panels of the 1st WHO International Standard (NIBSC code 09/162, genotype gB-1)[21] for CMV DNA diluted in CMV negative human plasma. 60 replicates of each dilution were tested with each of three reagent lots for a total of 180 replicates per dilution. The LLoQ results for the three reagent lots are shown in Table 8. The results from the reagent lot with the highest concentration meeting the TE requirements and ≥ 95% detection is summarized in Table 9. The LLoQ generated with the 1st WHO International Standard for CMV in plasma is 53 IU/mL.

Table 8: Determination of LLoQ Using the 1st WHO International Standard for CMV Diluted in Plasma

Reagent Lot	N	N Detected	Target Concentration	Aptima CMV Quant	SD	|Bias|	Calculated TE
			($\log_{10}$ IU/mL)	($\log_{10}$ IU/mL)	($\log_{10}$ IU/mL)	($\log_{10}$ IU/mL)	($\log_{10}$ IU/mL)
	60	56	1.48	1.64	0.36	0.16	0.87
1	60	59	1.54	1.72	0.29	0.18	0.76
	60	59	1.60	1.74	0.28	0.14	0.70
	60	59	1.70	1.85	0.19	0.15	0.53
	60	56	1.48	1.56	0.29	0.09	0.67
2	60	58	1.54	1.61	0.27	0.07	0.60
	60	58	1.60	1.69	0.28	0.09	0.64
	60	60	1.70	1.83	0.24	0.14	0.62
	60	56	1.48	1.67	0.26	0.19	0.71
3	60	58	1.54	1.67	0.24	0.13	0.60
	60	60	1.60	1.78	0.19	0.18	0.55
	60	60	1.70	1.87	0.22	0.17	0.61

SD=standard deviation
Panel members that met the accuracy goal (TE <= 1) and ≥ 95% detection for Reagent Lots 1, 2, and 3 are shaded.

Table 9: Summary of the LLoQ for Plasma Using the 1st WHO International Standard for CMV

Reagent Lot	(IU/mL)	(log_{10} IU/mL)
1	53	1.72
2	41	1.61
3	47	1.67

Lower Limit of Quantitation using the 1st WHO International Standard in Whole Blood

The LLoQ was determined by testing panels of the 1st WHO International Standard for CMV DNA diluted in CMV negative human whole blood. 60 replicates of each dilution were tested with each of three reagent lots for a total of 180 replicates per dilution. The results for the three reagent lots are shown in Table 10. The results from the reagent lot with the highest concentration meeting the TE requirements and ≥ 95% detection is summarized in Table 11. The LLoQ generated with the 1st WHO International Standard for CMV in whole blood is 176 IU/mL.

Table 10: Determination of LLoQ Using the 1st WHO International Standard for CMV Diluted in Whole Blood

Reagent Lot	N	N Detected	Target Concentration	Aptima CMV Quant	SD	|Bias|	Calculated TE
			(log_{10} IU/mL)	(log_{10} IU/mL)	(log_{10} IU/mL)	(log_{10} IU/mL)	(log_{10} IU/mL)
1	60	58	2.11	2.06	0.47	0.06	1.00
	60	59	2.16	2.04	0.51	0.12	1.14
	60	60	2.20	2.14	0.44	0.06	0.94
	60	59	2.24	2.28	0.26	0.04	0.56
2	60	60	2.11	2.02	0.42	0.09	0.93
	60	60	2.16	2.12	0.26	0.04	0.56
	60	59	2.20	2.14	0.30	0.07	0.67
	60	60	2.24	2.26	0.26	0.02	0.53
3	60	59	2.11	2.25	0.43	0.13	1.00
	60	59	2.16	2.34	0.27	0.18	0.72
	60	60	2.20	2.38	0.30	0.17	0.77
	60	60	2.24	2.39	0.30	0.15	0.74

SD=standard deviation
Panel members that met the accuracy goal (TE ≤ 1) and ≥ 95% detection for Reagent Lots 1, 2, and 3 are shaded.

Table 11: Summary of the LLoQ for Whole Blood Using the 1st WHO International Standard for CMV

Reagent Lot	(IU/mL)	(log_{10} IU/mL)
1	138	2.14
2	106	2.02
3	176	2.25

Determination of the Lower Limit of Quantitation of CMV Genotypes and Drug Resistant Mutants

Lower Limit of Quantitation of Genotypes and Drug Resistant Mutants in Plasma

The LLoQ established using the 1st WHO International Standard was verified by testing dilutions of CMV genotypes gB-2, gB-3, gB-4, and drug resistant mutants in CMV negative human plasma. 60 replicates of each panel member were tested with one reagent lot. The results are shown in Table 12. The calculated LLoQ for genotypes gB-2, gB-3, gB-4, and drug resistant mutants from the reagent lot with the highest concentration meeting the TE requirements and ≥ 95% detection is summarized in Table 13. The overall LLoQ for plasma in this assay is 53 IU/mL.

Note: *The performance of the Aptima CMV Quant assay with drug resistant mutations of CMV was only evaluated in plasma specimens.*

Table 12: Determination of LLoQ of Genotypes and Drug Resistant Mutants in Plasma

Genotypes/ Mutants	N	% Detected	Target Concentration (log_{10} IU/ml)	Aptima CMV Quant (log_{10} IU/ml)	SD (log_{10} IU/ml)	|Bias| (log_{10} IU/ml)	Calculated TE (log_{10} IU/ml)
gB-2	60	93.3	1.48	1.38	0.41	0.10	0.92
	60	96.7	1.54	1.39	0.39	0.16	0.95
	60	93.3	1.60	1.49	0.38	0.11	0.87
	60	96.7	1.65	1.70	0.24	0.04	0.51
	60	95.0	1.70	1.54	0.32	0.16	0.80
gB-3	60	91.7	1.48	1.27	0.38	0.20	0.97
	60	91.7	1.54	1.27	0.40	0.27	1.07
	60	88.3	1.60	1.31	0.47	0.29	1.23
	60	93.3	1.65	1.46	0.34	0.20	0.88
	60	91.7	1.70	1.57	0.29	0.13	0.71
	60	98.3	1.74	1.55	0.30	0.19	0.79

Table 12: Determination of LLoQ of Genotypes and Drug Resistant Mutants in Plasma (continued)

Genotypes/ Mutants	N	% Detected	Target Concentration (log_{10} IU/ml)	Aptima CMV Quant (log_{10} IU/ml)	SD (log_{10} IU/ml)	|Bias| (log_{10} IU/ml)	Calculated TE (log_{10} IU/ml)
gB-4	60	96.7	1.48	1.38	0.39	0.09	0.88
	60	98.3	1.54	1.51	0.33	0.03	0.69
	60	95.0	1.60	1.66	0.36	0.06	0.79
	60	98.3	1.65	1.66	0.29	0.01	0.59
	60	100.0	1.70	1.70	0.24	0.00	0.48
Drug resistant mutant (UL54 and UL97)	60	95.0	1.48	1.57	0.32	0.10	0.74
	60	98.3	1.54	1.58	0.32	0.04	0.68
	60	98.3	1.60	1.72	0.33	0.12	0.79
	60	100.0	1.65	1.74	0.22	0.08	0.51
	60	100.0	1.70	1.83	0.24	0.14	0.61
Drug resistant mutant (UL56)	60	95.0	1.48	1.54	0.28	0.07	0.64
	60	96.7	1.54	1.60	0.30	0.06	0.65
	60	100.0	1.60	1.69	0.26	0.08	0.60
	60	100.0	1.65	1.78	0.29	0.12	0.71
	60	100.0	1.70	1.74	0.27	0.05	0.58

SD=standard deviation
Panel members that met the accuracy goal (TE ≤ 1) and ≥ 95% detection for Reagent Lots 1, 2, and 3 are shaded.

Table 13: Summary of LLoQ of Genotypes and Drug Resistant Mutants in Plasma

Genotypes/Mutants	LLoQ	
	(IU/mL)	(log_{10} IU/mL)
gB-2	50	1.70
gB-3	35	1.55
gB-4	24	1.38
Drug resistant mutant UL54 and UL97*	38	1.57
Drug resistant mutant UL56**	35	1.54

*UL54 gene mutations can lead to cross resistance to several antivirals for treatment of CMV infection such as ganciclovir (GCV), cidofovir (CDV), and foscarnet (PFA). UL97 gene mutations also lead ganciclovir (GCV) resistance.

**UL56 gene mutations lead letermovir (LET) resistance.

Lower Limit of Quantitation Across Genotypes in Whole Blood

The LLoQ established using the 1st WHO International Standard was verified by testing dilutions of CMV genotypes gB-2, gB-3, and gB-4 in CMV negative human whole blood. 60 replicates of each panel member were tested with one reagent lot. The results are shown in Table 14. The LLoQ for genotypes gB-2, gB-3, and gB-4 from the reagent lot with the highest concentration meeting the TE requirements and ≥ 95% detection is summarized in Table 15. The overall LLoQ for whole blood in this assay is 176 IU/mL.

Table 14: Determination of LLoQ Across Genotypes in Whole Blood

Genotype	N	N Detected	Target Concentration	Aptima CMV Quant	SD	|Bias|	Calculated TE
			(log_{10} IU/ml)	(log_{10} IU/ml)	(log_{10} IU/ml)	(log_{10} IU/ml)	(log_{10} IU/ml)
gB-2	60	56	2.08	1.77	0.43	0.30	1.16
	60	56	2.15	1.87	0.39	0.27	1.06
	60	56	2.20	1.80	0.59	0.40	1.58
	60	58	2.26	1.97	0.41	0.28	1.11
	60	59	2.30	2.06	0.50	0.24	1.24
	60	57	2.34	2.01	0.52	0.33	1.38
	60	59	2.38	2.11	0.36	0.27	1.00
	60	60	2.41	2.19	0.30	0.23	0.84
gB-3	60	46	2.08	1.73	0.59	0.35	1.53
	60	54	2.15	1.78	0.50	0.36	1.37
	60	54	2.20	1.87	0.50	0.33	1.34
	60	58	2.26	2.02	0.52	0.23	1.27
	60	58	2.30	2.02	0.32	0.28	0.92
gB-4	60	55	2.08	1.78	0.53	0.30	1.37
	60	57	2.15	1.97	0.40	0.18	0.97
	60	58	2.20	2.09	0.39	0.12	0.89

SD=standard deviation

Table 15: Summary of LLoQ Across Genotypes in Whole Blood

Genotype	LLoQ	
	(IU/mL)	(log_{10} IU/mL)
gB-2	129	2.11
gB-3	104	2.02
gB-4	93	1.97

Traceability to the 1st WHO International Standard

A series of secondary standards with known concentrations were used throughout product development and product manufacturing to establish traceability to the 1st WHO International Standard. The CMV 1st WHO International Standard was diluted and tested along with the secondary standards, as well as assay controls, and calibrators used in the Aptima CMV Quant assay to evaluate traceability according to CLSI EP32-R.[16] The secondary standards ranged in concentration from 1.80 to 6.60 $\log_{10}$ IU/mL.

Traceability to the 1st WHO International Standard using Plasma

The concentrations tested for the CMV 1st WHO International Standard were between 2.18 to 4.70 $\log_{10}$ IU/mL. The WHO plasma panels, secondary standards, assay controls, and assay calibrators recovered as expected across the linear range of the assay, as can be seen from Figure 11.

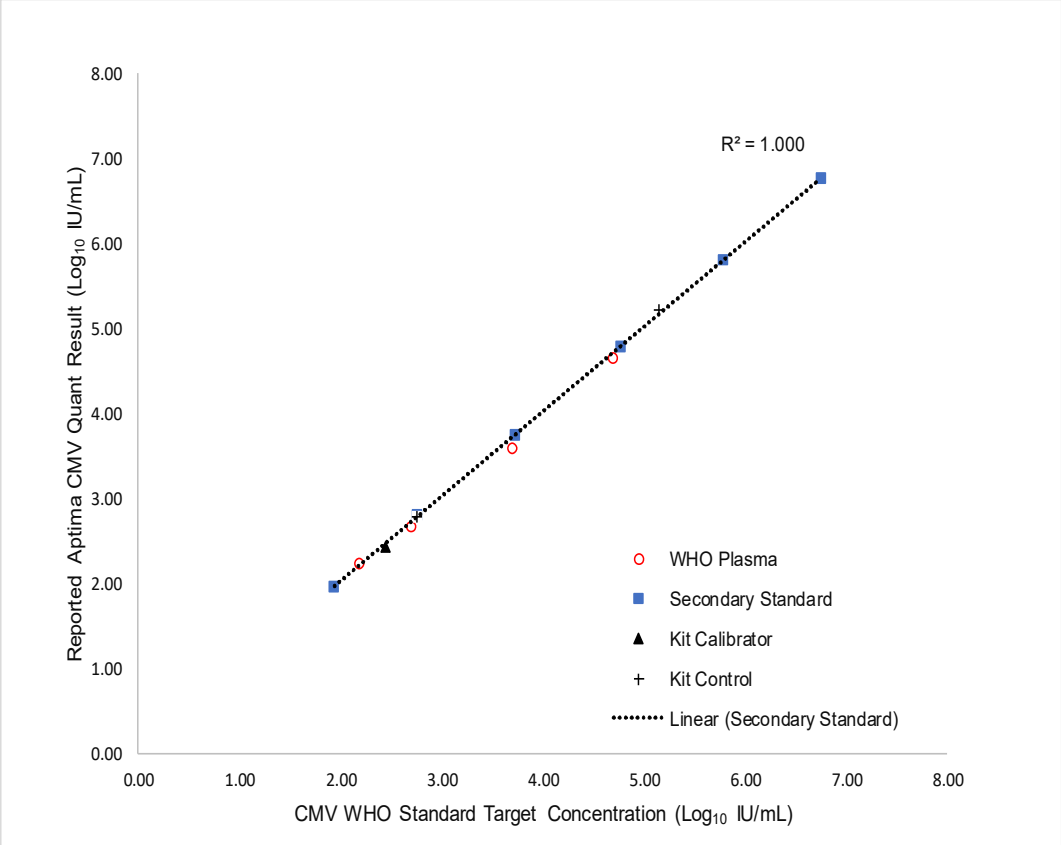

Figure 11. Traceability Between the 1st CMV WHO International Standard Target Concentrations and Reported Concentrations in the Aptima CMV Quant Assay (WHO Standard diluted in Plasma)

Traceability to the 1st WHO International Standard using Whole Blood

The concentrations tested for the CMV 1st WHO International Standard in whole blood were between 2.70 to 4.70 $\log_{10}$ IU/mL. The whole blood panels with WHO standards, secondary standards, assay controls, and assay calibrators recovered as expected across the linear range of the assay, as can be seen from Figure 12.

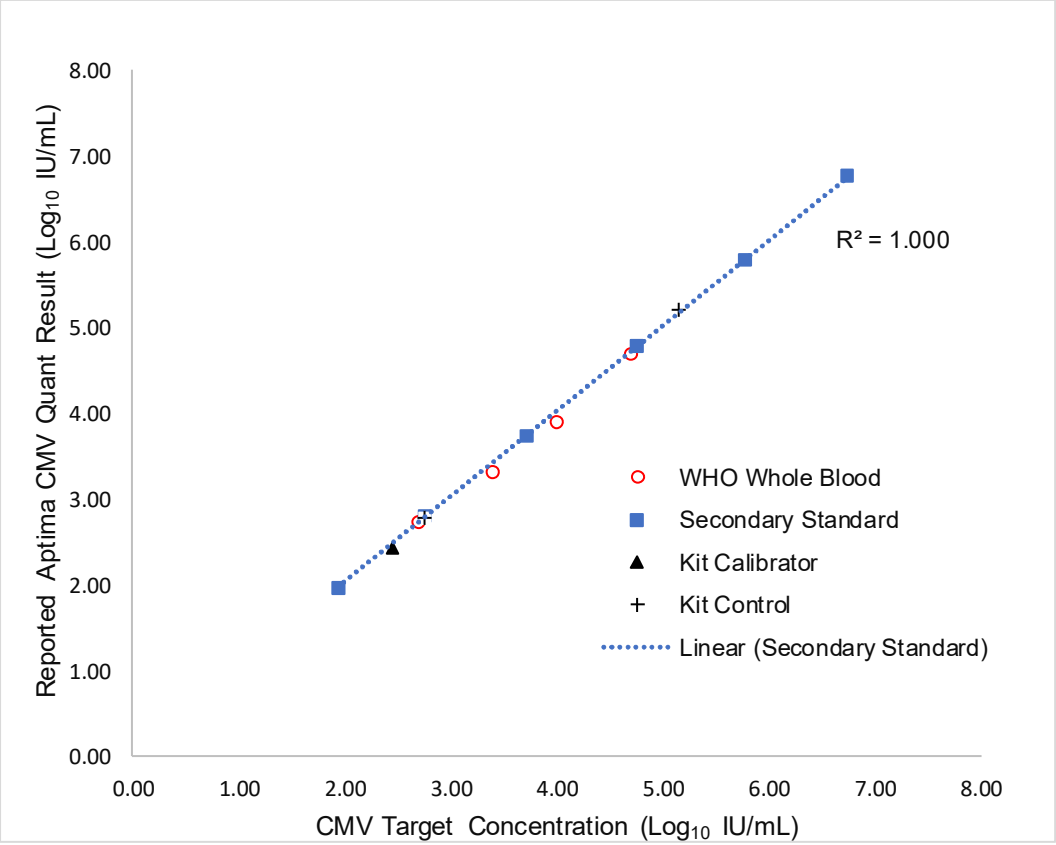

Figure 12. Traceability Between the 1st CMV WHO International Standard Target Concentrations and Reported Concentrations in the Aptima CMV Quant Assay (WHO Standard diluted in Whole Blood)

Precision

Plasma

To assess precision, a 6-member panel was made by diluting CMV positive clinical specimens or cultured CMV into CMV negative plasma. The panel was tested by three operators using three reagents lots on three Panther systems over 20 or more test days. Each operator performed two runs per day and each panel member was tested in duplicate in each run. The study was designed and analyzed following the recommendations of CLSI EP-05-A3.[17]

Table 16 shows the precision of assay results (in $\log_{10}$ IU/mL) between instruments, operators, reagent lots, runs, days, within runs, and overall. Total variability was primarily due to within-run variability (i.e. random error).

Table 16: Precision of the Aptima CMV Quant Assay in Plasma

N	Mean Concentration	Inter-Lot	Inter-Instrument	Inter-Operator	Inter-Day	Inter-Run	Intra-Run	Total
	($\log_{10}$ IU/mL)	SD	SD	SD	SD	SD	SD	SD
108	2.28	0.02	0.04	0.00	0.00	0.06	0.16	0.18
108	2.82	0.06	0.00	0.00	0.04	0.07	0.11	0.14
108	3.49	0.07	0.00	0.01	0.06	0.06	0.11	0.15
108	4.53	0.04	0.02	0.04	0.00	0.07	0.07	0.11
108	5.57	0.06	0.00	<0.001	0.04	0.02	0.09	0.12
108	6.67	0.06	0.03	0.00	0.00	0.00	0.10	0.12

SD=standard deviation
Note: Variability from some factors may be numerically negative, which can occur if the variability due to those factors is very small. When this occurs, SD is shown as 0.

Whole Blood

To assess precision, a 6-member panel was made by diluting CMV positive clinical specimens or spiking cultured CMV into CMV negative whole blood. The panel was tested by three operators using three reagents lots on three Panther systems over 20 or more test days. Each operator performed two runs per day and each panel member was tested in duplicate in each run.

Table 17 shows the precision of assay results (in $\log_{10}$ IU/mL) between instruments, operators, lots, runs, days, within runs, and overall. Total variability was primarily due to within-run variability (i.e., random error).

Table 17: Precision of the Aptima CMV Quant Assay in Whole Blood

N	Mean Concentration	Inter-Lot	Inter-Instrument	Inter-Operator	Inter-Day	Inter-Run	Intra-Run	Total
	(log_{10} IU/mL)	SD	SD	SD	SD	SD	SD	SD
108	2.78	0.00	0.01	0.05	0.00	0.08	0.14	0.17
108	3.38	0.03	0.00	0.04	0.00	0.00	0.13	0.14
108	3.95	0.06	0.00	0.07	0.05	0.05	0.13	0.18
108	4.76	0.03	0.01	0.08	0.00	0.07	0.12	0.16
108	5.64	0.01	0.00	0.07	0.00	0.00	0.11	0.13
108	6.74	0.03	0.00	0.05	0.00	0.04	0.09	0.12

SD=standard deviation
Note: Variability from some factors may be numerically negative, which can occur if the variability due to those factors is very small. When this occurs, SD is shown as 0.

Potentially Interfering Substances

The susceptibility of the Aptima CMV Quant assay to interference by elevated levels of endogenous substances, anticoagulants, and drugs commonly prescribed to transplant patients was evaluated. The test concentrations for each of the interfering substances were selected based on available literature references and guidance provided by CLSI EP07[18] and EP37[19]. CMV negative plasma samples and samples spiked with CMV to a concentration 2.22 log_{10} IU/mL and 3.30 log_{10} IU/mL were tested. CMV negative whole blood samples and samples spiked with CMV to a concentration of 2.72 and 4.00 log_{10} IU/mL of CMV DNA were tested for hemoglobin

No interference in the performance of the assay was observed in plasma samples in the presence of albumin (60 mg/mL), hemoglobin (10 mg/mL), triglycerides (15 mg/mL), unconjugated bilirubin (0.4 mg/mL) or human genomic DNA (2 µg/mL). No interference in wole blood samples in the performance of the assay was observed in the presence of 100 mg/mL of hemoglobin spiked into whole blood samples.

Clinical plasma specimens from patients with elevated levels of specific substances or from patients with the diseases listed in Table 18 tested with the Aptima CMV Quant assay. No interference in the performance of the assay was observed.

Table 18: Tested Clinical Specimen Types

	Clinical Specimen Types	Number of Clinical Specimens Tested
1	Antinuclear antibody (ANA)	10
2	Systemic lupus erythematosus (SLE)	10
3	Rheumatoid arthritis (RA)	10

No interference in the performance of the assay was observed in the presence of the exogenous substances listed in Table 19 at concentrations of least three times the C_{max} of drugs in human plasma.

Table 19: Exogenous Substances

Exogenous Substance Pool	Exogenous Substances Tested
1	Cefotetan, clavulanate potassium, Ticarcillin disodium, vancomycin
2	Piperacillin
3	Sulfamethoxazole
4	Tazobactam sodium, Trimethoprim, fluconazole
5	Ganciclovir, valganciclovir, cidofovir, Foscarnet, Valacyclovir, Acyclovir, Letermovir
6	Azathioprine, cyclosporine, Mycophenolate mofetil, Mycophenolic acid
7	Sirolimus, Tacrolimus, Prednisone, Everolimus
8	Sodium Citrate, EDTA, Heparin

Specificity

Specificity was determined by testing 780 frozen CMV negative clinical specimens. Specificity was calculated as the percentage of CMV negative samples with results of “Not Detected” versus the total number of samples tested for each sample type.

CMV DNA was not detected in 389 samples for plasma and 390 samples for whole blood. Specificity was 99.7% (389/390, 95% CI: 98.6 -100%) for plasma and 100% (390/390, 95% CI: 99.3-100%). The combined specificity of the Aptima CMV Quant assay for plasma and whole blood was 99.9% (779/780, 95% CI: 99.3-100%).

Table 20: Specificity in Plasma and Whole Blood Specimens

	Plasma	Whole Blood	Plasma & Whole Blood
Valid replicates (n)	390	390	780
Not Detected	389	390	779
Specificity (95% CI)	99.7% (98.6-100)	100% (99.3-100)	99.9% (99.3-100)

CI=confidence interval

Analytical Specificity

Potential cross-reactivity to the pathogens listed in Table 21 was evaluated in CMV negative human plasma in the presence or absence of 2.2 $\log_{10}$ IU/mL and 3.3 $\log_{10}$ IU/mL of CMV. Three blood parasites found in whole blood specimens were also evaluated in CMV negative whole blood in the presence or absence of 2.7 $\log_{10}$ IU/mL and 4.0 $\log_{10}$ IU/mL of CMV. Pathogens were tested at the highest concentration available. No cross-reactivity or interference was observed.

Table 21: Pathogens Tested for Analytical Specificity

Microorganism/Pathogen	Concentration		Microorganism/Pathogen	Concentration	
Adenovirus type 4	1,886	TCID50/mL[a]	*Mycobacterium intracellulare*	1,000,000	CFU/mL
BK Polyomavirus	1,000,000	cp/mL [b]	*Mycoplasma genitalium*	1,000,000	CFU/mL
Epstein-Barr virus	1,000,000	cp/mL	*Mycoplasma pneumoniae*	1,000,000	CFU/mL
Hepatitis B virus	1,000,000	IU/mL [c]	*Neisseria gonorrhoeae*	1,000,000	CFU/mL
Hepatitis C virus	1,000,000	cp/mL	*Propionibacterium acnes*	1,000,000	CFU/mL
Herpes Simplex virus type 1	1,428,571	TCID50/mL	*Salmonella enterica* serovar Typhimurium	1,000,000	CFU/mL
Herpes Simplex virus type 2	147,143	TCID50/mL	*Staphylococcus aureus*	1,000,000	CFU/mL
HIV-1 subtype B	1,000,000	cp/mL	*Staphylococcus epidermidis*	1,000,000	CFU/mL
Human Herpesvirus 6A	1,000,000	cp/mL	*Streptococcus agalactiae*	1,000,000	CFU/mL
Human Herpesvirus 7	1,428,571	TCID50/mL	*Streptococcus pneumoniae*	1,000,000	CFU/mL
Human Herpesvirus 8	1,000,000	cp/mL	*Streptococcus pyogenes*	1,000,000	CFU/mL
Human Metapneumovirus	192,857	TCID50/mL	*Aspergillus niger*	485,000	CFU/mL
Human Papillomavirus type 18	1,000,000	cp/mL	*Candida albicans*	1,000,000	CFU/mL
Human Parainfluenza virus	944	TCID50/mL	*Cryptococcus neoformans*	1,000,000	CFU/mL
Influenza virus	3,857	TCID50/mL	*Trichomonas vaginalis*	1,000,000	cells/mL
Rhinovirus	7,257	TCID50/mL	*Leishmania major**	1,000,000	cells/mL
Varicella Zoster virus	1,000,000	cp/mL	*Babesia microti**	1,000,000	cells/mL
Zika virus	29,286	TCID50/mL	*Plasmodium falciparum**	1,000,000	cells/mL
Chlamydia trachomatis	1,000,000	CFU/mL [d]			
Clostridium perfringens	1,000,000	CFU/mL			
Corynebacterium diphtheriae	1,000,000	CFU/mL			
Enterococcus faecalis	1,000,000	CFU/mL			
Escherichia coli	1,000,000	CFU/mL			
Klebsiella pneumoniae	1,000,000	CFU/mL			
Listeria monocytogenes	1,000,000	CFU/mL			

[a]TCID50/mL = Tissue culture infectious dose units per mL
[b]cp/mL = Viral copies per mL
[c]IU/mL = International units per mL
[d]CFU/mL = colony forming units per mL
*tested with whole blood sample type

Plasma Sample Dilution Using Aptima CMV Negative Control (1:3)

To assess the quantitation accuracy of CMV DNA in plasma samples diluted with Aptima CMV negative control, samples with concentrations distributed across the linear range were diluted 1:3 with Aptima CMV negative control (240 uL of sample combined with 480 uL of Aptima CMV negative control). Neat and diluted specimens were tested in triplicate. Testing was performed using one lot of reagents on one Panther system with two Aptima CMV negative control lots. The difference between the neat and diluted test results was calculated for each sample set as shown in Table 22. The sample concentrations were accurately recovered in the diluted samples after incorporating the dilution factor.

Table 22: Repeatability of Plasma Clinical Specimens Diluted in Negative Control

Neat Plasma Specimen Average Reported Concentration (log_{10} IU/mL) n=3	Diluted Plasma Specimen Average Reported Concentration (log_{10} IU/mL) n=6	Difference (log_{10} IU/mL)
2.30	2.42[a]	0.12
2.50	2.60	0.11
3.03	3.02	-0.01
3.46	3.45	-0.01
3.29	3.29	0.00
4.64	4.43	-0.21
5.32	5.31	-0.01
6.43	6.44	0.01
6.91[b]	6.95	0.05
>ULoQ[c]	7.41[d]	N/A

[a]Result from two replicates. Four results were "Detected" but not quantified.

[b]Result from two replicates. One result was "Detected" but not quantified because it was >ULoQ.

[c]Three results were "Detected" but not quantified because they were >ULoQ.

[d]Result from four replicates. Two results were "Detected" but not quantified because they were >ULoQ.

Confirmation of the LoD and LLoQ using CMV 1st WHO International Standards Diluted in Aptima CMV Negative Control

The LoD and LLoQ of the Aptima CMV Quant assay was confirmed with CMV 1st WHO International Standard (NIBSC Code 09/162) in plasma, diluted 1:3 using Aptima CMV negative control. Samples were prepared in CMV negative human plasma with CMV concentrations at 90, 105, 120, 135, 150 and 165 IU/mL. Each panel was diluted 1:3 in Aptima CMV negative control just prior to testing, to final concentrations of approximately 30, 35, 40, 45, 50, and 55 IU/mL. A total of 60 replicates of each panel member were tested with one reagent lot across three days. Total error was estimated using the Westgard Model: Total Error (TE) = |bias| + 2SD. All samples with a concentration of ≥45 IU/mL had ≥95% detection and total error (TE) of ≤1 $\log_{10}$ IU/mL as shown in Table 23. This confirms the LLoQ of CMV with samples diluted with negative control.

Table 23: LoD and LLoQ of Plasma Samples Diluted 1:3 Using Negative Control

N	% Detected	Target Concentration after 1:3 dilution	Target Concentration after 1:3 dilution	Aptima CMV Quant for diluted specimen	SD	|Bias|	Calculated TE
		(IU/mL)	($\log_{10}$ IU/mL)	($\log_{10}$ IU/mL)	($\log_{10}$ IU/mL)	($\log_{10}$ IU/mL)	($\log_{10}$ IU/mL)
60	98.3%	45	1.65	1.73	0.22	0.08	0.53

Carryover

Carryover contamination has been evaluated for the Panther system using plasma as a sample type using other viral load assays (Aptima HIV-1 Quant Dx assay, Aptima HCV Quant assay, Aptima HBV Quant assay). No carry-over contamination was observed in previous testing. To establish that the Panther system minimizes the risk of false positive results arising from carryover contamination in the whole blood sample type, a study was conducted using spiked panels on three Panther systems. Carryover was assessed using high titer CMV DNA spiked whole blood samples (6 $\log_{10}$ IU/mL) interspersed between CMV negative samples in a checkerboard pattern. Testing was carried out over twelve runs. The overall carryover rate was 0.24% (1/423).

Method Correlation

This study was designed in accordance with CLSI EP09c.[19]

Plasma Method Correlation

The performance of the Aptima CMV Quant assay was assessed against the comparator CMV assay by testing undiluted clinical specimens from CMV positive patients and contrived specimens made from various strains of cultured virus belonging to all four genotypes spiked in individual donor negative EDTA plasma. A total of 160 clinical specimens and 115 contrived specimens within the linear range common to both assays were used for the Deming regression as shown in Figure 13.

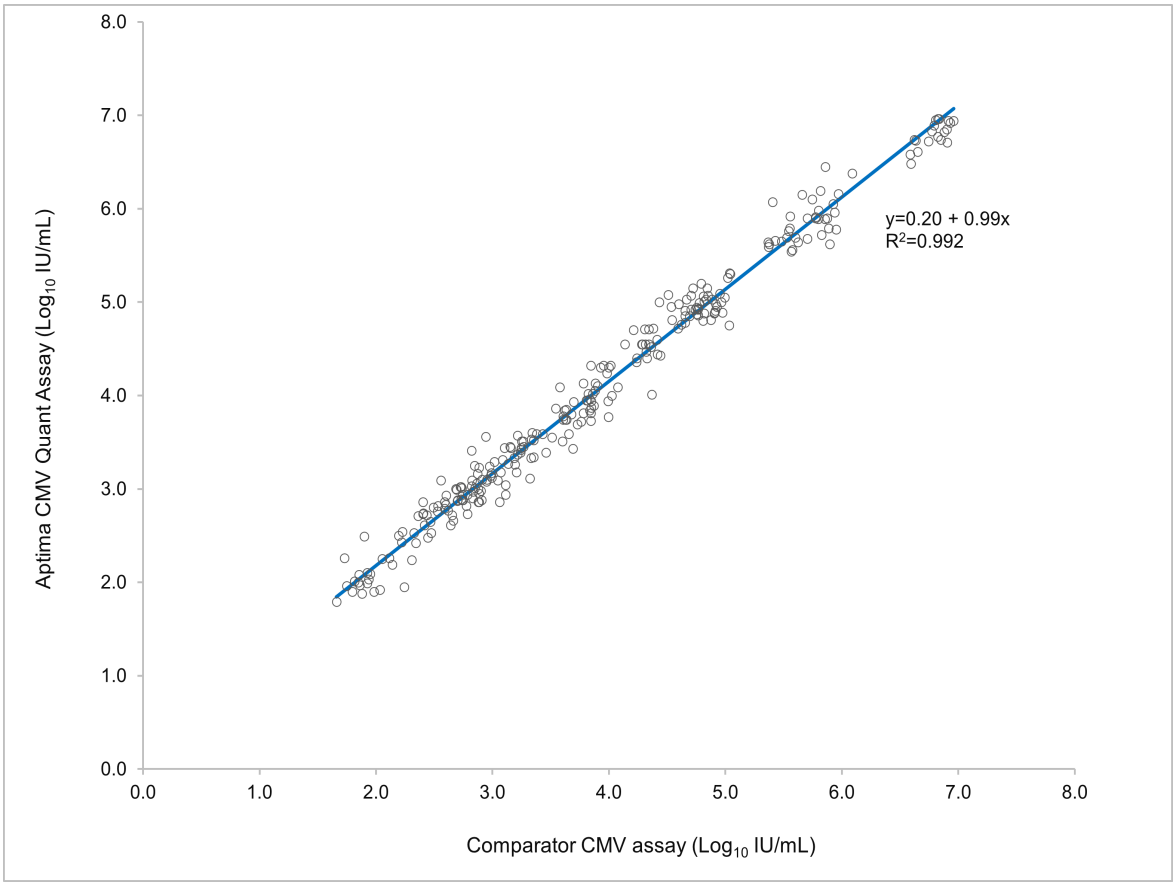

Figure 13. Correlation between CMV viral load in the Aptima CMV Quant assay and comparator CMV assay on testing plasma samples

Whole Blood Method Correlation

The performance of the Aptima CMV Quant assay was assessed against the comparator CMV assay by testing undiluted clinical specimens from CMV positive patients and contrived specimens made from cultured virus spiked in individual donor negative EDTA whole blood. A total of 159 clinical specimens and 83 contrived specimens within the linear range common to both assays were used for the Deming regression as shown in Figure 14.

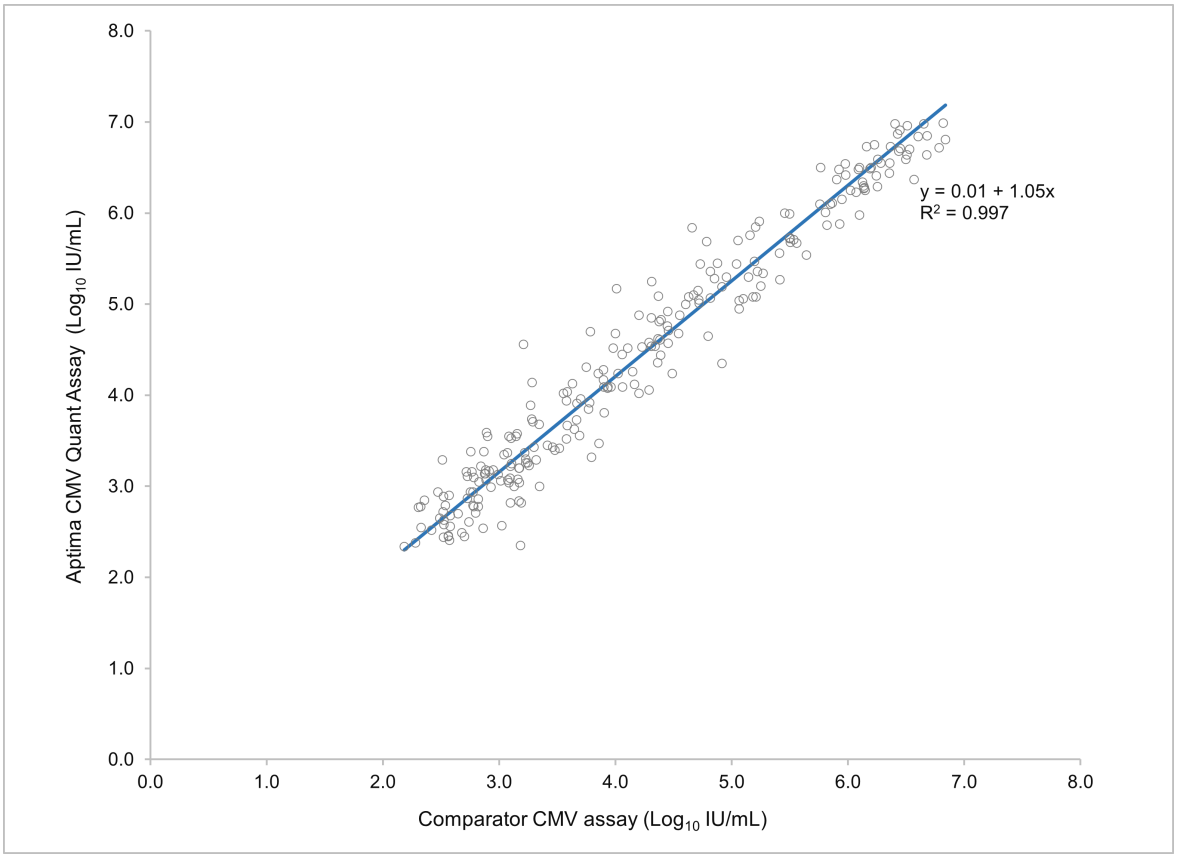

Figure 14. Correlation between CMV viral load in the Aptima CMV Quant assay and comparator CMV assay on testing whole blood samples

Reproducibility

Reproducibility in Plasma Samples

Reproducibility of the Aptima CMV Quant assay in plasma was evaluated at three external sites. Two operators performed testing at each site. Each operator performed one run per day over 5 days, using one reagent lot over the course of testing. Each run had three replicates of each panel member.

Reproducibility was tested using panel members prepared by diluting CMV positive clinical specimens or cultured CMV into CMV negative EDTA plasma. CMV DNA concentrations spanned the linear range of the assay.

Table 24 shows the reproducibility and precision of assay results for each positive panel member between sites, between operators, between days, between runs, within runs, and overall. The coefficient of variation was calculated using the following equation where σ^2 is the sample variance of the data after $\log_{10}$ transformation.

$$\%CV = 100 \times \sqrt{10^{\sigma^2 \times \ln(10)} - 1}$$

Table 24: Reproducibility of Aptima CMV Quant assay CMV DNA Levels on the Panther System in Positive Panel Members in Plasma

N	Observed Mean		Contribution to Total Variance SD (%CV²)					Total Variance SD (%CV)
	IU/mL	$\log_{10}$ IU/mL	Between Sites	Between Operators	Between Days	Between Runs	Within Runs	
90	198.33	2.26	0.05 (11.19)	0.00 (0)	0.06 (12.94)	0.00 (0)	0.17 (39.59)	0.18 (43.68)
90	603.27	2.76	0.02 (3.99)	<0.01 (2.22)	0.07 (15.68)	0.04 (10.25)	0.12 (27.04)	0.14 (33.67)
90	2428.54	3.36	0.06 (12.83)	0.00 (0)	0.09 (21.42)	0.06 (12.83)	0.11 (24.69)	0.16 (38.27)
90	27623.02	4.42	0.07 (15.98)	0.00 (0)	0.04 (9.29)	0.06 (13.85)	0.08 (19.38)	0.13 (30.63)
90	284107.74	5.44	0.07 (15.58)	0.00 (0)	0.04 (10.22)	0.00 (0)	0.09 (21.66)	0.12 (28.90)
90	3821364.62	6.57	0.08 (19.12)	0.00 (0)	0.06 (14.22)	0.02 (4.02)	0.08 (17.45)	0.13 (30.25)

%CV=log-normal coefficient of variation, SD=standard deviation ($\log_{10}$ IU/mL)

Note: Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, SD and %CV are shown as 0.

Reproducibility in Whole Blood Samples

Reproducibility of the Aptima CMV Quant assay in whole blood was evaluated at three external sites. Two operators performed testing at each site. Each operator performed one run per day over 5 days, using one reagent lot over the course of testing. Each run had three replicates of each panel member.

Reproducibility was tested using panel members prepared by diluting CMV positive clinical specimens or cultured CMV into CMV negative EDTA whole blood. CMV DNA concentrations spanned the linear range of the assay.

Table 25 shows the reproducibility and precision of assay results for each positive panel member between sites, between operators, between days, between runs, within runs, and overall excluding one observed outlier (0.2%, 1/533). The coefficient of variation was calculated using the following equation where σ^2 is the sample variance of the data after $\log_{10}$ transformation.

$$\%CV = 100 \times \sqrt{10^{\sigma^2 \times \ln(10)} - 1}$$

For all CMV positive and CMV negative panel members, the agreement values were 100%.

Table 25: Reproducibility of Aptima CMV Quant assay CMV DNA Levels on the Panther System in Positive Panel Members in Whole Blood

N	Observed Mean		Contribution to Total Variance SD (%CV²)					Total Variance SD (%CV)
	IU/mL	$\log_{10}$ IU/mL	Between Sites	Between Operators	Between Days	Between Runs	Within Runs	
89	604.32	2.73	0.00 (0)	0.00 (0)	0.00 (0)	0.11 (25.39)	0.18 (43.23)	0.21 (51.32)
89	2188.59	3.32	<0.01 (0)	0.00 (0)	0.00 (0)	0.07 (15.25)	0.11 (25.34)	0.13 (29.83)[a]
89	7830.84	3.87	0.04 (8.75)	0.04 (8.16)	0.00 (0)	0.08 (17.71)	0.13 (30.28)	0.16 (37.70)
88	48897.12	4.66	0.03 (7.11)	0.00 (0)	0.00 (0)	0.10 (22.47)	0.11 (24.99)	0.15 (34.89)
88	375626.91	5.56	0.04 (9.59)	0.04 (9.96)	0.00 (0)	0.05 (12.04)	0.09 (21.18)	0.12 (28.34)
89	4609046.44	6.64	0.08 (18.15)	0.00 (0)	0.05 (11.42)	0.03 (6.32)	0.10 (22.74)	0.14 (32.39)

%CV=log-normal coefficient of variation, SD=standard deviation ($\log_{10}$ IU/mL)

Note: Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, SD and %CV are shown as 0.

[a]Total variance result excluding the outlier that could potentially be a result of sample preparation issue.

Clinical Performance

Clinical Agreement

The clinical performance study was designed to assess the clinical agreement between the Aptima CMV Quant assay and an approved comparator test. During the prospective multi-center study at eight clinical sites, plasma specimens were collected from solid organ transplant recipients (SOTRs) and hematopoietic stem cell transplant recipients (HSCTRs) undergoing CMV monitoring in routine clinical practice. Additionally, residual frozen samples from SOTRs and HSCTRs were obtained from clinical specimen suppliers.

Of the 88 subjects that were enrolled in the prospective study, six subjects were not evaluable due to withdrawal (n = 5), or not having valid sample results with the Aptima CMV Quant assay and the approved test (n = 1). Table 26 shows the demographic and baseline clinical characteristics of the 82 evaluable subjects.

Table 26: Demographics and Baseline Clinical Characteristics of Evaluable Subjects Overall and by Transplant Type

Characteristics		SOTRs	HSCTRs	All
Total, N		62	20	82
Sex, n (%)	Male	28 (45.2)	14 (70.0)	42 (51.2)
	Female	34 (54.8)	6 (30.0)	40 (48.8)
Age (years)	Mean ± (SD)	52.1 ± (12.93)	51.9 ± (14.60)	52.1 ± (13.27)
	Median	53.0	54.5	54.0
	Minimum	20	22	20
	Maximum	81	69	81
Ethnicity, n (%)	Hispanic or Latino	2 (3.2)	3 (15.0)	5 (6.1)
	Not Hispanic or Latino	41 (66.1)	17 (85.0)	58 (70.7)
	Unknown	19 (30.6)	0 (0)	19 (23.2)
Race, n (%)	American Indian/Alaska Native	0 (0)	0 (0)	0 (0)
	Asian	1 (1.6)	1 (5.0)	2 (2.4)
	Black or African American	17 (27.4)	0 (0)	17 (20.7)
	Native Hawaiian/Pacific Islander	0 (0)	0 (0)	0 (0)
	White	37 (59.7)	18 (90.0)	55 (67.1)
	Other	0 (0)	1 (5.0)	1 (1.2)
	Unknown	7 (11.3)	0 (0)	7 (8.5)
Organ type, n (%)	Kidney	25 (40.3)	--	--
	Liver	15 (24.2)	--	--
	Lung	10 (16.1)	--	--
	Heart	12 (19.4)	--	--

Table 26: Demographics and Baseline Clinical Characteristics of Evaluable Subjects Overall and by Transplant Type *(continued)*

Characteristics		SOTRs	HSCTRs	All
Stem cell type, n (%)	Allogeneic	--	18 (90.0)	--
	Autologous	--	2 (10.0)	--
CMV serology status, n (%)	Donor Positive / Recipient Negative	34 (54.8)	3 (15.0)	37 (45.1)
	Donor Negative / Recipient Positive	6 (9.7)	8 (40.0)	14 (17.1)
	Donor Positive / Recipient Positive	22 (35.5)	9 (45.0)	31 (37.8)
On CMV antiviral therapy, n		50 (80.6)	13 (65.0)	63 (76.8)
Days on CMV antiviral therapy				
	n	41	12	53
	Mean	13.6	13.3	13.5
	Median	11	9.5	11
	Minimum	1	1	1
	Maximum	47	45	47

HSCTRs=hematopoietic stem cell transplant recipients, SD=standard deviation, SOTRs=solid organ transplant recipients

In the prospective study, 365 plasma samples were collected from the 82 evaluable subjects. Additionally, 261 residual frozen samples were obtained from clinical specimen suppliers. Of the 626 clinical plasma samples (i.e., samples collected in the prospective study and residual frozen samples combined), 597 paired (i.e., with a valid result both on the Aptima CMV Quant assay and the approved test) clinical plasma samples were included in agreement analyses. Of the 597 paired clinical samples, 339 samples were collected in the prospective study and 258 were residual frozen samples. Separately, agreement analyses were performed on 181 paired samples collected from subjects after they initiated CMV antiviral therapy as part of their routine care during the prospective study.

Table 27 shows the agreement analysis and percent agreement between the Aptima CMV Quant assay and the approved test at different thresholds (overall and by transplant group). Agreement analysis at different viral load intervals (overall and by transplant group) is shown in Table 28. Four out of 597 overall results were observed to be discrepant across more than the immediately adjacent category, of which 3 were from HSCTRs.

Table 27: Agreement Analysis and Percent Agreement at Different Thresholds (Overall and by Transplant Group)

Transplant Group Threshold	N[a]	Comparator[b] and Aptima CMV Quant Results				PPA %(n/N) [95% CI][c]	NPA % (n/N) [95% CI][c]
		Comp≥ ACMV≥	Comp< ACMV≥	Comp< ACMV<	Comp≥ ACMV<		
Overall							
TND	597	427	13	136	21	95.3 (427/448) [92.9, 96.9]	91.3 (136/149) [85.6, 94.8]
Detected, <2.1 log_{10} IU/mL (137 IU/mL)[d]	597	252	48	295	2	99.2 (252/254) [97.2, 99.8]	86.0 (295/343) [81.9, 89.3]
2.7 log_{10} IU/mL (500 IU/mL)	597	158	37	397	5	96.9 (158/163) [93.0, 98.7]	91.5 (397/434) [88.5, 93.8]
3.3 log_{10} IU/mL (1800 IU/mL)	597	93	20	483	1	98.9 (93/94) [94.2, 99.8]	96.0 (483/503) [93.9, 97.4]
3.9 log_{10} IU/mL (7943.3 IU/mL)	597	45	12	540	0	100 (45/45) [92.1, 100]	97.8 (540/552) [96.2, 98.8]
SOTRs							
TND	403	295	9	85	14	95.5 (295/309) [92.5, 97.3]	90.4 (85/94) [82.8, 94.9]
Detected, <2.1 log_{10} IU/mL (137 IU/mL)[d]	403	197	26	178	2	99.0 (197/199) [96.4, 99.7]	87.3 (178/204) [82.0, 91.2]
2.7 log_{10} IU/mL (500 IU/mL)	403	129	25	245	4	97.0 (129/133) [92.5, 98.8]	90.7 (245/270) [86.7, 93.6]
3.3 log_{10} IU/mL (1800 IU/mL)	403	78	16	308	1	98.7 (78/79) [93.2, 99.8]	95.1 (308/324) [92.1, 96.9]
3.9 log_{10} IU/mL (7943.3 IU/mL)	403	41	10	352	0	100 (41/41) [91.4, 100]	97.2 (352/362) [95.0, 98.5]
HSCTRs							
TND	194	132	4	51	7	95.0 (132/139) [90.0, 97.5]	92.7 (51/55) [82.7, 97.1]
Detected, <2.1 log_{10} IU/mL (137 IU/mL)[d]	194	55	22	117	0	100 (55/55) [93.5, 100]	84.2 (117/139) [77.2, 89.3]
2.7 log_{10} IU/mL (500 IU/mL)	194	29	12	152	1	96.7 (29/30) [83.3, 99.4]	92.7 (152/164) [87.6, 95.8]
3.3 log_{10} IU/mL (1800 IU/mL)	194	15	4	175	0	100 (15/15) [79.6, 100]	97.8 (175/179) [94.4, 99.1]
3.9 log_{10} IU/mL (7943.3 IU/mL)	194	4	2	188	0	100 (4/4) [51.0, 100]	98.9 (188/190) [96.2, 99.7]

ACMV=Aptima CMV Quant assay, CI=confidence interval, Comp=comparator assay, HSCTRs=hematopoietic stem cell transplant recipients, NPA=negative percent agreement, PPA=positive percent agreement, SOTRs=solid organ transplant recipients, TND=target not detected

Notes:

≥: Result is greater than or equal to the given threshold value

<: Result is less than the given threshold value

PPA summarizes results greater than or equal to the given threshold; NPA summarizes results less than the given threshold.

[a] Number of paired clinical samples (samples collected in the prospective study and frozen residual frozen samples obtained from clinical specimen suppliers combined).

[b] approved test

[c] Score CI

[d] LLoQ of an alternate approved test

Table 28: Agreement Analysis at Different Viral Load Intervals (Overall and by Transplant Group)

Transplant Group Aptima CMV assay Result	Comparator[b] Result (log_{10} IU/mL)						
	Total[a], N	TND	Detected, <2.1	≥2.1 to <2.7	≥2.7 to <3.3	≥3.3 to <3.9	≥3.9
Overall							
Total number of paired samples, N	597	149	194	91	69	49	45
TND	157	136	21	0	0	0	0
Detected, <2.1 log_{10} IU/mL[c]	140	13	125	2	0	0	0
≥2.1 to <2.7 log_{10} IU/mL	105	0	46	54	5	0	0
≥2.7 to <3.3 log_{10} IU/mL	82	0	2[d]	34	45	1	0
≥3.3 to <3.9 log_{10} IU/mL	56	0	0	1[d]	18	37	0
≥3.9 log_{10} IU/mL	57	0	0	0	1[d]	11	45
SOTRs							
Total number of paired samples, N	403	94	110	66	54	38	41
TND	99	85	14	0	0	0	0
Detected, <2.1 log_{10} IU/mL[c]	81	9	70	2	0	0	0
≥2.1 to <2.7 log_{10} IU/mL	69	0	26	39	4	0	0
≥2.7 to <3.3 log_{10} IU/mL	60	0	0	25	34	1	0
≥3.3 to <3.9 log_{10} IU/mL	43	0	0	0	15	28	0
≥3.9 log_{10} IU/mL	51	0	0	0	1[d]	9	41
HSCTRs							
Total number of paired samples, N	194	55	84	25	15	11	4
TND	58	51	7	0	0	0	0
Detected, <2.1 log_{10} IU/mL[c]	59	4	55	0	0	0	0
≥2.1 to <2.7 log_{10} IU/mL	36	0	20	15	1	0	0
≥2.7 to <3.3 log_{10} IU/mL	22	0	2[d]	9	11	0	0
≥3.3 to <3.9 log_{10} IU/mL	13	0	0	1[d]	3	9	0
≥3.9 log_{10} IU/mL	6	0	0	0	0	2	4

HSCTRs=hematopoietic stem cell transplant recipients, SOTRs=solid organ transplant recipients, TND=target not detected

[a] Number of paired clinical samples (samples collected in the prospective study and frozen residual frozen samples obtained from clinical specimen suppliers combined).

[b] approved test

[c] LLoQ of an alternate approved test

[d] 4 out of 597 overall results were observed to be discrepant across more than the immediately adjacent category; 1 of the 4 was from an SOTR, and 3 of the 4 were from HSCTRs. Of the 2 HSCTRs that underwent testing with an alternate NAAT, 1 was found in agreement with the Aptima CMV Quant assay results.

Table 29 shows the agreement analysis and percent agreement at different thresholds (overall and by transplant group) for samples collected from subjects after they initiated CMV antiviral therapy as part of routine care in the prospective study. The agreement analysis at different viral load intervals using all time points post-treatment initiation combined (overall and by transplant group) are shown in Table 30. One out of 181 overall results were observed to be discrepant across more than the immediately adjacent category, which was observed in an SOTR.

Table 29: Agreement Analysis and Percent Agreement at Different Thresholds using all Time Points Post-Treatment Initiation Combined (Overall and by Transplant Group)

Transplant Group Threshold	N[a]	Comparator[b] and Aptima CMV Quant Results				PPA % (n/N) [95% CI][c]	NPA % (n/N) [95% CI][c]
		Comp≥ ACMV≥	Comp< ACMV≥	Comp< ACMV<	Comp≥ ACMV<		
Overall							
TND	181	121	4	47	9	93.1 (121/130) [87.4, 96.3]	92.2 (47/51) [81.5, 96.9]
Detected, <2.1 log_{10} IU/mL (137 IU/mL)[d]	181	69	15	97	0	100 (69/69) [94.7, 100]	86.6 (97/112) [79.1, 91.7]
2.7 log_{10} IU/mL (500 IU/mL)	181	42	9	129	1	97.7 (42/43) [87.9, 99.6]	93.5 (129/138) [88.1, 96.5]
3.3 log_{10} IU/mL (1800 IU/mL)	181	23	5	153	0	100 (23/23) [85.7, 100]	96.8 (153/158) [92.8, 98.6]
3.9 log_{10} IU/mL (7943.3 IU/mL)	181	12	3	166	0	100 (12/12) [75.8, 100]	98.2 (166/169) [94.9, 99.4]
SOTRs							
TND	136	102	2	26	6	94.4 (102/108) [88.4, 97.4]	92.9 (26/28) [77.4, 98.0]
Detected, <2.1 log_{10} IU/mL (137 IU/mL)[d]	136	57	15	64	0	100 (57/57) [93.7, 100]	81.0 (64/79) [71.0, 88.1]
2.7 log_{10} IU/mL (500 IU/mL)	136	34	8	93	1	97.1 (34/35) [85.5, 99.5]	92.1 (93/101) [85.1, 95.9]
3.3 log_{10} IU/mL (1800 IU/mL)	136	18	5	113	0	100 (18/18) [82.4, 100]	95.8 (113/118) [90.5, 98.2]
3.9 log_{10} IU/mL (7943.3 IU/mL)	136	10	3	123	0	100 (10/10) [72.2, 100]	97.6 (123/126) [93.2, 99.2]
HSCTRs							
TND	45	19	2	21	3	86.4 (19/22) [66.7, 95.3]	91.3 (21/23) [73.2, 97.6]
Detected, <2.1 log_{10} IU/mL	45	12	0	33	0	100 (12/12) [75.8, 100]	100 (33/33) [89.6, 100]
2.7 log_{10} IU/mL (500 IU/mL)	45	8	1	36	0	100 (8/8) [67.6, 100]	97.3 (36/37) [86.2, 99.5]
3.3 log_{10} IU/mL (1800 IU/mL)	45	5	0	40	0	100 (5/5) [56.6, 100]	100 (40/40) [91.2, 100]
3.9 log_{10} IU/mL (7943.3 IU/mL)	45	2	0	43	0	100 (2/2) [34.2, 100]	100 (43/43) [91.8, 100]

ACMV=Aptima CMV Quant assay, CI=confidence interval, Comp=comparator assay, HSCTRs=hematopoietic stem cell transplant recipients, NPA=negative percent agreement, PPA=positive percent agreement, SOTRs=solid organ transplant recipients, TND=target not detected

Notes:

- ≥: Result is greater than or equal to the given threshold value
- <: Result is less than the given threshold value
- PPA summarizes results greater than or equal to the given threshold; NPA summarizes results less than the given threshold.

[a] Number of paired samples that were collected from subjects who were on CMV antiviral therapy at enrollment or initiated CMV antiviral therapy during the prospective study.

[b] approved test

[c] Score CI

[d] LLoQ of an alternate approved test

Table 30: Agreement Analysis at Different Viral Load Intervals using all Time Points Post-Treatment Initiation Combined (Overall and by Transplant Group)

Transplant Group Aptima CMV Quant Result	Comparator[b] Result (log_{10} IU/mL)						
	Total[a], N	TND	Detected, <2.1	≥2.1 to <2.7	≥2.7 to <3.3	≥3.3 to <3.9	≥3.9
Overall							
Total number of paired	181	51	61	26	20	11	12
TND	56	47	9	0	0	0	0
Detected, <2.1 log_{10} IU/mL[c]	41	4	37	0	0	0	0
≥2.1 to <2.7 log_{10} IU/mL	33	0	15	17	1	0	0
≥2.7 to <3.3 log_{10} IU/mL	23	0	0	9	14	0	0
≥3.3 to <3.9 log_{10} IU/mL	13	0	0	0	4	9	0
≥3.9 log_{10} IU/mL	15	0	0	0	1[d]	2	12
SOTRs							
Total number of paired	136	28	51	22	17	8	10
TND	32	26	6	0	0	0	0
Detected, <2.1 log_{10} IU/mL[c]	32	2	30	0	0	0	0
≥2.1 to <2.7 log_{10} IU/mL	30	0	15	14	1	0	0
≥2.7 to <3.3 log_{10} IU/mL	19	0	0	8	11	0	0
≥3.3 to <3.9 log_{10} IU/mL	10	0	0	0	4	6	0
≥3.9 log_{10} IU/mL	13	0	0	0	1[d]	2	10
HSCTRs							
Total number of paired	45	23	10	4	3	3	2
TND	24	21	3	0	0	0	0
Detected, <2.1 log_{10} IU/mL[c]	9	2	7	0	0	0	0
≥2.1 to <2.7 log_{10} IU/mL	3	0	0	3	0	0	0
≥2.7 to <3.3 log_{10} IU/mL	4	0	0	1	3	0	0
≥3.3 to <3.9 log_{10} IU/mL	3	0	0	0	0	3	0
≥3.9 log_{10} IU/mL	2	0	0	0	0	0	2

HSCTRs=hematopoietic stem cell transplant recipients, SOTRs=solid organ transplant recipients, TND=target not detected

[a] Number of paired samples that were collected from subjects who were on CMV antiviral therapy at enrollment or initiated CMV antiviral therapy during the prospective study.

[b] approved assay

[c] LLoQ of an alternate approved test

[d] 1 out of 181 overall results were observed to be discrepant across more than the immediately adjacent category.

Method Comparison

The method comparison study was conducted to assess the performance of the Aptima CMV Quant assay as compared to an approved test. A total of 309 paired CMV positive clinical samples consisting of 165 samples collected in the prospective study and 144 residual frozen samples with results in the common linear range for both assays were included in the method comparison analyses. Additionally, a total of 105 contrived samples were prepared by spiking cultured CMV virus into CMV-negative EDTA plasma of which 103 were in the common linear range for both assays. Contrived samples were analyzed separately.

Table 31 presents Deming regression parameter estimates (log_{10} IU/mL). Figure 15 through Figure 18 show Deming regression of the viral load results (log_{10} IU/mL) from the Aptima CMV Quant assay and the approved test.

Table 31: Deming Regression Parameter Estimates by Sample Type and Transplant Group

Sample Type	Transplant Group	Viral Load Unit	Parameter	N[a]	Estimate	Jackknife Method[b]		Bootstrap Method[c]		r
						SE	95% CI	SE	95% CI	
Clinical	Overall	log_{10} IU/mL	Intercept	309	0.20	0.038	(0.12, 0.27)	0.021	(0.15, 0.24)	0.97
			Slope		1.00	0.011	(0.98, 1.03)	0.007	(0.99, 1.02)	
	SOTRs	log_{10} IU/mL	Intercept	227	0.17	0.043	(0.09, 0.26)	0.025	(0.12, 0.22)	0.98
			Slope		1.01	0.012	(0.98, 1.03)	0.008	(0.99, 1.02)	
	HSCTRs	log_{10} IU/mL	Intercept	82	0.16	0.101	(-0.04, 0.36)	0.048	(0.07, 0.26)	0.95
			Slope		1.03	0.037	(0.96, 1.11)	0.017	(1.00, 1.07)	
Contrived	n/a	log_{10} IU/mL	Intercept	103	0.06	0.058	(-0.05, 0.18)	0.059	(-0.05, 0.18)	1.00
			Slope		1.01	0.011	(0.98, 1.03)	0.012	(0.98, 1.03)	

CI=confidence interval, HSCTRs=hematopoietic stem cell transplant recipients, r=correlation coefficient, SE=standard error, SOTRs=solid organ transplant recipients

[a] Number of paired samples with results in the common linear range for both assays.

[b] Independence assumed between all samples; jackknife method used to estimate SE and CI.

[c] Clinical samples were adjusted for within-subject correlation using the bootstrap re-sampling method with 500 iterations; this method was also used for contrived samples, but without stratifying by subject.

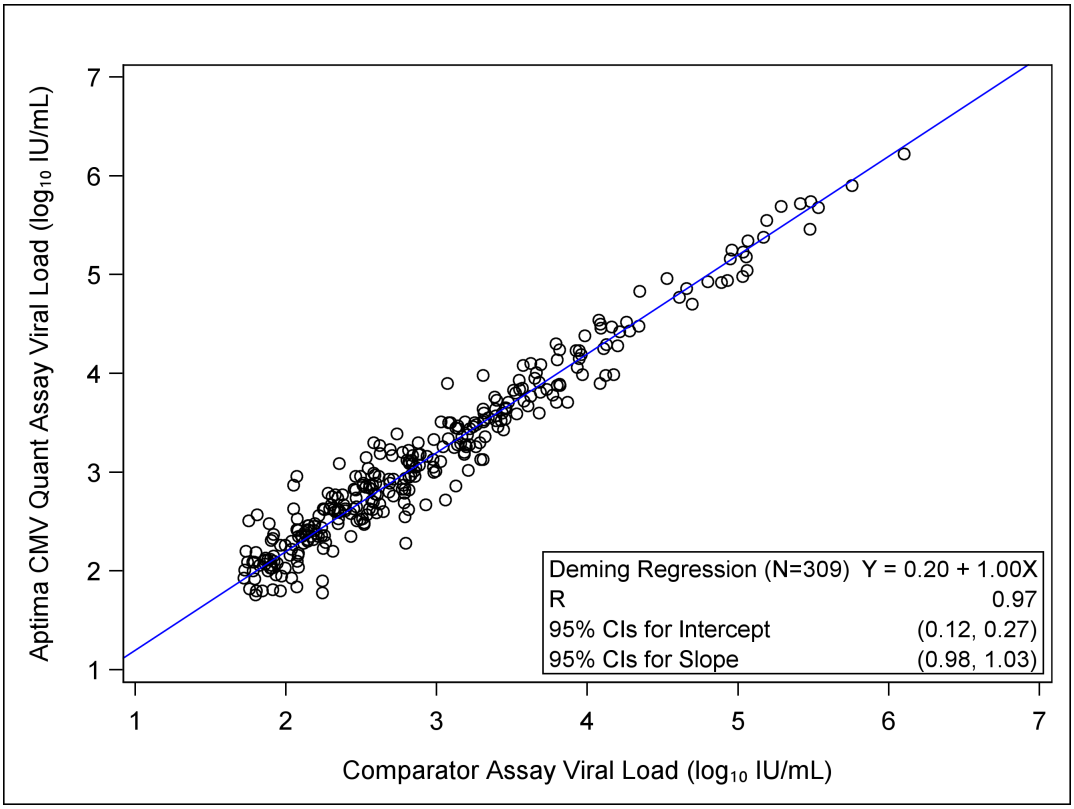

Figure 15. Deming Linear Regression Plot (Clinical Samples: SOTRs and HSCTRs Combined)

CI=confidence interval, HSCTRs=hematopoietic stem cell transplant recipients, R=correlation coefficient, SOTRs=solid organ transplant recipients

Notes:

- Paired samples with results in the common linear range for both assays included.
- Deming regression model assumes independence between all samples; jackknife method used to estimate CIs.

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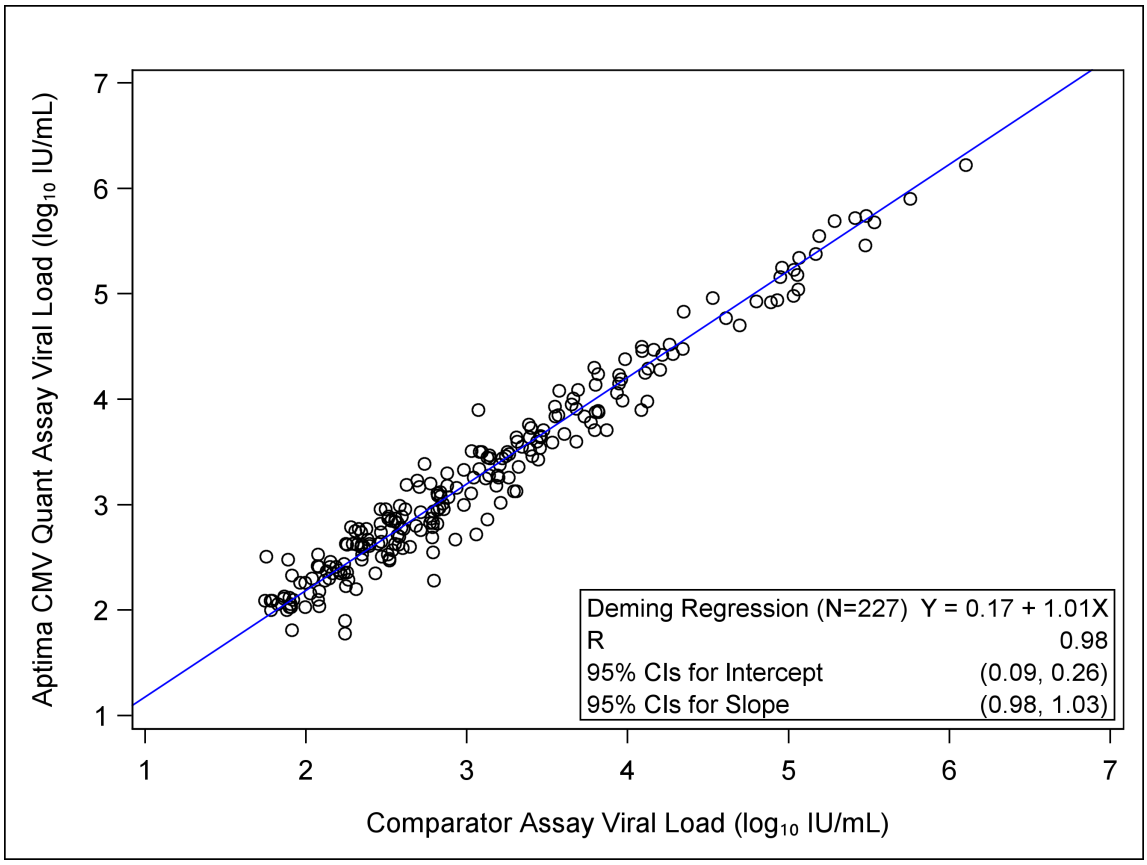

Figure 16. Deming Linear Regression Plot of Viral Loads (Clinical Samples: SOTRs only)

CI=confidence interval, SOTRs=solid organ transplant recipients, R=correlation coefficient

Notes:

- Paired samples with results in the common linear range for both assays included.
- Deming regression model assumes independence between all samples; jackknife method used to estimate CIs.

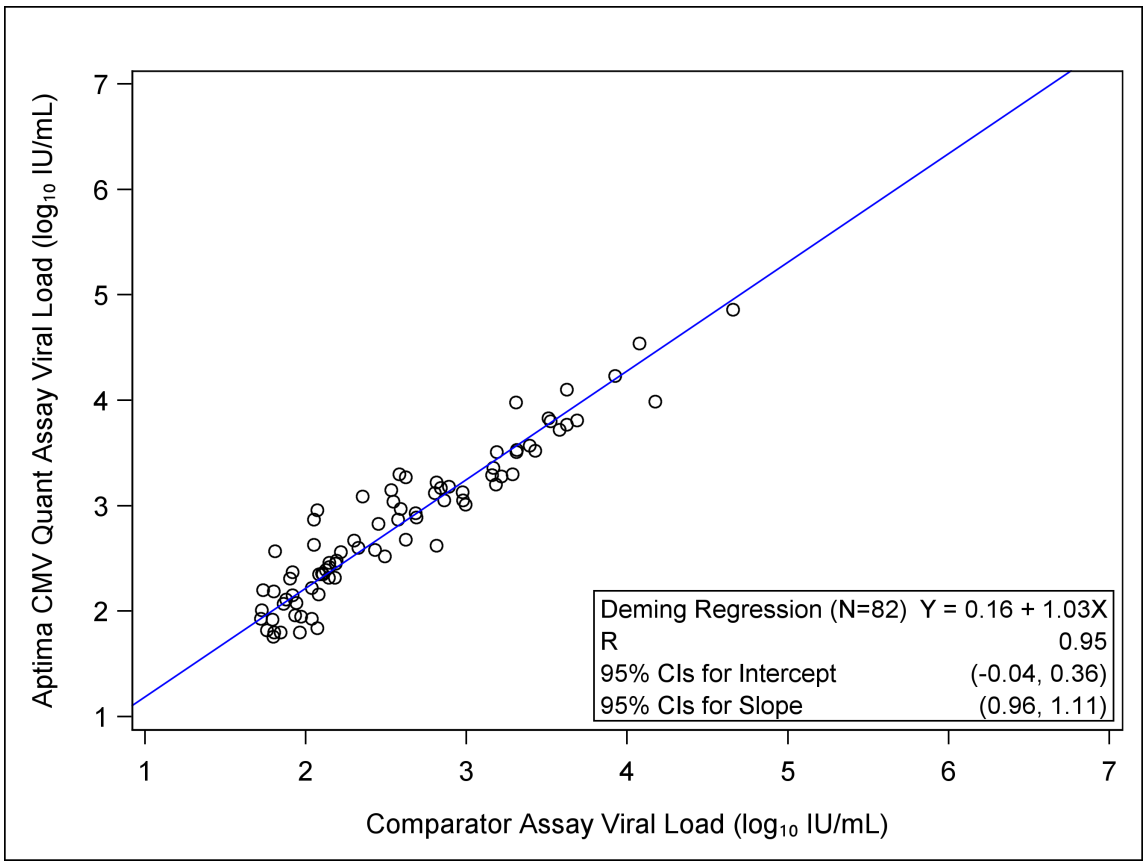

Figure 17. Deming Linear Regression Plot of Viral Loads (Clinical Samples: HSCTRs only)

CI=confidence interval, HSCTRs=hematopoietic stem cell transplant recipients, R=correlation coefficient

Notes:

- Paired samples with results in the common linear range for both assays included.
- Deming regression model assumes independence between all samples; jackknife method used to estimate CIs.

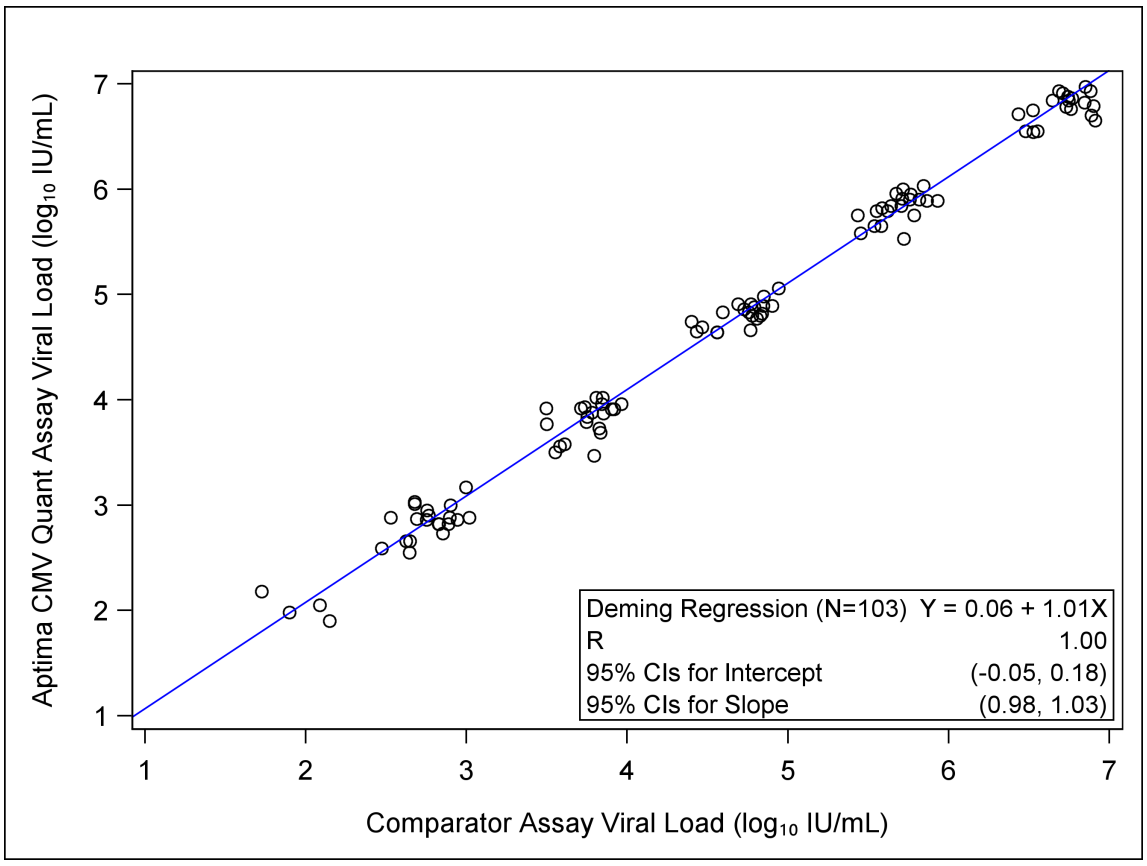

Figure 18. Deming Linear Regression Plot of Viral Loads (Contrived Samples)

CI=confidence interval, R=correlation coefficient

Notes:

- Paired samples with results in the common linear range for both assays included.
- Deming regression model assumes independence between all samples; jackknife method used to estimate CIs.

Mean Paired Difference

Table 32 below presents the mean paired difference between the Aptima CMV Quant assay and the approved test at representative decision intervals.

Table 32: Mean of Paired Viral Load Differences at Representative Decision Intervals by Sample Type and Transplant Group

Sample Type	Transplant Group	Representative Decision Intervals[a] (log_{10} IU/mL)	Total number of paired samples[b] (N)	Mean (SE)	95% CI
Clinical	Overall	All	254	0.20 (0.012)	(0.17, 0.22)
		≥2.1 to <3.0	129	0.21 (0.018)	(0.18, 0.25)
		≥3.0 to <4.0	87	0.19 (0.021)	(0.15, 0.23)
		≥4.0 to <5.0	24	0.17 (0.039)	(0.09, 0.25)
		≥5.0	14	0.18 (0.037)	(0.10, 0.26)
	SOTRs	All	199	0.18 (0.014)	(0.16, 0.21)
		≥2.1 to <3.0	95	0.19 (0.021)	(0.14, 0.23)
		≥3.0 to <4.0	69	0.18 (0.024)	(0.13, 0.23)
		≥4.0 to <5.0	21	0.17 (0.038)	(0.09, 0.25)
		≥5.0	14	0.18 (0.037)	(0.10, 0.26)
	HSCTRs	All	55	0.26 (0.026)	(0.20, 0.31)
		≥2.1 to <3.0	34	0.29 (0.034)	(0.22, 0.36)
		≥3.0 to <4.0	18	0.22 (0.039)	(0.13, 0.30)
		≥4.0 to <5.0	3	0.16 (0.188)	(-0.65, 0.97)
		≥5.0	0	NC (NC)	NC
Contrived	n/a	All	100	0.08 (0.014)	(0.05, 0.11)
		≥2.1 to <3.0	20	0.07 (0.037)	(0.00, 0.15)
		≥3.0 to <4.0	21	0.05 (0.036)	(-0.03, 0.12)
		≥4.0 to <5.0	20	0.10 (0.025)	(0.04, 0.15)
		≥5.0	39	0.10 (0.022)	(0.06, 0.14)

CI=confidence interval, HSCTRs=hematopoietic stem cell transplant recipients, NC = not calculable, SE=standard error, SOTRs=solid organ transplant recipients

[a] Paired samples are allocated into decision intervals based on the approved test result.

[b] Number of paired samples with results in the common linear range for both assays.

Bias at Select Viral Load Levels

Table 33 below presents the bias between the Aptima CMV Quant assay and the approved test at five select viral load levels from 2.1 log_{10} IU/mL to 7.0 log_{10} IU/mL with associated non-transformed equivalents.

Table 33: Bias/Systematic Difference at Select Viral Load Levels by Sample Type and Transplant Group

Sample Type	Transplant Group	Select Viral Load Levels log_{10} IU/mL (IU/mL)	Systemic Difference[a] log_{10} IU/mL (IU/mL)
Clinical	Overall	2.1 (137)	0.20 (1797.1)
		2.7 (500)	0.20 (1948.2)
		3.3 (1800)	0.21 (2489.1)
		3.9 (7943.3)	0.21 (5045.3)
		7.0 (10000000)	0.22 (4162789.2)
	SOTRs	2.1 (137)	0.18 (2251.8)
		2.7 (500)	0.19 (2402.4)
		3.3 (1800)	0.19 (2941.7)
		3.9 (7943.3)	0.19 (5490.5)
		7.0 (10000000)	0.21 (4151107.2)
	HSCTRs	2.1 (137)	0.23 (180.1)
		2.7 (500)	0.25 (430.5)
		3.3 (1800)	0.27 (1327.2)
		3.9 (7943.3)	0.29 (5564.7)
		7.0 (10000000)	0.40 (6897935.4)
Contrived	n/a	2.1 (137)	0.07 (33420.4)
		2.7 (500)	0.08 (33467.9)
		3.3 (1800)	0.08 (33638.0)
		3.9 (7943.3)	0.08 (34442.0)
		7.0 (10000000)	0.10 (1342167.4)

HSCTRs=hematopoietic stem cell transplant recipients, SOTRs=solid organ transplant recipients

[a] The systematic difference is the difference between the outcome variable (Y) and the viral load (X) derived at each of the select viral load levels using the Deming regression estimates for slope and intercept.

Allowable Total Difference (ATD)

Table 34 along with Figure 19 through Figure 22 below present the ATD results using the paired differences between the Aptima CMV Quant assay and the approved test versus their average at representative thresholds and the percentage of paired results in the ATD zone.

Table 34: Percentage of Paired Sample Differences Within Allowable Total Difference (ATD) Zone at Different Viral Load Intervals by Sample Type and Transplant Group

Sample Type	Transplant Group	Viral Load Intervals[a] (log_{10} IU/mL)	N[b]	Paired sample differences within ATD zone				
				n (%)	Percentiles			
					2.5%	5%	95%	97.5%
Clinical	Overall	All	271	234 (86.3)	-0.19	-0.14	0.40	0.42
		Low (≥2.1 to <3.3)	171	147 (86.0)	-0.24	-0.16	0.41	0.44
		Medium (≥3.3 to <3.9)	52	48 (92.3)	-0.08	-0.08	0.38	0.38
		High (≥3.9 to <7)	48	39 (81.3)	-0.18	-0.18	0.37	0.40
	SOTRs	All	207	183 (88.4)	-0.19	-0.14	0.40	0.42
		Low (≥2.1 to <3.3)	123	109 (88.6)	-0.26	-0.18	0.41	0.44
		Medium (≥3.3 to <3.9)	40	38 (95.0)	-0.16	-0.08	0.38	0.40
		High (≥3.9 to <7)	44	36 (81.8)	-0.18	-0.14	0.37	0.40
	HSCTRs	All	64	51 (79.7)	-0.18	0.01	0.38	0.41
		Low (≥2.1 to <3.3)	48	38 (79.2)	-0.19	0.01	0.41	0.45
		Medium (≥3.3 to <3.9)	12	10 (83.3)	0.09	0.09	0.32	0.32
		High (≥3.9 to <7)	4	3 (75.0)	-0.18	-0.18	0.31	0.31
Contrived	n/a	All	99	96 (97.0)	-0.19	-0.14	0.29	0.34
		Low (≥2.1 to <3.3)	20	20 (100)	-0.14	-0.13	0.35	0.35
		Medium (≥3.3 to <3.9)	14	13 (92.9)	-0.32	-0.32	0.27	0.27
		High (≥3.9 to <7)	65	63 (96.9)	-0.19	-0.11	0.24	0.29

HSCTRs=hematopoietic stem cell transplant recipients, SOTRs=solid organ transplant recipients

[a] Paired samples are allocated into decision intervals based on the approved test result.

[b] Number of paired samples with results in the common linear range for both assays.

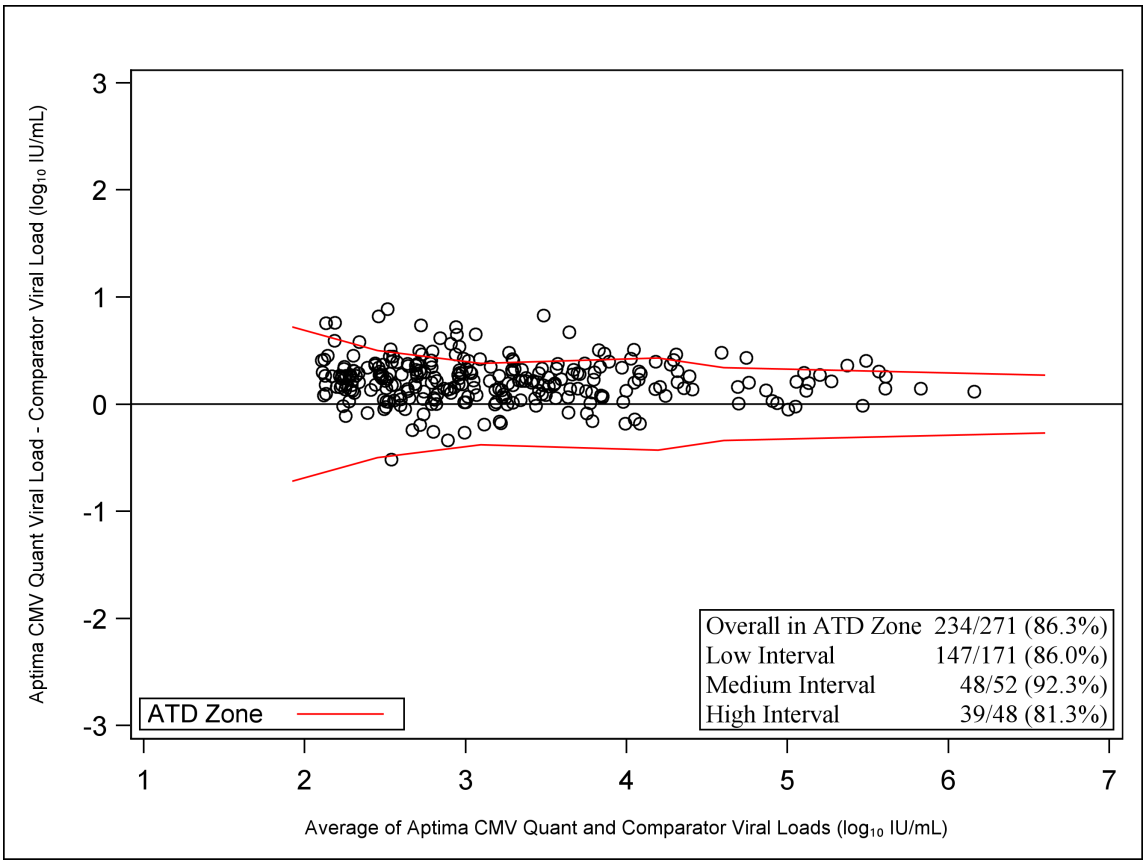

Figure 19. Difference Plot of Paired Samples and ATD Zone (Clinical Samples: SOTRs and HSCTRs Combined)

ATD=allowable total difference, HSCTRs=hematopoietic stem cell transplant recipients, SOTRs=solid organ transplant recipients

Note: Paired samples with results in the common linear range for both assays included.

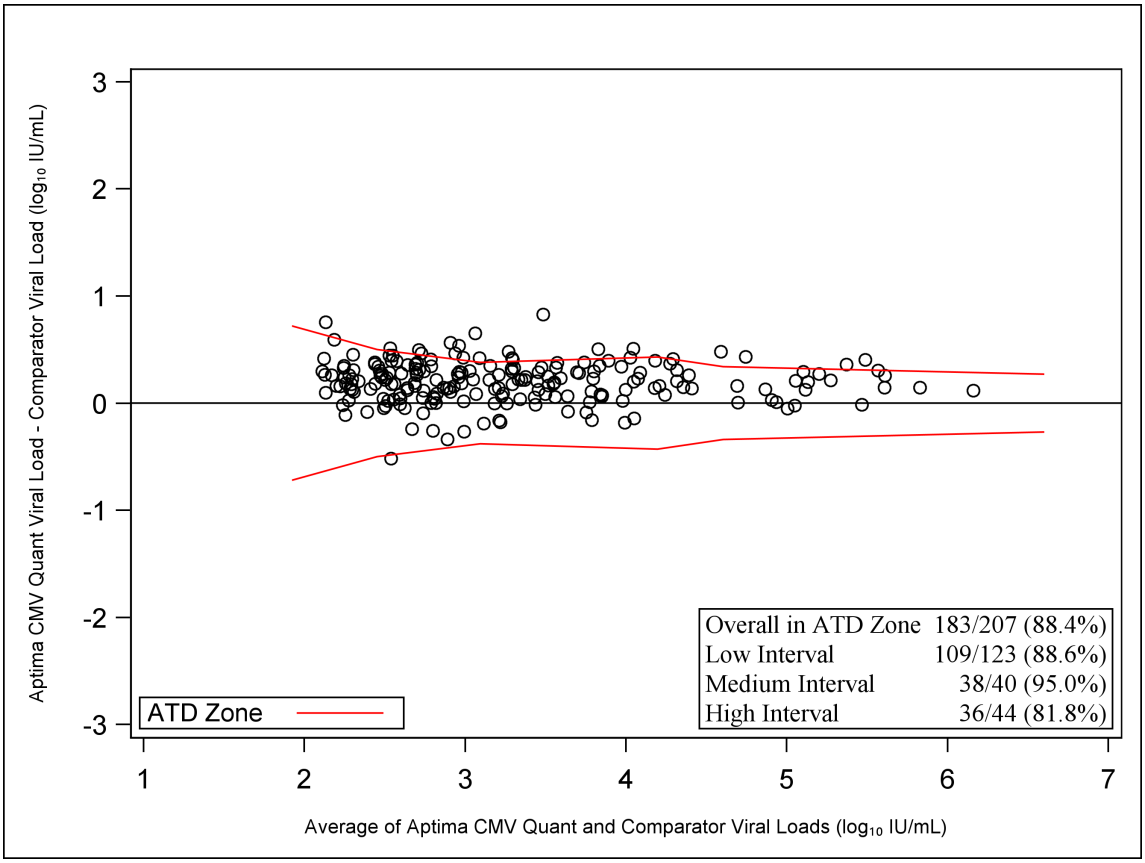

Figure 20. Difference Plot of Paired Samples and ATD Zone (Clinical Samples: SOTRs only)

ATD=allowable total difference, SOTRs=solid organ transplant recipients

Note: Paired samples with results in the common linear range for both assays included.

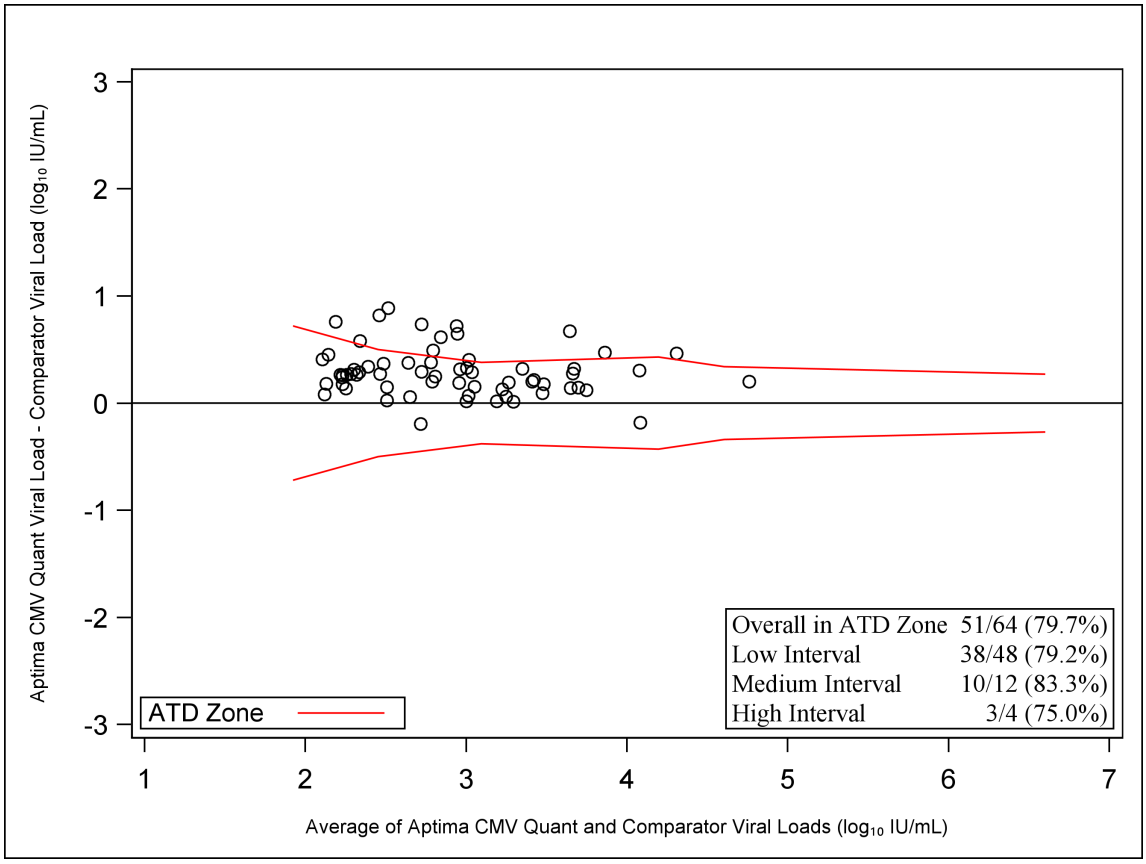

Figure 21. Difference Plot of Paired Samples and ATD Zone (Clinical Samples: HSCTRs only)

ATD=allowable total difference, HSCTRs=hematopoietic stem cell transplant recipients

Note: Paired samples with results in the common linear range for both assays included.

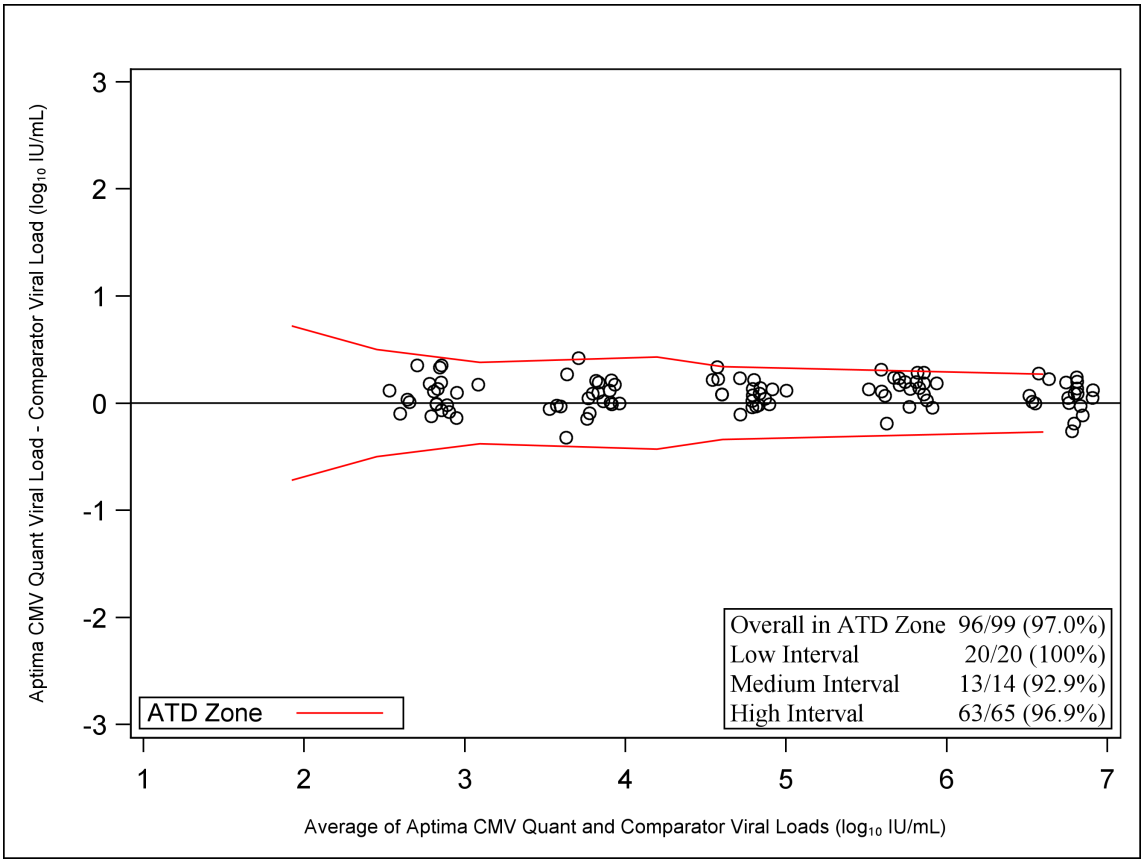

Figure 22. Difference Plot of Paired Samples and ATD Zone (Contrived Samples)

ATD=allowable total difference

Note: Paired samples with results in the common linear range for both assays included.

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21. **1st WHO International Standard for Human Cytomegalovirus (HCMV) for Nucleic Acid Amplification Techniques (NIBSC 09/162)**, Merlin strain

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Serious incidents occurring in relation to the device in the European Union should be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

AW-27747-001 Rev. 002
2024-06

Revision History	Date	Description
AW-27747 Rev. 001	June 2023	• Created Aptima CMV Quant assay IFU AW-27747 Rev. 001 based on AW-25509 Rev. 003 for regulatory compliance with IVDR. • Added Summary of Safety and Performance • Updated General Information • Updated hazard information. • Updated sections of Analytical Performance. and Materials Provided Table, • Added Clinical Performance: Clinical Agreement, Method Comparison, Mean Paired Difference, Bias at Select Viral Load Levels, and Allowable Total Difference (ATD). • Updated contact information including: EC Rep, CE Mark, Australian Rep information, and technical support. • Miscellaneous style and formatting updates.
AW-27747 Rev. 002	June 2024	• Revised to incorporate plasma specimen dilution workflow • Updated sections listed below: • Warnings and Precautions • Specimen Collection and Storage • Materials Required but Available Separately • Panther System Test Procedure • Interpretation of Results • Analytical Specificity