January 17, 2025

Hologic, Inc.
Maria Jose Cortes-Mateos
Regulatory Affairs Specialist III
10210 Genetic Center Dr.
San Diego, California 92121

Re: K243935
Trade/Device Name: Aptima CMV Quant Assay
Regulation Number: 21 CFR 866.3180
Regulation Name: Quantitative Cytomegalovirus Nucleic Acid Tests For Transplant Patient Management
Regulatory Class: Class II
Product Code: PAB
Dated: December 19, 2024
Received: December 20, 2024

Dear Maria Jose Cortes-Mateos:

We have reviewed your section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (the Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device" (https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality System (QS) regulation (21 CFR Part 820), which includes, but is not limited to, 21 CFR 820.30, Design controls; 21 CFR 820.90, Nonconforming product; and 21 CFR 820.100, Corrective and preventive action. Please note that regardless of whether a change requires premarket review, the QS regulation requires device manufacturers to review and approve changes to device design and production (21 CFR 820.30 and 21 CFR 820.70) and document changes and approvals in the device master record (21 CFR 820.181).

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR Part 803) for devices or postmarketing safety reporting (21 CFR Part 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

All medical devices, including Class I and unclassified devices and combination product device constituent parts are required to be in compliance with the final Unique Device Identification System rule ("UDI Rule"). The UDI Rule requires, among other things, that a device bear a unique device identifier (UDI) on its label and package (21 CFR 801.20(a)) unless an exception or alternative applies (21 CFR 801.20(b)) and that the dates on the device label be formatted in accordance with 21 CFR 801.18. The UDI Rule (21 CFR 830.300(a) and 830.320(b)) also requires that certain information be submitted to the Global Unique Device Identification Database (GUDID) (21 CFR Part 830 Subpart E). For additional information on these requirements, please see the UDI System webpage at https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/unique-device-identification-system-udi-system.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See

the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

MARIA I. GARCIA -S

Maria Garcia, Ph.D.
Assistant Director
Division of Microbiology Devices
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Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 07/31/2026
See PRA Statement below.

Submission Number *(if known)*

K243935

Device Name

Aptima CMV Quant Assay

Indications for Use *(Describe)*

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.

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

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

510(k) SUMMARY

Aptima CMV Quant Assay

I. SUBMITTER

Hologic, Inc.
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Date Prepared: December 17, 2024

II. DEVICE

Proprietary Name:	Aptima CMV Quant Assay
Classification Name:	Quantitative cytomegalovirus nucleic acid tests for transplant patient management.
Regulation Number:	21 CFR 866.3180
Regulatory Class:	Class II
Product Code:	PAB

III. PREDICATE DEVICE

The predicate device is the Aptima CMV Quant Assay (P210029; approved May 9, 2022), marketed as a 100-test kit configuration.

IV. 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/Panther Fusion 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 WHO International Standard for human cytomegalovirus. 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.

The addition of a protocol to treat specific plasma samples with proteinase K in the assay package insert will allow the retesting of those samples to obtain a valid result.

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 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 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.

Assay Components

The Aptima CMV Quant Assay provides enough reagents to run 100 tests. The assay kit contains 4 boxes: Box 1, Main Assay Kit Reagents; Box 2, Target Enhancer Reagent (TER); Box 3, Calibrator Kit; and Box 4, Controls Kit. All these components are required for sample processing.

Box 1, Main Assay Reagents, and Box 2, TER, are master lot related and cannot be ordered separately. They are provided in different boxes due to the different reagent storage temperature conditions. Box 3 contains the Calibrator Kit, and Box 4 contains the Controls Kit when

provided as part of the master kit. These two kits may also be procured separately if customers need additional calibrators or controls. A listing of the components that are required to perform the Aptima CMV Quant Assay is detailed in **Table 1.** The ancillary reagents are listed in **Table 2.**

Table 1 - Reagents Required to Perform the Aptima CMV Quant Assay, 100 test kit

Box	Description	Components Description
BOX 1	**Aptima CMV Quant Main Assay Reagents Kit (2°C to 8°C)**	Amplification Reagent
		Enzyme Reagent
		Promoter Reagent
		Amplification Reconstitution Solution
		Enzyme Reconstitution Solution
		Promoter Reconstitution Solution
		Target Capture Reagent (TCR)
BOX 2	**Aptima CMV Quant Target Enhancer Reagent (15°C to 30°C)**	Target Enhancer Reagent (TER)
BOX 3	**Aptima CMV Quant Calibrator Kit (-15°C to -35°C)**	Positive Calibrator
BOX 4	**Aptima CMV Quant Controls Kit (-15°C to -35°C)**	Negative Control
		Low Positive Control
		High Positive Control

Table 2 - Ancillary Reagents

Aptima Assay Fluids Kit
Proteinase K (RNA grade), 20 mg/mL, DNase-Free, RNase-Free (Thermo Fisher Scientific)

Instrumentation

The Aptima CMV Quant Assay has been designed for and validated on the Panther/Panther Fusion system. The Panther/Panther Fusion system is an integrated hardware and software system that together with the Aptima CMV Quant Assay fully automates all the steps necessary to perform the assay from sample preparation through amplification of nucleic acid, detection, data reduction, and amplicon inactivation.

Note: Retesting plasma samples with an ML2 flag will require manual addition of proteinase K reagent to the plasma specimen prior to loading the sample onto the Panther/Panther Fusion System for testing. The processing steps are fully automated as indicated above.

V. 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.

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

VI. COMPARISON OF TECHNOLOGICAL CHARACTERISTICS WITH THE PREDICATE DEVICE

Table 3 displays the updates made to the assay's package insert to include the protocol for retesting plasma samples with an associated ML2 flag. This protocol involves the manual pretreatment of plasma specimens with ML2 flag invalid results associated with ML2 flags with proteinase K reagent prior to testing on Panther/Panther Fusion System.

Table 3 - Comparison of Predicate and Subject Device

Feature	Aptima CMV Quant Assay	Aptima CMV Quant Assay (including proteinase K protocol)	Predicate and Subject Device Comparison
Intended 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.	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. The Aptima CMV Quant Assay is not intended for use as a screening assay for the presence of CMV in blood or blood products.	Same
Organisms Detected	Human cytomegalovirus (CMV)	Human cytomegalovirus (CMV)	Same
Test Quantity	100 Tests	100 Tests	Same
Patient Population	Solid-organ transplant and hematopoietic stem cell transplant patients	Solid-organ transplant and hematopoietic stem cell transplant patients	Same
Specimen Types	Plasma	Plasma	Same

Feature	Aptima CMV Quant Assay	Aptima CMV Quant Assay (including proteinase K protocol)	Predicate and Subject Device Comparison
Analyte	CMV DNA, genotypes 1, 2, 3, and 4	CMV DNA, genotypes 1, 2, 3, and 4	Same
Technology Principle of Operation	Real-time Transcription Mediated Amplification (TMA)	Real-time Transcription Mediated Amplification (TMA)	Same
User Complexity	High	High	Same
Platform	Panther/Panther Fusion system	Panther/Panther Fusion system	Same
Platform Software	Panther/Panther Fusion system software	Panther/Panther Fusion system software	Same
Assay Software	Aptima CMV Quant Assay software	Aptima CMV Quant Assay software	Same.
Warnings and Precautions – Assay Related	N/A	O. In case of an invalid result with ML2 flag, do not retest the neat specimen with the Aptima CMV Quant Assay. Refer to *Panther System Test Procedure*, step F, in this package insert for instructions to treat the plasma specimen with proteinase K prior to retesting. ***Note:*** *For ML2 flag, refer to the appropriate Panther/Panther Fusion System Operator's Manual for Mag Wash Clean Instructions.* P. Avoid contact of proteinase K with the Aptima CMV Quant Assay reagents and reagent preparation area. The performance of the assay may be affected if the reagents come in contact with proteinase K. Change gloves if they come in contact with proteinase K.	Section added to indicate how to handle samples with an ML2 flag and proper use of the proteinase K reagent.

Feature	Aptima CMV Quant Assay	Aptima CMV Quant Assay (including proteinase K protocol)	Predicate and Subject Device Comparison
Samples Onboard the Panther System	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.	Samples may be left on the Panther system uncapped for up to 8 hours, **excluding specimens treated with proteinase K that need to be tested immediately after proteinase K treatment.** 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.	Added a clarification to test samples treated with proteinase K immediately after treatment.
Materials Required but Available Separately	Cat. No. Panther® System —	Cat. No. Panther® System 303095 Panther Fusion System PRD-04172 Panther System Continuous Fluids and Waste (Panther Plus) PRD-06067 Proteinase K (RNA grade), 20 mg/mL, DNase-Free, RNase-Free (Thermo Fisher Scientific) 25530-049 Thermo FisherScientific	The catalog numbers of the Panther/Panther Fusion system were added. The proteinase K catalog number was added.
Optional Materials	*Aptima Specimen Aliquot Tubes (SATs) (100 pack) 503762*	*Aptima Specimen Aliquot Tubes (SATs) (100 pack) FAB-18184*	The SATs catalog number was updated

Feature	Aptima CMV Quant Assay	Aptima CMV Quant Assay (including proteinase K protocol)	Predicate and Subject Device Comparison
Processing of CMV plasma specimen with an ML2 flag for retesting	N/A	F. Processing of CMV plasma specimen with an ML2 flag for retesting 1. Allow the specimens to reach 15°C to 30°C prior to processing. 2. Transfer 1000 µL of patient specimen into a labeled secondary tube. 3. Add 50 µL of proteinase K to aliquoted plasma specimen. 4. Cap the labeled secondary tube. 5. Vortex for 15 seconds. 6. Incubate for 10 minutes at 65°C. 7. Cool down for 1 minute at room temperature (15°C to 30°C). 8. Briefly centrifuge the specimen to gather all droplets at the bottom of the tube before testing on the Panther system. See System Preparation, Step G.2 below, for information about loading the rack and removing the caps. *Note: After a specimen is treated with proteinase K, it should be tested immediately.*	The proteinase K protocol has been added to allow the retesting of samples with an ML2 flag (Step F)
Quality Control	N/A	In case of an invalid result with ML2 flag, do not retest the neat specimen with the Aptima CMV Quant Assay. Refer to *Panther System Test Procedure*, step F, in this package insert for instructions to treat the plasma specimen with proteinase K prior to retesting.	Text was added to indicate how to handle samples with a ML2 flag and Mag Wash cleaning instructions.

Feature	Aptima CMV Quant Assay	Aptima CMV Quant Assay (including proteinase K protocol)	Predicate and Subject Device Comparison
		Note: *For ML2 flag, refer to the appropriate Panther/Panther Fusion System Operator's Manual for Mag Wash Clean Instructions.*	
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 log10 IU/mL.	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 log10 IU/mL.	Same
Bibliography	N/A	21. Youn J-H, Walker L, Carlson S, Soutar C, Frank K, Zelazny A, Das S. Mitigation of errors on an FDA-approved platform for cytomegalovirus viral load assay. J Clin Microbiol. 2024 Jul 16;62(7)	Addition of a reference

VI. PERFORMANCE DATA

Plasma samples containing abnormally high presence of globulin proteins can coagulate on exposure to alkaline shock (addition of the Target Enhancer Reagent, a component of the assay kit) during the nucleic acid extraction step of the assay, forming a gel in the assay tube. This gel clogs the aspirators in the magnetic wash station on the Panther/Panther Fusion system, during the wash steps for nucleic acid purification, causing the instrument sensors to invalidate the results of the sample and report it with a ML2 flag. Pre-treatment of the invalidated ML2 specimens with proteinase K reagent enables generation of valid results for these specimens by digesting the proteins and preventing the formation of gels when the sample is exposed to alkaline shock.

The validated proteinase K protocol has been incorporated into the assay's package insert as follows:

Processing of CMV plasma specimen with an ML2 flag for retesting:

1. Allow the specimens to reach 15°C to 30°C prior to processing.
2. Transfer 1000 µL of patient specimen into a labeled secondary tube.
3. Add 50 µL of proteinase K to aliquoted plasma specimen.
4. Cap the labeled secondary tube.
5. Vortex for 15 seconds.
6. Incubate for 10 minutes at 65°C.
7. Cool down for 1 minute at room temperature (15°C to 30°C).
8. Briefly centrifuge the specimen to gather all droplets at the bottom of the tube before testing on the Panther system.

Note: After a specimen is treated with proteinase K, it should be tested immediately.

This protocol was validated using plasma specimens collected from the transplant patient population by the National Institute of Health (NIH). The prevalence of specimens invalidated with ML2 flags at the NIH decreased from 0.87% (on testing 1039 plasma specimens without proteinase K treatment) to 0% (on testing 4098 specimens with proteinase K treatment). The NIH also demonstrated that using proteinase K for pretreatment of specimens did not impact the

accuracy of CMV quantification in the Aptima Assay (Youn J-H, Walker L, Carlson S, Soutar C, Frank K, Zelazny A, Das S. Mitigation of errors on an FDA-approved platform for cytomegalovirus viral load assay. J Clin Microbiol. 2024 Jul 16;62(7). The Hologic risk assessment to evaluate the impact of changing the specimen handling workflow to incorporate proteinase K pretreatment of specimens prior to performing the Aptima CMV Quant Assay concluded that the benefits provided by the updates to the Hologic specimen handling workflow at customer sites outweigh the risks.

Using this protocol will enable clinical laboratories to generate a valid result on the Panther/Panther Fusion system for plasma specimens that initially reported an invalid result with ML2 flag.

VII. CONCLUSIONS

Implementation of pre-treatment of plasma specimens with proteinase K prior to testing in the Aptima CMV Quant Assay enables clinical laboratories to generate a valid retest result, for plasma specimens that initially reported invalid results with ML2 flag. Prompt generation of valid retest results minimizes delays in patient treatment decisions.

This protocol does not negatively impact the safety and effectiveness of the Aptima CMV Quant Assay because the assay performance characteristics including accuracy are maintained with incorporation of this modification.