SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: CMV DNA Quantitative test

Device Trade Name: Aptima® CMV Quant Assay

Device Procode: PAB

Applicant's Name and Address: Hologic, Inc.
10210 Genetic Center Drive
San Diego, CA 92121

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P210029

Date of FDA Notice of Approval: May 9, 2022

II. INDICATIONS FOR USE

The Aptima CMV Quant Assay is an in vitro nucleic acid amplification test for the quantitation of human cytomegalovirus (CMV) DNA in human EDTA plasma on the fully automated Panther system.

The Aptima CMV Quant Assay is intended for use to aid in the management of solid-organ transplant patients and hematopoietic stem cell transplant patients. In patients receiving anti-CMV therapy, serial DNA measurements can be used to assess viral response to treatment. The results from Aptima CMV Quant Assay must be interpreted within the context of all relevant clinical and laboratory findings.

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

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the Aptima CMV Quant Assay labeling.

V. DEVICE DESCRIPTION

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 (including variants of 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 World Health Organization (WHO) International Standard for human cytomegalovirus (NIBSC code: 09/162).

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.

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 and a primer containing a T7 promoter sequence is used to generate a DNA copy of the target sequence containing a promoter sequence for T7 RNA polymerase. T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy of the CMV DNA in the specimen.

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.

Components of the Aptima CMV Quant Assay Kit

The Aptima CMV Quant Assay kit (100 tests) for the Panther system consists of 4 reagent kits:

Box 1: Aptima CMV Quant Assay kit which contains the following reagents:

- Amplification Reagent

- Enzyme Reagent
- Promoter Reagent
- Target Capture Reagent
- Amplification Reconstitution Reagent
- Enzyme Reconstitution Reagent
- Promoter Reconstitution Reagent

Box 2: Aptima CMV Quant Controls kit which contains the following reagents:

- Negative Control
- Low Positive Control
- High Positive Control

Box 3: Aptima CMV Quant Calibrator kit which contains the following reagent:

- Positive Calibrator

Box 4: Aptima CMV Quant Target Enhancer Reagent Box

- Target Enhancer Reagent

There is one ancillary kit required to perform the assay but available separately: Aptima Assay Fluids kit (also known as Universal Fluids Kit) which contains the following reagents:

- Wash Solution
- Buffer for Deactivation Fluid
- Oil Reagent

Quality Control Procedures

The Aptima CMV Quant Assay contains three quality controls:

1. 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.

Various acceptance criteria for each replicate of calibrator are embedded in the Panther software. 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.

2. 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.

Various acceptance criteria for each control are embedded in the software on the Panther system. 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.

3. Internal Calibrator/Internal Control

Each sample, irrespective of whether it is a calibrator, control, or specimen, contains an internal calibrator/internal control (IC). Various acceptance criteria for IC are embedded in the software on the Panther system. 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 appropriate Panther/Panther Fusion System Operator's Manual.

4. Process Controls

The Panther system has various process controls that verify that the correct volume of each reagent is dispensed for each reaction and also that the various modules including incubators meet their temperature specifications. Specimens may be invalidated by the Panther system if the instrument process control specifications are not met.

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.

Table 1: 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
> 10,000,0000	> 7.00	CMV DNA concentration is above the upper limit of quantification (ULoQ)
Invalid[a]	Invalid[a]	Error indicated in the generation of the result. Specimen should be retested

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

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are currently multiple commercially available assays to quantitate CMV DNA. Quantification of the level of CMV DNA aids in the management of solid-organ transplant patients and hematopoietic stem cell transplant patients and assessing the viral response in patients receiving anti-CMV therapy. Assays that quantitate viral loads can provide a glimpse of patient's response to treatment. In addition to transcription mediated amplification (TMA), polymerase chain reaction (PCR) technology is used for CMV DNA quantitation (viral load) which is used as an aid in the assessment of viral response to antiviral treatment as measured by changes in CMV DNA levels.

Additional information about a patient's CMV infection can be determined based on their medical history and serology results, with consideration of the advantages and disadvantages associated with each of these procedures.

VII. MARKETING HISTORY

The Aptima CMV Quant Assay, and accessory kit are marketed in multiple countries. The device has not been withdrawn from marketing for any reasons related to its safety or effectiveness. The following is list of countries where the product is distributed:

- Austria
- Belgium
- Denmark
- Finland
- France
- Germany
- Ireland
- Italy
- Liechtenstein
- Luxembourg
- Malta
- The Netherlands
- Norway
- Portugal
- Spain
- Sweden
- Switzerland
- United Kingdom

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Potential adverse effects (e.g., complications) associated with the use of the device are discussed below. The Aptima CMV Quant Assay is intended to aid in the management of solid-organ transplant patients and hematopoietic stem cell transplant patients, and patients receiving anti-CMV therapy. The results from the Aptima CMV Quant Assay must be interpreted within the context of all relevant clinical and laboratory findings.

The risks associated with the device, when used as intended, are those related to the risk of false test results, failure to correctly interpret the test results, and failure to correctly operate the device. The risk of false positive or falsely elevated CMV viral loads in patients undergoing preemptive therapy or monitoring of treatment for known CMV DNA in the blood is related to the risks of initiation or continuation of antiviral therapy when it is not necessary or the reduction of immunosuppression in transplant patients in whom reduction of immunosuppression is not indicated. The initiation or continuation of antiviral therapy can result in known drug toxicities, including suppression of bone marrow, in particular leukopenia, which can add to the patient's risk of contracting opportunistic infections. Other known drug toxicities include thrombocytopenia, diarrhea, and bloodstream infections if a central venous catheter is used to administer therapy. Reduction of immunosuppression can increase a transplant patient's risk of rejection of the transplanted organ or graft-versus-host disease, the latter of which can result in maculopapular rash, persistent nausea and vomiting, diarrhea, lichen planus, scleroderma, and ulcerations and sclerosis of the gastrointestinal tract.

The risk of false negative or falsely low CMV viral loads in patients undergoing preemptive therapy or monitoring of treatment for known CMV DNA in the blood include failure to initiate or premature discontinuation of appropriate antiviral treatment or reduction of immunosuppression, thus increasing the risk of CMV disease. The sequelae of untreated CMV disease because of false negative or falsely low CMV DNA include CMV Syndrome and tissue-invasive CMV disease with end-organ damage, including colitis, hepatitis, nephritis, pneumonitis, meningitis, and retinitis. CMV infection and disease is associated with morbidity, failure of the transplanted organ, and death in transplant patients. False negative or falsely low CMV DNA results can yield an increased rate of late CMV, selective drug use, and increased drug cost and subsequent drug toxicities.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

Limit of Detection (LoD) Using the 1st CMV 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 Clinical and Laboratory Standards Institute (CLSI) EP17-A2. The LoD was determined by testing panels of the 1st WHO International Standard (NIBSC code 09/162, genotype gB-1)

for CMV diluted in CMV negative human plasma. Sixty 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 for the Aptima CMV Quant Assay using the 1st WHO International Standard is 40.7 IU/mL for plasma.

Limit of Detection Across CMV Genotypes

The LoD was verified for three different genotypes based on Glycoprotein B sequence (gB-2, gB-3, gB-4, and drug resistant mutants) by testing various concentrations of CMV around the established LoD for plasma using the WHO Standard. 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. The highest overall LoD is 40.7 IU/mL.

Table 2: Limit of Detection Across CMV Genotypes

Genotype	Concentration (IU/mL)
gB-2	40
gB-3	40
gB-4	35
Drug resistant mutant U54 and UL97*	35
Drug resistant mutant UL56**	35

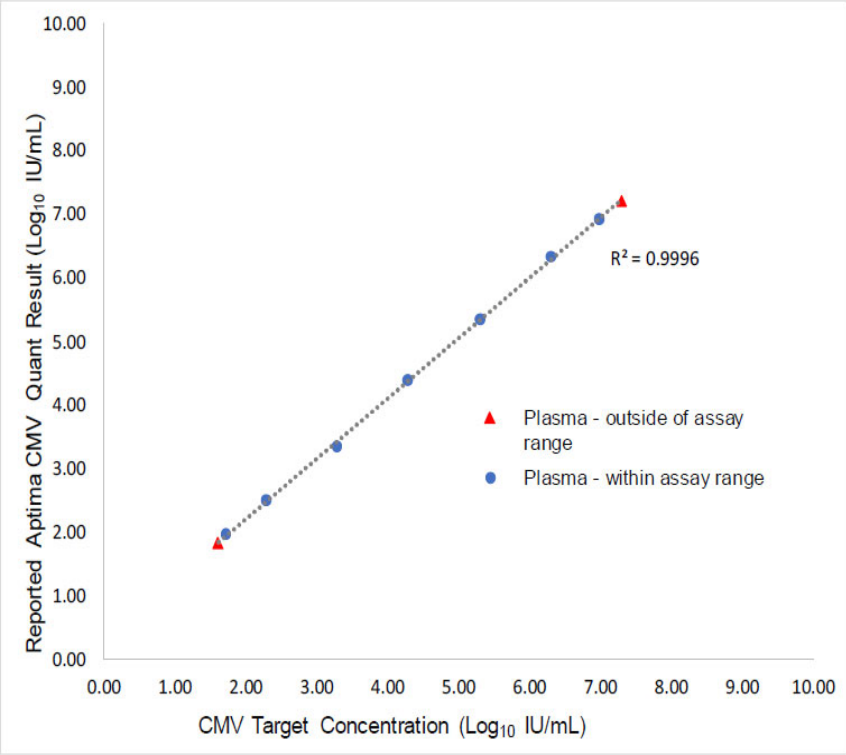
*UL54 gene mutations can lead to cross resistance to several antivirals for treatment of CMV infection such as ganciclovir (GCV), cidofovir (CDV), and foscarnet (PFA). UL97 gene mutations also lead to ganciclovir (GCV) resistance.

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

Linear Range

The linear range was established by testing panels of CMV diluted in CMV negative human plasma according to CLSI EP06-A. 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 1.

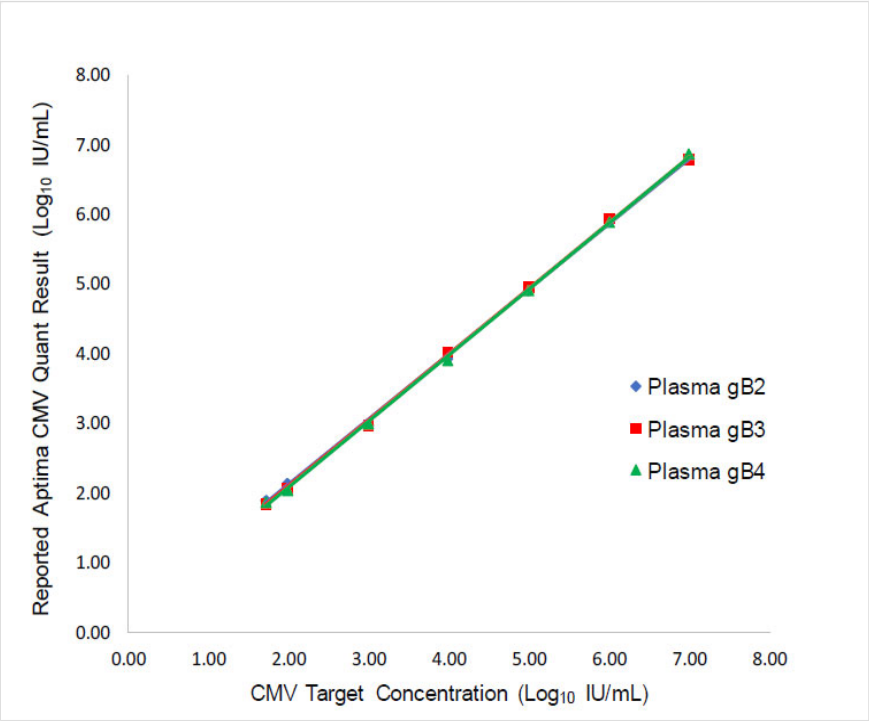
Figure 1: Linearity in Plasma Samples



Linearity Across CMV Genotypes

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 2.

Figure 2: Linearity Across CMV Genotypes gB-2, gB-3, and gB-4 (Plasma)



Lower Limit of Quantitation (LLoQ) Using the 1st CMV 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. Total error was estimated using various models including the Westgard Model: Total Error (TE) = |bias| + 2SD and Total Error (TE) = SQRT(2) x 2 SD where SD = Standard Deviation. 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).

The LLoQ was determined by testing panels of the 1st WHO International Standard (NIBSC code 09/162, genotype gB-1) for CMV DNA diluted in CMV negative human plasma. Sixty replicates of each dilution were tested with each of three reagent lots for a total of 180 replicates per dilution.

The LLoQ generated with the 1st WHO International Standard for CMV in plasma is 53 IU/mL.

Determination of LLoQ Across CMV Genotype:
The LLoQ was established by testing dilutions of CMV genotypes gB-2, gB-3, gB-4, and drug resistant mutants in CMV negative human plasma. Sixty replicates of each panel member were tested with one reagent lot. 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 3. The overall LLoQ for plasma in this assay is 53 IU/mL.

Table 3: LLoQ Result Summary for CMV Genotypes and Drug Resistant Mutants

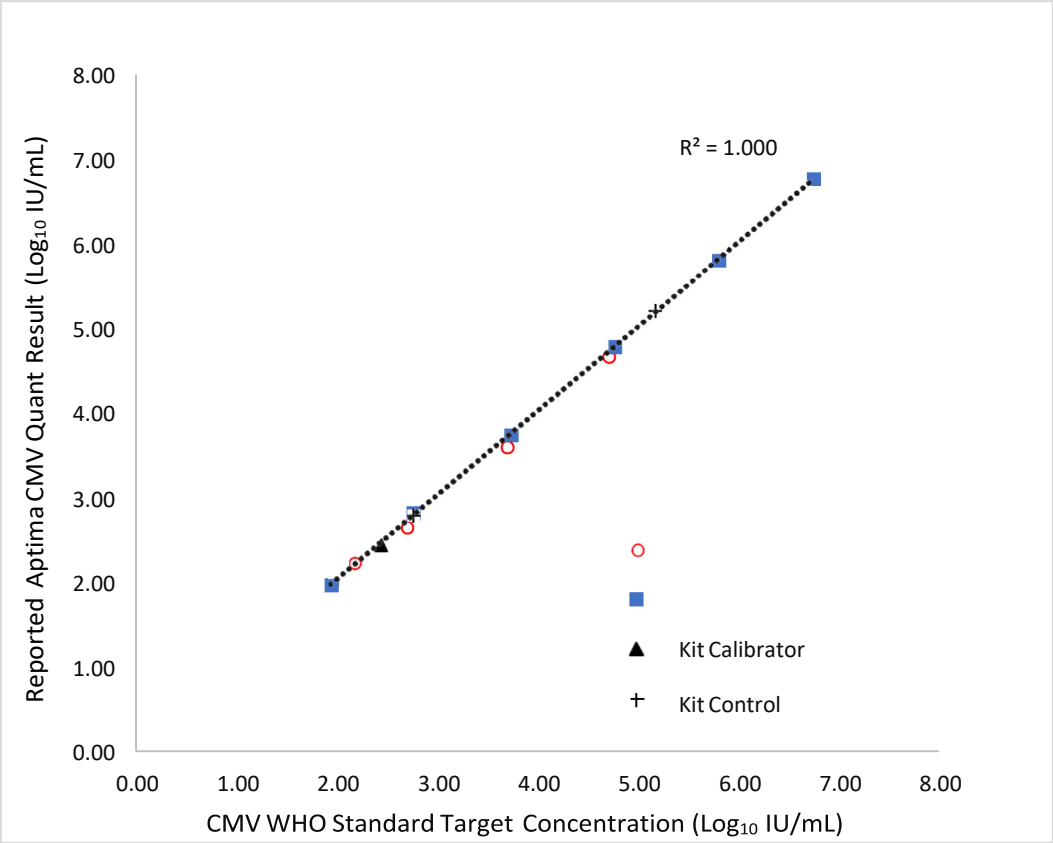
Genotype	LLoQ (IU/mL)	LLoQ (log_{10} IU/mL)
gB2	50	1.70
gB3	35	1.55
gB4	46	1.66
Drug resistant mutant UL54 and UL97*	38	1.57
Drug resistant mutant UL56**	35	1.54

*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.

Traceability to the 1st CMV WHO International Standard:
A series of secondary standards with known concentrations were used throughout product development and product manufacturing to establish traceability to the WHO standard. The CMV WHO 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. The secondary standards ranged in concentration from 1.80 to 6.60 log_{10} IU/mL.

The concentrations tested for the CMV WHO standard were between 2.18 to 4.70 log_{10} IU/mL. The WHO plasma panels, secondary standards, assay controls, and assay calibrator recovered as expected across the linear range of the assay, as can be seen from Figure 3.

Figure 3: Traceability Between the 1st CMV WHO Standard Target Concentrations and Reported Concentrations in the Aptima CMV Quant Assay (WHO Standard)



Precision

To assess reproducibility, a 6-member panel was made by diluting CMV positive clinical specimens or cultured CMV into CMV negative ethylenediamine tetraacetate (EDTA) 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.

Table 4 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 4: Precision of the Aptima CMV Quant Assay

N	Mean Concentration (log_{10} IU/mL)	Inter-Lot SD	Inter-Instrument SD	Inter-Operator SD	Inter-Day SD	Inter-Run SD	Intra-Run SD	Total 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.

Analytical Specificity-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 and EP37. CMV negative plasma samples and samples spiked with CMV to concentrations of 2.22 log_{10} IU/mL and 3.30 log_{10} IU/mL were tested.

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).

Clinical plasma specimens from patients with elevated levels of specific substances or from patients with the diseases listed in Table 5 were tested with the Aptima CMV Quant Assay. No interference in the performance of the assay was observed.

Table 5: Clinical Specimens Tested for Interference

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

No interference in the performance of the assay was observed in the presence of the exogenous substances listed in Table 6 at concentrations of least three times the Cmax of drugs in human plasma.

Table 6: Exogenous Substances Tested for Interference

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

Analytical Specificity

Specificity was determined by testing 390 frozen CMV negative EDTA plasma clinical specimens. Specificity was calculated as the percentage of CMV negative samples with results of "Not Detected" versus the total number of samples tested.

CMV DNA was not detected in 389 samples. Specificity was 99.7% (389/390, 95% CI: 98.6 -100%).

Table 7: Specificity in CMV Negative EDTA Plasma Clinical Specimens

Aptima CMV Quant	Plasma Total
Valid (n)	390
Non Reactive (n)	389
Initial Reactive (n)	1
True Positive (n)	0
False Positive (n)	1
Specificity	99.7%
95% CI, Lower Limit	98.6%
95% CI, Upper Limit	100%

CI = Confidence Interval

Analytical Specificity – Cross Reactivity

Potential cross-reactivity to the pathogens listed in Table 8 was evaluated in CMV negative human plasma the presence or absence of 2.2 $\log_{10}$ IU/mL and 3.3 $\log_{10}$ IU/mL of CMV. Pathogens were tested at the highest concentration available. No cross-reactivity or interference was observed.

Table 8: Pathogens Tested for Analytical Specificity - Cross-Reactivity

Microorganism/Pathogen	Concentration	
Adenovirus type 4	1,886	TCID50/mL[a]
BK Polyomavirus	1,000,000	cp/mL[b]
Epstein-Barr virus	1,000,000	cp/mL
Hepatitis B virus	1,000,000	IU/mL[c]
Hepatitis C virus	1,000,000	cp/mL
Herpes Simplex virus type 1	1,428,571	TCID50/mL
Herpes Simplex virus type 2	147,143	TCID50/mL
HIV-1 subtype B	1,000,000	cp/mL
Human Herpesvirus 6A	1,000,000	cp/mL
Human Herpesvirus 7	1,428,571	TCID50/mL
Human Herpesvirus 8	1,000,000	cp/mL
Human Metapneumovirus	192,857	TCID50/mL
Human Papillomavirus type 18	1,000,000	cp/mL
Human Parainfluenza virus	944	TCID50/mL
Influenza virus	3,857	TCID50/mL
Rhinovirus	7,257	TCID50/mL
Varicella Zoster virus	1,000,000	cp/mL
Zika virus	29,286	TCID50/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
Mycobacterium intracellulare	1,000,000	CFU/mL
Mycoplasma genitalium	1,000,000	CFU/mL
Mycoplasma pneumoniae	1,000,000	CFU/mL
Neisseria gonorrhoeae	1,000,000	CFU/mL
Propionibacterium acnes	1,000,000	CFU/mL
Salmonella enterica serovar Typhimurium	1,000,000	CFU/mL
Staphylococcus aureus	1,000,000	CFU/mL
Staphylococcus epidermidis	1,000,000	CFU/mL
Streptococcus agalactiae	1,000,000	CFU/mL
Streptococcus pneumoniae	1,000,000	CFU/mL
Streptococcus pyogenes	1,000,000	CFU/mL

Microorganism/Pathogen	Concentration	
Aspergillus niger	485,000	CFU/mL
Candida albicans	1,000,000	CFU/mL
Cryptococcus neoformans	1,000,000	CFU/mL
Trichomonas vaginalis	1,000,000	cells/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

Specimen Stability
Specimen stability studies demonstrated that, for Aptima CMV Quant Assay, specimens should be stored as follow:

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 K_2EDTA and K_3EDTA 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.

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,
- In the PPT at -20°C or -70°C for up to 60 days.

Samples On Board: 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.

Real-Time Reagent (including Controls) Stability
Expiration dating for the Aptima CMV Quant Assay reagents (Table 9) has been established as listed below, *w*hen stored under recommended conditions for use. Stability studies are progressing towards the 24-month claim for all components of the Aptima CMV Quant Assay.

Table 9: Real-Time Reagent Stability

Kit Description	Shelf Life
Aptima CMV Quant Assay Kit	24 months at 2°C to 8°C
Aptima CMV Quant Target Enhancer Reagent Kit	24 months at 15°C to 30°C
Aptima CMV Quant Calibrator Kit	15 months at -15°C to -35°C
Aptima CMV Quant Control Kit	12 months at -15°C to -35°C

B. Animal Studies

Not applicable

C. Additional Studies

None

X. SUMMARY OF PRIMARY CLINICAL STUDIES

The applicant performed clinical studies to establish a reasonable assurance of safety and effectiveness of the Aptima CMV Quant Assay using samples that would routinely be tested for CMV in the US. Data for this clinical study were the basis for the PMA approval decision. A summary of the clinical study is presented below.

A. Study Design

The clinical performance study was designed to assess the clinical agreement between the Aptima CMV Quant Assay and an FDA-approved comparator test. During the prospective multi-center study at eight clinical sites, 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.

Below are the detailed criteria for subject inclusion, exclusion and withdrawal of the study:

Subject Inclusion:

- Subject and/or legally authorized representative was willing and able to provide consent prior to study participation.
- Subject was ≥18 years of age.
- Subject was undergoing or a candidate for routine SOC monitoring per the collection sites' CMV management protocol.
- Subject met one of the following two criteria:
  -Subject was a kidney, liver, lung, or heart transplant recipient; or
  -Subject was an allogeneic or autologous HSCTR.
- Subject was serotype D+/R-, D-/R+ or D+/R+.
- Subject had a positive CMV result of equal to or above the LLoQ (≥ LLoQ) within
  10 days prior to enrollment with no intervening negative CMV result below the LLoQ (<LLoQ) and/or TND by SOC viral load testing.

Subject Exclusion:

- Subject had already participated in this study.
- Subject was unsuitable for study participation based on the PI's decision (eg, unlikely to comply with study procedure(s), significant medical complication).

- Subject was participating in another investigational study that the PI believed might interfere with the subject's participation in this study.

Subject Withdrawal:

- Subject did not meet eligibility criteria and was erroneously enrolled;
- Subject chose to terminate study participation;
- No specimens were collected from a subject; or
- All of the subject's specimens were withdrawn.

B. Accountability of PMA Cohort

A total of 82 subjects participated in the prospective clinical study. Of these subects, 65 were SOTRs and 20 were HSCTRs providing 259 SOTR specimens and 80 HSCTR specimens for the agreement analyses. For the method comparison analyses, 140 SOTR and 25 HSCTR prospective specimens were provided. There were also banked specimens included in the studies: 144 SOTR and 114 HSCTR specimens were provided for the agreement analyses. There were 87 SOTR and 57 HSCTR banked specimens available for method comparison analyses. All specimens were assessed for entry into the clinical performance evaluation of the Aptima CMV Quant Assay.

C. Study Population Demographics and Baseline Parameters

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 FDA approved test (n = 1). Table 10 shows the demographic and baseline clinical characteristics of the 82 evaluable subjects.

Table 10: 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	51.9	52.1
	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)	-	-
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

D. Safety and Effectiveness Results

1. Safety Results

There were no adverse effects of the device reported while the study was conducted.

2. Effectiveness Results

The non-clinical and clinical studies performed in support of the effectiveness of the Aptima CMV Quant Assay demonstrated that the Aptima CMV Quant Assay can be used to aid in the management of solid-organ transplant patients and hematopoietic stem cell transplant patients. In patients receiving anti-CMV therapy, serial DNA measurements can be used to assess viral response to treatment. The results from the Aptima CMV Quant Assay must be interpreted within the context of all relevant clinical and laboratory findings.

Agreement Analysis

In the prospective study, 365 samples were collected from the 82 evaluable subjects. Additionally, 261 frozen residual frozen samples were obtained from clinical specimen suppliers. Of the 626 clinical 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 FDA approved test) clinical 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 11 shows the agreement analysis and percent agreement between the Aptima CMV Quant Assay and the FDA-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 12. 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 11: 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 Comp ≥ ACMV ≥	Comp < ACMV ≥	Comp < ACMV <	Comp ≥ ACMV <	PPA % (n/N) [95% CI][c]	NPA % (n/N) [95% CI][c]
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	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] FDA-approved test; [c] Score CI; [d] LLoQ of an alternate FDA-approved test

Table 12: Agreement Analysis at Different Viral Load Intervals (Overall and by Transplant Group)

Transplant Group	Comparator[b] Result (log_{10} IU/mL)						
Aptima CMV Assay Result	**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 S amples, 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] FDA-approved test

[c] LLoQ of an alternate FDA-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 13 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 14. 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 13: 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 Comp ≥ ACMV ≥	Comp < ACMV ≥	Comp < ACMV <	Comp ≥ ACMV <	PPA % (n/N) [95% CI][c]	NPA% (n/N) [95% CI][c]
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 (137 IU/mL)[d]	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.

≥: 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] FDA-approved test; [c] Score CI; [d] LLoQ of an alternate FDA-approved test.

Table 14: Agreement Analysis at Different Viral Load Intervals using all Time Points Post-Treatment Initiation Combined (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	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] FDA-approved assay

[c] LLoQ of an alternate FDA-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 FDA-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.

The method comparison study included the analysis listed below:

a) Deming Regression Analysis
b) Mean Paired Difference
c) Allowable Total Difference (ATD)

a) Deming Regression Analysis

Table 15 presents Deming regression parameter estimates (log_{10} IU/mL). Figure 4 through Figure 7 show Deming regression of the viral load results (log_{10} IU/mL) from the Aptima CMV Quant Assay and the FDA-approved test.

Table 15: Deming Regression Parameter Estimates by Sample Type and Transplant Group

						Jackknife Method[b]		Bootstrap Method[c]		
Sample Type	**Transplant Group**	**Viral Load Unit**	**Parameter**	**N[a]**	**Estimate**	**SE**	**95% CI**	**SE**	**95% CI**	**r**
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)	

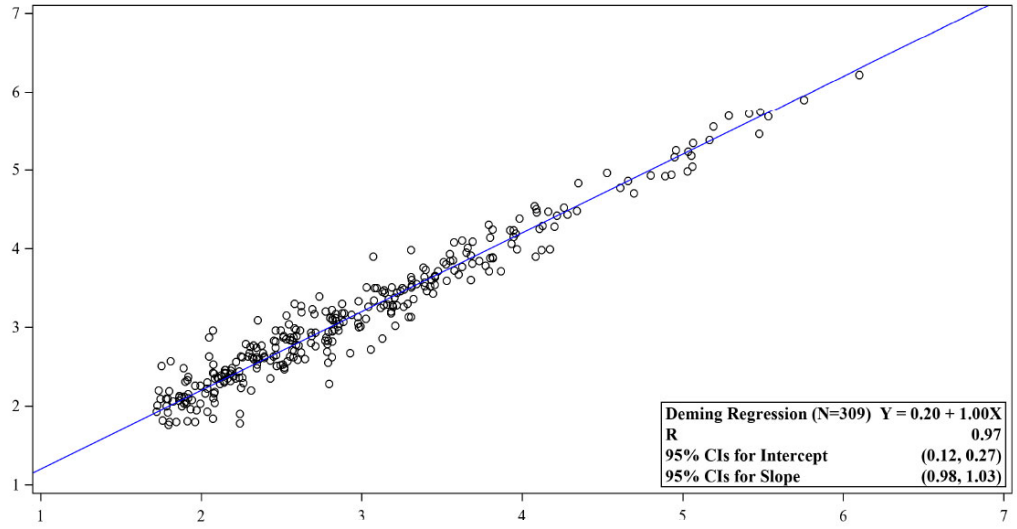
CI=confidence interval, HSCTRs=hematopoietic stem cell transplant recipients, r=correlation coefficient, SE=standard error, SOTRs=solid organ transplant recipients

[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 4. Deming Linear Regression Plot (Clinical Samples: SOTRs and HSCTRs Combined)

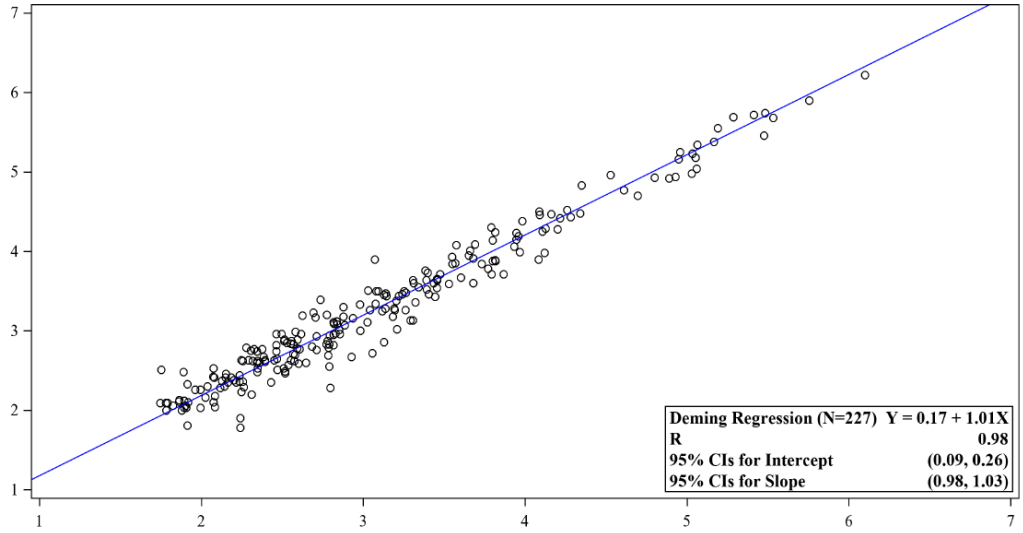


CI=confidence interval, HSCTRs=hematopoietic stem cell transplant recipients, r=correlation coefficient, SOTRs=solid organ transplant recipients

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.

Figure 5. Deming Linear Regression Plot of Viral Loads (Clinical Samples: SOTRs only)

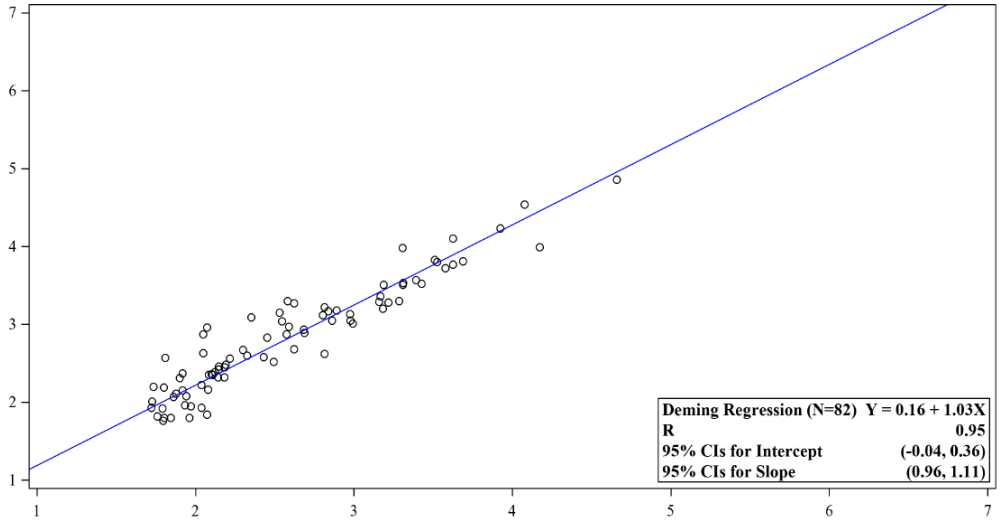


CI=confidence interval, SOTRs=solid organ transplant recipients, r=correlation coefficient

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

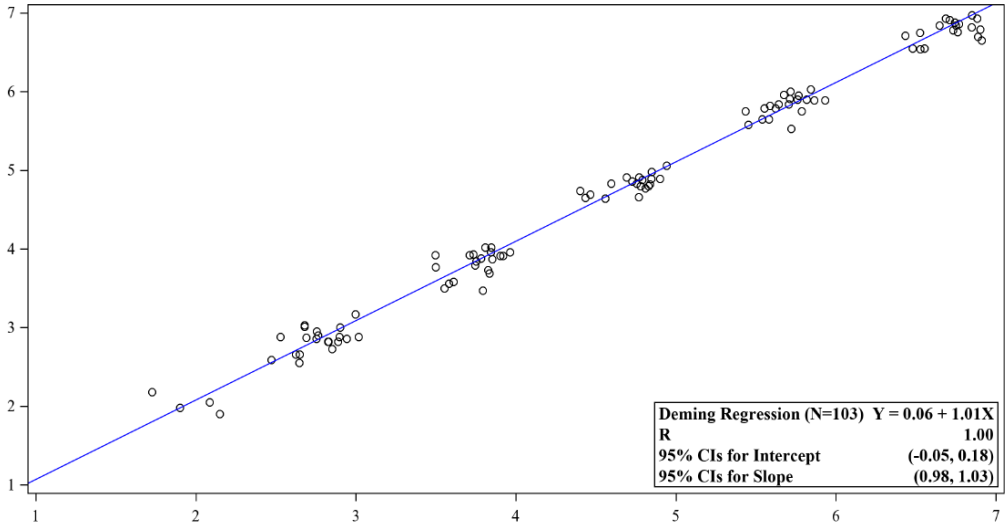


CI=confidence interval, HSCTRs=hematopoietic stem cell transplant recipients, r=correlation coefficient

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 7. 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

b) Mean Paired Difference

Table 16 below presents the mean paired difference between the Aptima CMV Quant Assay and the FDA-approved test at representative decision intervals.

Table 16: 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 FDA-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 17 below presents the bias between the Aptima CMV Quant Assay and the FDA-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 17: 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 Differemce[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.

c) Allowable Total Difference (ATD)

Table 18 along with Figure 8 through Figure 11 below present the ATD results using the paired differences between the Aptima CMV Quant Assay and the FDA-approved test versus their average at representative thresholds and the percentage of paired results in the ATD zone.

Table 18: Percentage of Paired Sample Differences Within Allowable Total Difference (ATD) Zone at Different Viral Load Intervals by Sample Type and Transplant Group

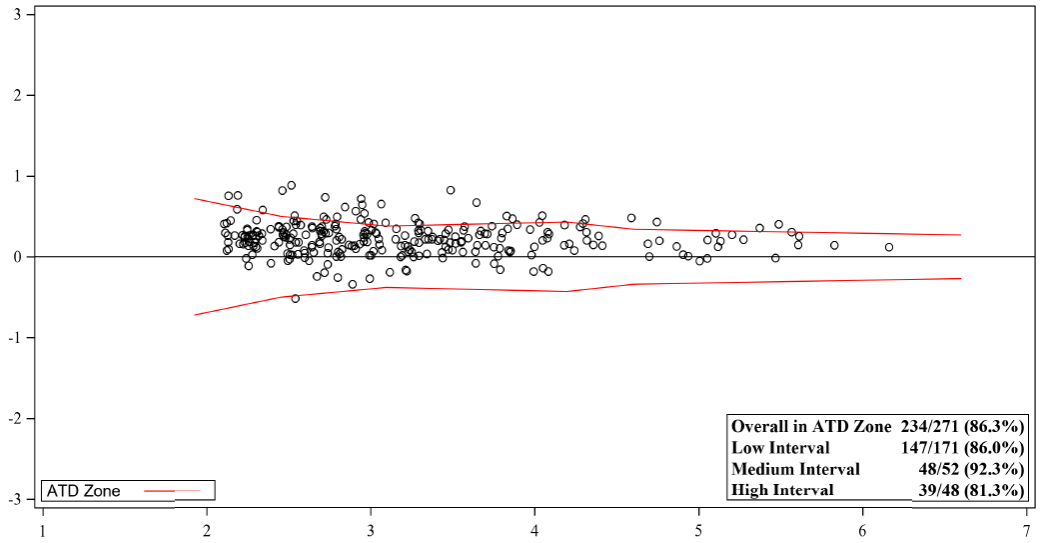
Sample Type	Transplant Group	Viral Load Intervals[a] (log_{10} IU/mL)	N[b]	Paired Sample Differences Within ATD Zone				
				n (%)	Percentiles			
					2.5%	5%	95%	97.50%
Clinical	Overall	All	271	234 (86.3)	-0.19	-0.14	0.40	0.42
		Low (≥2.1 to <3.3)	171	147 (86.0)	-0.24	-0.16	0.41	0.44
		Medium (≥3.3 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 FDA-approved test result.

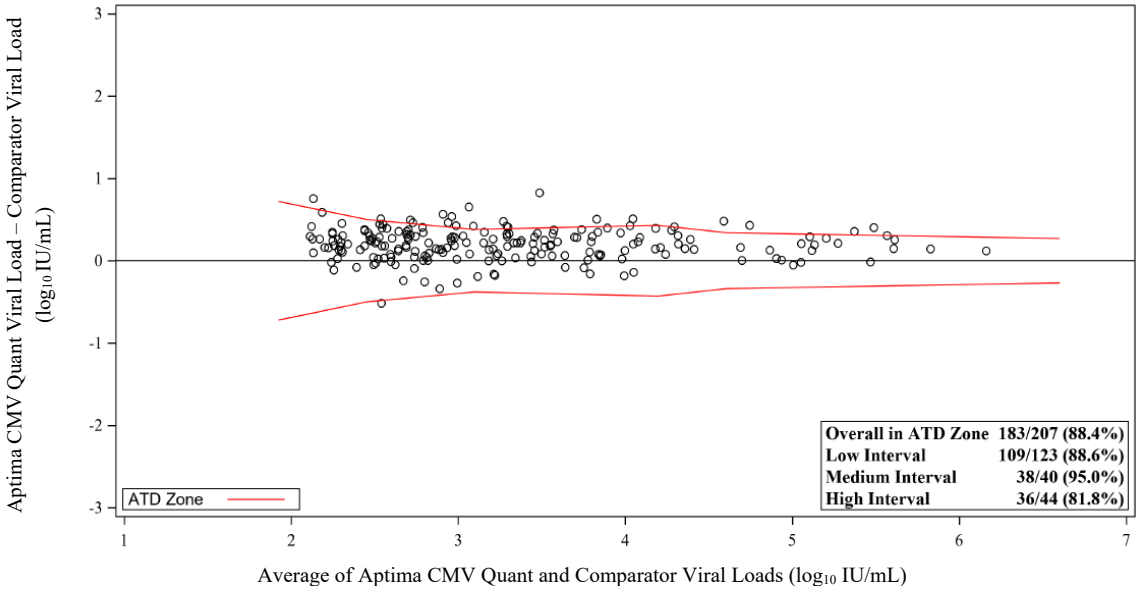
[b] Number of paired samples with results in the common linear range for both assays.

Figure 8. Difference Plot of Paired Samples and ATD Zone (Clinical Samples: SOTRs and HSCTRs Combined)



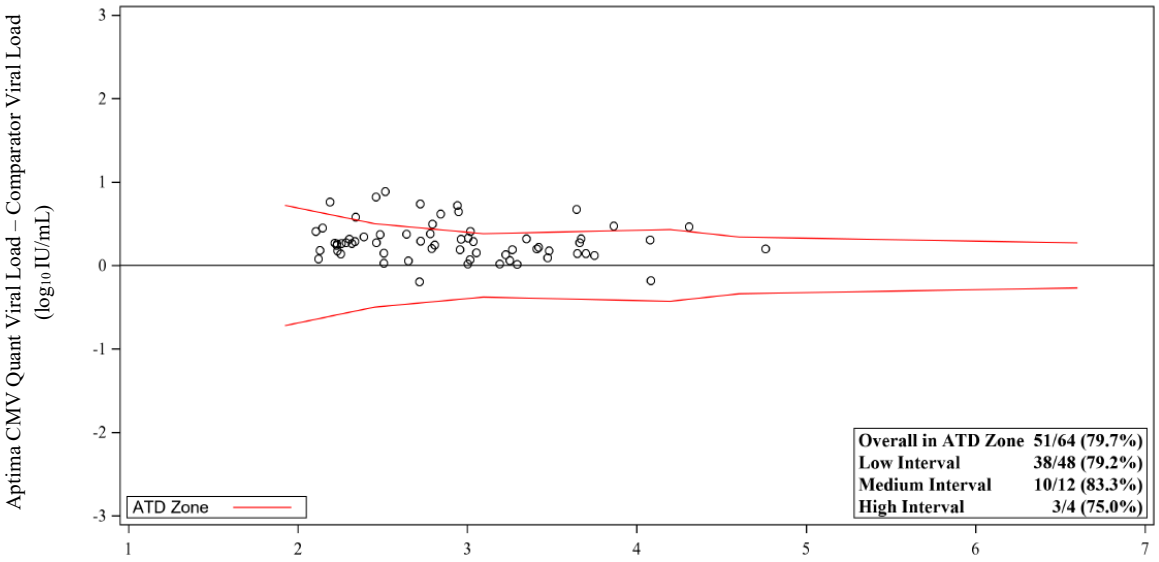
ATD=allowable total difference, HSCTRs=hematopoietic stem cell transplant recipients, SOTRs=solid organ transplant recipients Note: Paired samples with results in the common linear range for both assays included.

Figure 9. Difference Plot of Paired Samples and ATD Zone (Clinical Samples: SOTRs only)



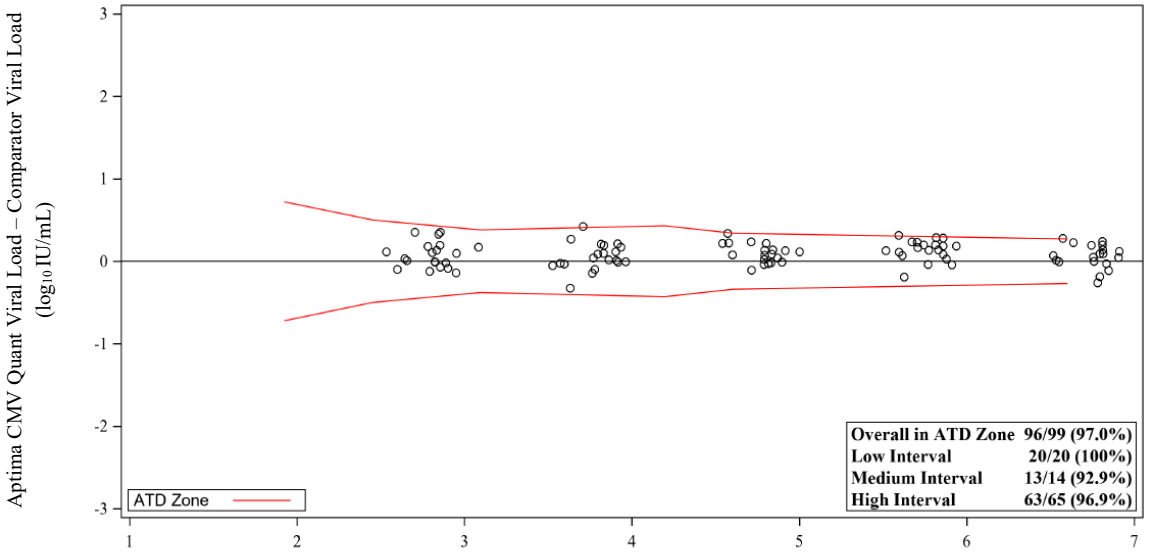
ATD=allowable total difference, SOTRs=solid organ transplant recipients
Note: Paired samples with results in the common linear range for both assays included.

Figure 10. 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 11. 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.

Conclusion
This study evaluated the clinical performance of the Aptima CMV Quant Assay on the Panther system in EDTA plasma samples from SOTRs and HSCTRs. Method comparison analyses (ie, Deming regression) and clinical agreement analyses at clinical decision points were performed to evaluate the clinical performance of the Aptima CMV Quant Assay relative to the comparator.

High agreement was demonstrated across 6 viral load intervals between the Aptima CMV Quant Assay and the comparator assay for all clinical samples and for samples collected after CMV antiviral therapy initiation.

Overall, the results demonstrated that Aptima CMV Quant Assay can be used to assess CMV DNA viral load in subjects undergoing solid-organ transplant and hematopoietic stem cell transplant. In patients receiving anti-CMV therapy, serial DNA measurements can be used to assess viral response to treatment.

Reproducibility (3 sites)

Reproducibility of the Aptima CMV Quant Assay 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 19 shows the reproducibility and precision of assay results for each positive panel member between sites, between operators/runs, between days, within runs, and overall. The coefficient of variation was calculated using the following equation where σ2 is the sample variance of the data after $\log_{10}$ transformation.

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

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

Table 19: Reproducibility of Aptima CMV Quant Assay CMV DNA Levels on the Panther System in Positive Panel Member

N	Observed Mean		Contribution to Total Variance SD (%CV)				Total Variance SD (%CV)
	IU/mL	log_{10} IU/mL	Between-Site	Between-Operator/Run[a]	Between-Day	Within-Run	
90	198.33	2.26	0.05 (11.19)	0.00 (0)	0.06 (12.94)	0.17 (39.59)	0.18 (43.68)
90	603.27	2.76	0.02 (3.99)	0.05 (10.49)	0.07 (15.68)	0.12 (27.04)	0.14 (33.67)
90	2428.54	3.36	0.06 (12.83)	0.06 (12.83)	0.09 (21.42)	0.11 (24.69)	0.16 (38.27)
90	27623.02	4.42	0.07 (15.98)	0.06 (13.85)	0.04 (9.29)	0.08 (19.38)	0.13 (30.63)
90	284107.74	5.44	0.07 (15.58)	0.00 (0)	0.04 (10.22)	0.09 (21.66)	0.12 (28.90)
90	3821364.62	6.57	0.08 (19.12)	0.02 (4.02)	0.06 (14.22)	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.
[a] Between-Operators may be confounded with Between-Runs; therefore, Between-Operators and Between-Runs estimates are combined in Between-Operators/Runs.

Conclusion
The Aptima CMV Quant Assay demonstrated 100% agreement with the expected qualitative results for all panel members. The total signal variability of the CMV DNA levels (as measured by SD), was less than or equal to 0.18 for positive panel members. The results indicate that the repeatability and reproducibility of the quantitative Aptima CMV Quant Assay using the Panther system are robust in plasma samples. These findings support the proposed intended use.

3. Subgroup Analyses

Not Applicable.

4. Pediatric Extrapolation

In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population younger than 18 years old.

E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included 12 investigators. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c),

and (f). The information provided does not raise any questions about the reliability of the data.

XI PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Microbiology Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM NONCLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The effectiveness of the Aptima CMV Quant Assay has been demonstrated when used for the quantitation of CMV DNA in human EDTA plasma for the management of solid-organ transplant patients and hematopoietic stem cell transplant patients.

B. Safety Conclusions

Based on the results of the analytical and clinical laboratory studies, Aptima CMV Quant Assay, when used according to the provided directions and in conjunction with other laboratory results and clinical information, should be safe and pose minimal risk to the patient due to false test results.

C. Benefit-Risk Determination

The benefit of the device is the ability to manage Hematopoetic Stem Cell Transplant and Solid Organ Transplant patients who are undergoing anti-cytomegalovirus therapy and to assess virological response to anti-cytomegalovirus therapy as part of a strategy to prevent CMV reactivation in patients who have undergone organ transplantation. Preemptive therapy involves monitoring for CMV in blood at regular intervals to detect early viral replication in transplant patients and initiating appropriate anti-viral treatment or reduction of immunosuppression. This type of prevention strategy can augment transplant clinical success and outcomes by decreasing the risk of CMV disease. The sequelae of untreated CMV disease because of undetected CMV DNA include CMV Syndrome and tissue-invasive CMV disease with end-organ damage, including colitis, hepatitis, nephritis, pneumonitis, meningitis, and retinitis. CMV infection and disease is associated with morbidity, failure of the transplanted organ, and death in transplant patients. The advantages of preemptive therapy include a reduced rate of late CMV, selective drug use, and decreased drug cost and drug toxicities. For patients with CMV DNA in the blood undergoing treatment, a decrease in the viral load, usually monitored weekly, generally correlates with a clinical response to treatment.

The lack of a widely accepted viral load threshold for diagnosis and preemptive therapy introduces notable uncertainty in the benefit of quantitative CMV nucleic

acid testing because of variability of CMV nucleic acid testing. The variability in viral load values among tests has hindered studies aimed at establishing the appropriate viral load cutoff for the diagnosis of clinically significant CMV infection. Clinically relevant cut-off values would likely depend upon a variety of factors, including: the assay used, specimen type (plasma v. whole blood), recipient and donor CMV serostatus, organ transplanted, and type of immunosuppression. Still, patients and clinicians can reap the benefits described above by detecting any CMV DNA in the blood and evaluating relative increases or decreases in detected viral load over time.

The risks associated with the device, when used as intended, are those related to the risk of false test results, failure to correctly interpret the test results, and failure to correctly operate the device.

The risk of false positive or falsely elevated CMV viral loads in patients undergoing preemptive therapy or monitoring of treatment for known CMV DNA in the blood is related to the risks of initiation or continuation of antiviral therapy when it is not necessary or the reduction of immunosuppression in transplant patients in whom reduction of immunosuppression is not indicated. The initiation or continuation of antiviral therapy can result in known drug toxicities, including suppression of bone marrow, in particular leukopenia, which can add to the patient's risk of contracting opportunistic infections. Other known drug toxicities include thrombocytopenia, diarrhea, and bloodstream infections if a central venous catheter is used to administer therapy. Reduction of immunosuppression can increase a transplant patient's risk of rejection of the transplanted organ or graft-versus-host disease, the latter of which can result in maculopapular rash, persistent nausea and vomiting, diarrhea, lichen planus, scleroderma, and ulcerations and sclerosis of the gastrointestinal tract.

The risk of false negative or falsely low CMV viral loads in patients undergoing preemptive therapy or monitoring of treatment for known CMV DNA in the blood include failure to initiate or premature discontinuation of appropriate antiviral treatment or reduction of immunosuppression, thus increasing the risk of CMV disease. The sequelae of untreated CMV disease because of false negative or falsely low CMV DNA include CMV Syndrome and tissue-invasive CMV disease with end-organ damage, including colitis, hepatitis, nephritis, pneumonitis, meningitis, and retinitis. CMV infection and disease is associated with morbidity, failure of the transplanted organ, and death in transplant patients. False negative or falsely low CMV DNA results can yield an increased rate of late CMV, selective drug use, and increased drug cost and subsequent drug toxicities.

The clinical benefits outweigh the risks for the proposed assay considering the performance of the device in the clinical study and the risk mitigations afforded by the premarket application. The proposed assay labeling will facilitate accurate assay implementation and interpretation of results. The clinical performance observed in the analytical and clinical studies suggests that errors will be uncommon and that the assay may provide substantial benefits to patients when used with other laboratory

results and clinical information as an aid in the management of Hematopoetic Stem Cell Transplant and Solid Organ Transplant patients who are undergoing anti-cytomegalovirus therapy and to assess virological response to anti-cytomegalovirus therapy.

1. Patient Perspective

   This submission either did not include specific information on patient perspectives or the information did not serve as part of the basis of the decision to approve or deny the PMA for this device.

In conclusion, given the available information above, the data support that for the claimed intended use the probable benefits outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. The data from the preclinical studies demonstrated acceptable analytical sensitivity, traceability, linearity, precision, and analytical specificity of the Aptima CMV Quant Assay when used according to the instructions for use. The clinical utility study results obtained with the Aptima CMV Quant Assay are informative for assessing the response to antiviral treatment in solid organ transplant patients and hematopoietic stem cell transplant patients undergoing anti-CMV therapy, and that the test is safe and effective when used according to the directions for use in the labeling.

XIII CDRH DECISION

CDRH issued an approval order on May 9, 2022

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.