February 13, 2025

Hologic, Inc.
Katerina Capkova
Regulatory Affairs Manager
10210 Genetic Center Dr.
San Diego, California 92121

Re: K243396
Trade/Device Name: Aptima SARS-CoV-2 Assay
Regulation Number: 21 CFR 866.3981
Regulation Name: Device To Detect And Identify Nucleic Acid Targets In Respiratory Specimens From Microbial Agents That Cause The Sars-Cov-2 Respiratory Infection And Other Microbial Agents When In A Multi-Target Test
Regulatory Class: Class II
Product Code: QQX
Dated: October 31, 2024
Received: October 31, 2024

Dear Katerina Capkova:

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,

Anna M. Mielech -S

Anna Mielech, PhD.
Deputy Branch Chief (Acting)
Viral Respiratory and HPV Branch
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
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.

510(k) Number *(if known)*
K243396

Device Name
Aptima SARS-CoV-2 Assay

Indications for Use *(Describe)*

The Aptima® SARS-CoV-2 Assay is a nucleic acid amplification in vitro diagnostic test intended for the qualitative detection of RNA from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) isolated and purified from nasopharyngeal (NP) swab and anterior nasal (AN) swab specimens obtained from patients with signs and symptoms of COVID-19.

Positive results are indicative of the presence of SARS-CoV-2 RNA. The Aptima SARS-CoV-2 Assay is intended for use as an aid in the diagnosis of COVID-19 if used in conjunction with other clinical, epidemiological, and laboratory findings. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

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)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) SUMMARY

Aptima SARS-CoV-2 Assay

I. SUBMITTER: Hologic, Inc.
10210 Genetic Center Drive
San Diego, CA 92121

Contact Person: Katerina Capkova, PhD
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Date Prepared: February 11, 2025

II. DEVICE

Proprietary Name:	Aptima SARS-CoV-2 Assay
Classification Name:	Respiratory Specimen Nucleic Acid SARS-CoV-2 Test
Regulation Number:	21 CFR 866.3981
Regulatory Class:	Class II
Product Code:	QQX

III. PREDICATE DEVICE

The predicate device for the Aptima SARS-CoV-2 Assay is the BioFire COVID-19 Test 2 (K211079; cleared November 1, 2021, BioFire Defense, LLC).

IV. DEVICE DESCRIPTION

The Aptima SARS-CoV-2 Assay is a nucleic acid amplification *in vitro* diagnostic test developed for use on the fully automated Panther/Panther Fusion system to detect RNA from SARS-CoV-2 isolated and purified from nasopharyngeal and anterior nasal swab specimens collected into UTM/VTM or with the RespDirect Collection Kit.

The Aptima SARS-CoV-2 Assay combines the technologies of target capture, Transcription Mediated Amplification (TMA), and Dual Kinetic Assay (DKA).

Specimens are collected and transferred into their respective specimen transport tubes. The transport solutions in these tubes release the RNA target and protect them from degradation during storage. When the Aptima SARS-CoV-2 Assay is performed in the laboratory, the target RNA molecules are isolated from specimens by use of capture oligomers via target capture that utilizes magnetic microparticles. The capture oligomers contain sequences complementary to specific regions of the target molecules as well as a string of deoxyadenosine residues. A separate capture oligomer is used for each target. During the hybridization step, the sequence specific regions of the capture oligomers bind to specific regions of the target molecules. The capture oligomer:target complex is then captured out of solution by decreasing the temperature of the reaction to room temperature. This temperature reduction allows hybridization to occur between the deoxyadenosine region on the capture oligomer and the poly-deoxythymidine molecules that are covalently attached to the magnetic particles. The microparticles, including the captured target molecules bound to them, are pulled to the side of the reaction vessel using magnets and the supernatant is aspirated. The particles are washed to remove residual specimen matrix that may contain amplification reaction inhibitors. After the target capture steps are completed, the specimens are ready for amplification.

Target amplification assays are based on the ability of complementary oligonucleotide primers to specifically anneal and allow enzymatic amplification of the target nucleic acid strands. The Aptima SARS-CoV-2 Assay replicates specific regions of the RNA from SARS-CoV-2 virus. Detection of the RNA amplification product sequences (amplicon) is achieved using nucleic acid

hybridization. Single-stranded chemiluminescent nucleic acid probes, which are unique and complementary to a region of each target amplicon and Internal Control (IC) amplicon, are labeled with different acridinium ester (AE) molecules. The AE-labeled probes combine with the amplicon to form stable hybrids. The Selection Reagent differentiates hybridized from unhybridized probe, eliminating the generation of signal from the unhybridized probe. During the detection step, light emitted from the labeled hybrids is measured as photon signals in a luminometer and are reported as Relative Light Units (RLU). In DKA, differences in the kinetic profiles of the labeled probes allow for the differentiation of signal; kinetic profiles are derived from measurements of photon output during the detection read time.

The Aptima SARS-CoV-2 Assay amplifies and detects 2 conserved regions of the ORF1ab gene in the same reaction, using the "glower" kinetic type. The 2 regions are not differentiated and amplification of either or both regions lead to RLU signal. The assay results are determined by a cut-off based on the total RLU and the kinetic curve type.

Assay Components

The Aptima SARS-CoV-2 Assay is available in 100-test and 250-test kit configuration. In both cases, the assay kit consists of two boxes containing nine reagents which are required for sample processing. In addition, the controls kit contains the positive and negative controls. RespDirect Collection Kit is an ancillary component of the assay. The Panther Fusion Specimen Lysis Tubes are also an ancillary kit to this assay required for processing of Viral Transport Media/Universal Transport Media (VTM/UTM) specimens prior to testing on the Panther/Panther Fusion system. In addition, there is one ancillary kit (Aptima Assay Fluids Kit) which is universally used with other commercialized Aptima assays.

Table 1: Reagents Required to Perform the Aptima SARS-CoV-2 Assay

Box	Components Description
Assay Kit-Refrigerated	Amplification Reagent
	Enzyme Reagent
	Promoter Reagent
	Internal Control
Assay Kit-Room Temperature	Amplification Reconstitution Solution
	Enzyme Reconstitution Solution
	Promoter Reconstitution Solution
	Selection Reagent
	Target Capture Reagent
Controls Kit-Refrigerated	Negative Control
	Positive Control

Table 2: Ancillary and Collection Kits Required to Perform the Aptima SARS-CoV-2 Assay

RespDirect Collection Kit
Panther Fusion Specimen Lysis Tubes
Aptima Assay Fluids Kit

Instrumentation

The Aptima SARS-CoV-2 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 SARS-CoV-2 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.

V. INDICATIONS FOR USE

The Aptima® SARS-CoV-2 Assay is a nucleic acid amplification in vitro diagnostic test intended for the qualitative detection of RNA from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) isolated and purified from nasopharyngeal (NP) swab and anterior nasal (AN) swab specimens obtained from patients with signs and symptoms of COVID-19. Positive results are indicative of the presence of SARS-CoV-2 RNA. The Aptima SARS-CoV-2 Assay is intended for use as an aid in the diagnosis of COVID-19 if used in conjunction with other clinical, epidemiological, and laboratory findings. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses.
Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

VI. COMPARISON OF TECHNOLOGICAL CHARACTERISTICS WITH THE PREDICATE DEVICE

A comparison of the Aptima SARS-CoV-2 Assay to the predicate BioFire COVID-19 Test 2 (K211079) is summarized in **Table 3**.

Table 3: Comparison of Similarities and Differences Between the Subject Device (Aptima SARS-CoV-2 Assay) and the Predicate Device (BioFire COVID-19 Test 2)

Item	Aptima SARS-CoV-2 Assay (Subject Device)	BioFire COVID-19 Test 2 (Predicate Device) K211079
Prescription/over-the-counter use	Prescription Only	Same
Patient Population	Individuals with signs and symptoms of COVID-19	Same
Specimen Types	Nasopharyngeal (NP) and nasal swab specimens	Nasopharyngeal (NP) swab specimens
Intended User	Professional use	Same
Technology Principle of Operation	Transcription-mediated amplification NAAT	Reverse transcriptase multiplexed polymerase chain reaction test
Organisms Detected	SARS-CoV-2	Same
Assay Controls	Internal and run controls	Internal and external controls
Platform	Automated nucleic acid amplification platform. Uses Panther/Panther Fusion system for all steps including nucleic acid extraction, amplification, detection, and result processing.	Automated nucleic acid amplification platform. Uses BioFire FilmArray 2.0 or BioFire FilmArray Torch systems including integrated sample preparation, amplification, detection, and analysis.

Intended Use	The Aptima® SARS-CoV-2 Assay is a nucleic acid amplification in vitro diagnostic test intended for the qualitative detection of RNA from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) isolated and purified from nasopharyngeal (NP) swab and anterior nasal (AN) swab specimens obtained from patients with signs and symptoms of COVID-19. Positive results are indicative of the presence of SARS-CoV-2 RNA. The Aptima SARS-CoV-2 Assay is intended for use as an aid in the diagnosis of COVID-19 if used in conjunction with other clinical, epidemiological, and laboratory findings. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.	The BioFire COVID-19 Test 2 is a qualitative nested multiplexed RT-PCR in vitro diagnostic test intended for use with the BioFire FilmArray 2.0 and BioFire FilmArray Torch Systems. The BioFire COVID-19 Test 2 detects nucleic acids from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in nasopharyngeal swabs (NPS) from symptomatic individuals suspected of COVID-19 by their healthcare provider. Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in NPS specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out co-infection with other pathogens. Results are meant to be used in conjunction with other clinical, epidemiologic, and laboratory data, in accordance with the guidelines provided by the relevant public health authorities. The BioFire COVID-19 Test 2 is intended for use by trained medical and laboratory professionals in a laboratory setting or under the supervision of a trained laboratory professional.
Time to Obtain Test Results	Approximately 2.5 hours	Approximately 45 minutes

VII. PERFORMANCE DATA

The following performance data (analytical and clinical) were provided in support of the substantial equivalence determination.

Brief Description of Analytical (Non-Clinical) Studies

The following analytical studies (non-clinical) were conducted to support the clearance of the Aptima SARS-CoV-2 Assay on the Panther/Panther Fusion System.

Analytical Sensitivity - Limit of Detection (LoD)

The analytical sensitivity (limit of detection or LoD) of the Aptima SARS-CoV-2 Assay was determined by testing dilutions of processed negative clinical NP swab VTM/UTM matrix spiked with inactivated cultured SARS-CoV-2 virus (USA-WA1/2020; BEI Resources; NR-52281) and WHO International Standard for SARS-CoV-2, NIBSC (20/146). For the cultured virus, ten replicates of each serial dilution were evaluated for each of two assay reagent lots across two Panther systems. The LoD was determined to be 0.01 $TCID_{50}$/mL in the test sample (0.026 $TCID_{50}$/mL in the neat, unprocessed sample) and verified by testing an additional minimum 20 replicates with one assay reagent lot. For the WHO International Standard, a minimum of 24 replicates were tested with each of the three reagent lots using Probit analysis for each lot and was confirmed with an additional 24 replicates using a single lot. The lowest concentration at which ≥95% detection was observed was 87.5 IU/mL . LoD confirmation was also performed with the RespDirect Collection Kit at 24 replicates with a single reagent lot and ≥95% detection was determined to be 87.5 IU/mL.

Note: The stated LoDs pertain to the concentrations in the tubes loaded onto the instrument. For samples collected in VTM/UTM, this is the concentration in the processed sample in an SLT. For samples collected using the RespDirect Collection kit, this is the concentration in the Enhanced Direct Load tube (RespDirect Collection Kit).

Reactivity – Wet Testing

The reactivity of the Aptima SARS-CoV-2 Assay was determined by testing virus strains in processed negative clinical NP swab VTM/UTM matrix. Each strain was tested in triplicate at 3X LoD with one reagent lot. For strains not detected at 3X LoD, additional testing at higher concentrations was performed until 100% positivity was observed. Table 4 shows the lowest concentration of each strain in which 100% positivity was observed.

Table 4: Analytical Reactivity Summary for SARS-CoV-2

Description	Concentration
USA-WA1/2020*	0.03 $TCID_{50}$/mL
USA-CA1/2020	0.03 $TCID_{50}$/mL
USA-AZ1/2020	0.10^{1} $TCID_{50}$/mL
USA-WI1/2020	0.03 $TCID_{50}$/mL
USA/OR-OHSU-PHL00037/2021 | B.1.1.7	0.03 $TCID_{50}$/mL
Uganda/MUWRP-20200195568/2020 | A.23.1	0.03 $TCID_{50}$/mL
USA/PHC658/2021 | B.1.617.2	0.03 $TCID_{50}$/mL
USA/MD-HP05285/2021 | B.1.617.2	0.03 $TCID_{50}$/mL
USA/CA/VRLC009/2021 | B.1.427	0.03 $TCID_{50}$/mL
USA/CA/VRLC012/2021 | P.2	0.03 $TCID_{50}$/mL
USA/MD-HP03056/2021 | B.1.525	0.03 $TCID_{50}$/mL
USA/CA-Stanford-15_S02/2021 | B.1.617.1	0.03 $TCID_{50}$/mL
Peru/un-CDC-2-4069945/2021 | C.37	0.03 $TCID_{50}$/mL
USA/MD-HP20874/2021 | B.1.1.529	0.03 $TCID_{50}$/mL
USA/GA-EHC-2811C/2021 | B.1.1.529	0.03 $TCID_{50}$/mL
USA/MD-HP30386/2022 | BA.4	0.03 $TCID_{50}$/mL
USA/COR-22-063113/2022 | BA.5	0.03 $TCID_{50}$/mL
South Africa/CERI-KRISP-K040013/2022 | BA.5	0.03 $TCID_{50}$/mL
USA/MD-HP38861/2022 | BQ.1.1	0.03 $TCID_{50}$/mL
USA/MD-HP40900/2022 | XBB.1.5	0.10^{1} $TCID_{50}$/mL
USA/MD-HP47865/2023 | XXB.2.3	0.03 $TCID_{50}$/mL
USA/MD-HP46933/2023 | EG.1.2	0.03 $TCID_{50}$/mL
USA/MD-HP47946/2023 | EG.5.1	0.03 $TCID_{50}$/mL

USA/CA-Stanford-139_S35/2023 | XBB.1.9	0.10^{1}	$TCID_{50}$/mL
USA/CA-Stanford-139_S23/2023 | XBB.1.16	0.10^{1}	$TCID_{50}$/mL
USA/MI-UM-10052670540/2023 | BA.2.86	0.10^{2}	$TCID_{50}$/mL
USA/New York-PV96109/2023 | JN.1	0.15^{1}	$TCID_{50}$/mL
USA/MD-HP49152/2023 | HV.1	0.015	$TCID_{50}$/mL

*Strain used to establish LoD.

[1]In silico analysis showed 100% homology to amplification regions.

[2]In silico analysis identified a single mismatch in the probe oligo for one region. Due to the location of the mismatch and 100% homology to the second region, detection is not expected to be impacted. *Virus stock degradation or error in $TCID_{50}$/mL quantification may have impacted the concentration at 100% detection.*

Reactivity-*In silico* Analysis

The inclusivity of the Aptima SARS-CoV-2 Assay was evaluated using in silico analysis of the assay target capture oligos, amplification primers, and detection probes for the SARS-CoV-2 target systems in relation to sequences available in the NCBI and GISAID gene databases. Any sequence with missing or ambiguous sequence information was removed from the analysis for that region. Based on the *in silico* analysis of GISAID and NCBI sequences available for SARS-CoV-2 (10% random sampling of 16,553,661 million sequences up to July, 31, 2023 and all 508,436 sequences August 1, 2023 – January 31, 2024), the Aptima SARS-CoV-2 Assay is predicted to detect 99.98% (2,136,815/2,137,175 sequences) of all sequences evaluated.

The sequences evaluated included lineages and variants of concern (VOC) or variants under investigation (VUI) that may have important epidemiological, immunological, or pathogenic properties from the public health perspective. All lineages and variants of public health interest identified as of January 31, 2024 are predicted to be detected; new sequences and variants will continue to be monitored for impacts on detection by the Aptima SARS-CoV-2 Assay.

Analytical Specificity and Microbial Interference

Analytical specificity (cross-reactivity) and microbial interference with the Aptima SARS-CoV-2 Assay were evaluated in the presence of closely related and non-targeted organisms. Panels consisting of 48 organisms (6) were tested in processed negative clinical NP swab VTM/ UTM matrix in the absence or presence of 3X LoD SARS-CoV-2. Bacteria were tested at 10^6 CFU/mL and viruses were tested at 10^5 $TCID_{50}$/mL, except where noted. No cross-reactivity or microbial interference was observed for any of the 48 organisms tested on the Aptima SARS-CoV-2 Assay at the indicated concentrations.

Table 5: Aptima SARS-CoV-2 Analytical Specificity and Microbial Interference Microorganisms

Microorganism	Concentration[1]	Microorganism	Concentration[1]
Adenovirus 1	$1x10^5$ $TCID_{50}$/mL	*Aspergillus fumigatus*	$1x10^6$ CFU/mL
Adenovirus 7a	$1x10^5$ $TCID_{50}$/mL	*Bordetella parapertussis*	$1x10^6$ CFU/mL
CMV Strain AD 169	$5x10^3$ $TCID_{50}$/mL	*Bordetella pertussis*	$1x10^6$ CFU/mL
EBV	$1x10^5$ $TCID_{50}$/mL	*Candida albicans*	$1x10^6$ CFU/mL
Enterovirus Type 71	$1x10^5$ $TCID_{50}$/mL	*Chlamydia pneumoniae*	$1x10^6$ CFU/mL
Human coronavirus 229E	$1x10^5$ $TCID_{50}$/mL	*Corynebacterium diphtheriae*	$1x10^6$ CFU/mL
Human coronavirus OC43	$1x10^5$ $TCID_{50}$/mL	*Escherichia coli*	$1x10^6$ CFU/mL
Human coronavirus HKU1[2]	$1x10^6$ copies/mL	*Fusobacterium necrophorum*	$1x10^6$ CFU/mL
Human coronavirus NL63	$1x10^4$ $TCID_{50}$/mL	*Haemophilus influenzae*	$1x10^6$ CFU/mL
Human Metapneumovirus (hMPV)	$1x10^6$ $TCID_{50}$/mL	*Lactobacillus plantarum*	$1x10^6$ CFU/mL
Influenza A	$1x10^5$ $TCID_{50}$/mL	*Legionella pneumophila*	$1x10^6$ CFU/mL
Influenza B	$2x10^3$ $TCID_{50}$/mL	*Moraxella catarrhalis*	$1x10^6$ CFU/mL
Measles	$1x10^5$ $TCID_{50}$/mL	*Mycobacterium tuberculosis*	$1x10^6$ CFU/mL
MERS-coronavirus	$1x10^4$ $TCID_{50}$/mL	*Mycoplasma genitalium*	$1x10^6$ CFU/mL
Mumps	$1x10^5$ $TCID_{50}$/mL	*Mycoplasma pneumoniae*	$1x10^6$ CFU/mL
Parainfluenza virus 1	$1x10^5$ $TCID_{50}$/mL	*Neisseria gonorrhoeae*	$1x10^6$ CFU/mL
Parainfluenza virus 2	$1x10^5$ $TCID_{50}$/mL	*Neisseria meningitides*	$1x10^6$ CFU/mL
Parainfluenza virus 3	$1x10^5$ $TCID_{50}$/mL	*Neisseria mucosa*	$1x10^6$ CFU/mL
Parainfluenza virus 4	$1x10^3$ $TCID_{50}$/mL	*Pneumocystis jirovecii (PJP)*	$1x10^6$ nuclei/mL

Respiratory syncytial virus	$1x10^5$ $TCID_{50}$/mL	*Pseudomonas aeruginosa*	$1x10^6$ CFU/mL
Rhinovirus	$1x10^4$ $TCID_{50}$/mL	*Staphylococcus aureus*	$1x10^6$ CFU/mL
SARS-coronavirus[2]	$1x10^6$ copies/mL	*Staphylococcus epidermis*	$1x10^6$ CFU/mL
Varicella Zoster Virus	$1x10^4$ $TCID_{50}$/mL	*Streptococcus pneumoniae*	$1x10^6$ CFU/mL
Pooled human nasal wash[3] – *to represent diverse microbial flora in human respiratory tract*	N/A	*Streptococcus pyogenes* *Streptococcus salivaris*	$1x10^6$ CFU/mL $1x10^6$ CFU/mL

[1]CFU = Colony Forming Units; $TCID_{50}$ = Median Tissue Culture Infectious Dose

[2]Cultured virus and whole genome purified nucleic acid for Human coronavirus HKU1 and SARS-coronavirus were not readily available at the time testing was performed. Human coronavirus HKU1 and SARS-coronavirus IVTs corresponding to the ORF1ab gene regions targeted by the assay were used to evaluate cross-reactivity and microbial interference.

[3]In place of evaluating pooled human nasal wash, testing of 30 individual negative clinical NP swab specimens was performed to represent diverse microbial flora in the human respiratory tract.

Interfering Substances

Interfering endogenous and exogenous substances (mucin, whole blood, potential medications and over-the-counter products) that may be present in the samples were evaluated in the Aptima SARS-CoV-2 assay. Clinically relevant concentrations of potentially interfering substances were added to pooled clinical negative NP swab VTM/UTM matrix and tested in the absence and presence of SARS-CoV-2 inactivated virus at 3X LoD. The substances and concentrations are shown in Table 6. No impact on the performance of the Aptima SARS-CoV-2 Assay was seen for any of the substances at the concentration tested.

Table 6: Potentially Interfering Substances

Substance Type	Substance Name	Active Ingredient(s)	Highest Test Concentration*
Endogenous	Mucin	Purified mucin protein	60 µg/mL
	Blood (human)	N/A	2% v/v
Nasal sprays or drops	Neo-Synephrine®	Phenylephrine	15% v/v
	Anefrin	Oxymetazoline	15% v/v
	Saline	Sodium chloride	15% v/v
	Ventolin HFA[2]	Albuterol	45 ng/mL
Nasal corticosteroids	QVAR® Beconase AQ[2]	Beclomethasone	15 ng/mL
	Dexacort[2]	Dexamethasone	12 µg/mL
	Flonase	Fluticasone	5% v/v
	Nasacort	Triamcinolone	5% v/v
	Rhinocort	Budesonide	5% v/v
	Nasonex[2]	Mometasone	0.5 ng/mL
	AEROSPAN®[2]	Flunisolide	9.9 µg/mL
Nasal gel	Zicam® (Allergy Relief)	Luffa opperculata, Galphimia, Glauca, Histaminum hydrochloricum, Sulfur	5% v/v
Throat lozenges	Cepacol Extra Strength	Benzocaine, Menthol	0.7 mg/mL
	Cold-Eeze throat lozenge	Zinc gluconate	0.7 mg/mL
Anti-viral drugs	Relenza®[2]	Zanamivir	3.3 mg/mL
	TamiFlu[2]	Oseltamivir	399 ng/mL
	Virazole[2]	Ribavirin	10.5 µg/mL
Antibiotic, nasal	Bactroban cream[2]	Mupirocin	1.6 µg/mL
Antibacterial,	Tobramycin[2]	Tobramycin	33.1 µg/mL
Solvent Control	Water	N/A	5% v/v
	Dimethyl Sulfoxide (DMSO)	N/A	5% v/v

[1]v/v: volume by volume
[2]Active ingredient tested, not substance
[3]Two out of three positive results were observed for the positive pool containing Nasacort and Rhinocort. Substances were separated and retested and all replicates (3/3) were positive.

Carryover Contamination

The carryover contamination rate of the Aptima SARS-CoV-2 Assay for samples tested was assessed by testing high titer panels consisting of SARS-CoV-2 virus in negative clinical NP swab VTM/UTM matrix spiked 100 $TCID_{50}$/mL (10,000 times the assay LoD). Positive panels were tested in a checkerboard pattern, alternating with negative panels. Testing consisted of 588

negative and positive valid tests across three Panther systems. The Aptima SARS-CoV-2 Assay observed a carryover rate of 0% (0/294).

Assay Precision

The Aptima SARS-CoV-2 Assay within-lab precision was evaluated with a 4-member panel consisting of virus in negative clinical NP swab VTM/UTM matrix. The 4-member panel included a Negative, a High Negative (0.1X LoD), a Low Positive (1X LoD) and a Moderate Positive (5X LoD) panel. The panels were tested by two operators, using three reagent lots on three Panther systems over six days, at one site. Two runs were performed per operator per day for a total minimum of 36 runs. Each of the four panels was tested in three replicates per run for a total of 108 replicates per panel. The agreement with expected results was 100% in the Negative, Low Positive and Moderate Positive panel members. The High Negative panel member was 10X below the assay LoD, therefore a mix of positive and negative results were expected. This panel had 68/108 (63%) positive results. Agreement with expected results for all four panels is shown in Table 7.

Table 7: Agreement of Aptima SARS-CoV-2 Assay Results with Expected Results

Panel Description	Panel Composition	Panel Conc. $TCID_{50}$/mL	Expected Result	N Positive	N Tested	Mean kRLU	Agreement w/Expected (95% CI)
Negative	N/A	N/A	Negative	0	108	289	100% (96.6-100)
High Negative	0.1xLoD	0.001	N/A	68	108	627	N/A
Low Positive	1.0xLoD	0.01	Positive	108	108	1131	100% (96.6-100)
Moderate Positive	5.0xLoD	0.05	Positive	108	108	1147	100% (96.6-100)

The total SARS-CoV-2 signal variability measured as %CV ranged from 2.75% to 3.84% in Negative, Low Positive, and Moderate Positive panel members. For the sources of variation all six factors evaluated had %CV values <3.0% as shown in Table 8. The High Negative panel

member is 10x below the assay LoD and the %CV for this panel is expected to be higher than the others. The highest source of variability for this panel was within-run variability.

Table 8: kRLU Signal Variability of the Aptima SARS-CoV-2 Assay by Panel Member

Panel	Between Days		Between Instruments		Between Operators		Between Lots		Between Runs		Within Runs		Total	
	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%0	SD	CV (%)
Negative	0.91	0.31	4.97	1.72	0.0	0.0	4.04	1.40	0.0	0.0	6.75	2.33	9.35	3.23
High Negative*	30.45	4.85	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	244.08	38.91	245.97	39.21
Low Positive	6.46	0.57	6.74	0.60	0.0	0.0	28.10	2.48	0.0	0.0	31.77	2.81	43.43	3.84
Moderate Positive	8.53	0.74	5.59	0.49	0.0	0.0	22.98	2.00	11.06	0.96	15.59	1.36	31.59	2.75

*Panel was built to 10x below the assay LoD. Higher variability is expected in this panel.
Note: In the event that variability from some factors is numerically negative, SD and CV are shown as 0.0.

Collection Device Equivalency

Equivalence between NP specimens collected into VTM/UTM and NP and AN swab specimens collected in RespDirect (eSTM) was evaluated by testing individual negative specimens and contrived positive panels prepared from paired negative clinical samples collected from patients with symptoms of COVID-19. Contrived panels were prepared by spiking individual donor paired NP specimens, and AN swab specimens for RespDirect only, with SARS-CoV-2 to 2X and 5X LoD. The results of the negative and contrived panels demonstrated similar agreement comparable between the two collection devices (Table 9).

Table 9: Results of negative and contrived panels composed of paired individual donor clinical specimens (NP for VTM/UTM and NP/nasal swab for RespDirect), collected with each collection device spiked with SARSCoV-2

Analyte	Sample Concentration	N per Collection Device	VTM/UTM-NP % Positive	RespDirect-NP % Positive	RespDirect-nasal swab % Positive
None (Negative Sample)	0	150	0	0	0
SARS-CoV-2	2X LoD	50	100	100	100
	5X LoD	50	100	100	100

Reproducibility

Aptima SARS-CoV-2 Assay reproducibility was evaluated at three US sites using one negative and two positive panel members. Testing was performed using one lot of assay reagents and six operators (two at each site). At each site, testing was performed for at least five days. Each run had three replicates of each panel member.

A negative panel member was created using pooled negative clinical NP swab specimens in VTM/UTM processed into STM (i.e., negative matrix). Positive panel members were created by spiking 1-2X LoD (low positive) or 3-5X LoD (moderate positive) concentrations of SARS-CoV-2 inactivated virus into the negative matrix. The agreement with expected results was 100% for all panel members. The total SARS-CoV-2 signal variability, measured as %CV, was ≤7.93% (SD less than or equal to 91.35) for all positive panel members (Table 10).

Table 10: kRLU Signal Variability of the Aptima SARS-CoV-2 Assay by Panel Member

			Between Sites		Between Operators/ Runs[1]		Between Days		Within Runs		Total	
Panel Description	N	Mean kRLU	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Negative	90	286.0	27.04	9.45	25.42	8.89	0.45	0.16	6.55	2.29	37.69	13.18
SARS-CoV-2 Low Pos	90	1152.2	67.79	5.88	15.16	1.32	25.06	2.18	53.77	4.67	91.35	7.93
SARS-CoV-2 Mod Pos	90	1163.7	77.30	6.64	36.60	3.15	4.10	0.35	26.67	2.29	89.68	7.71

CV = coefficient of variation, Mod = moderate, Pos = positive, kRLU = relative light unit × 1000, SD = standard deviation.
[1] Between Operator may be confounded with Between Run; therefore, Between Operator and Between Run estimates are combined in Between Operator/Run.

Brief Description of Clinical Studies

Two clinical studies were performed. Aptima SARS-CoV-2 Assay clinical performance was estimated in prospectively collected NP specimens in Clinical Study 1 and in prospectively collected AN swab specimens in Clinical Study 2.

Clinical Study 1: Prospective Clinical Study - Nasopharyngeal Swab Specimens in UTM/VTM

This study was performed to demonstrate clinical performance characteristics for the Aptima SARS-CoV-2 Assay in NP swab specimens. A prospective multicenter study was conducted using remnant NP swab specimens from male and female individuals of all ages exhibiting signs and/or symptoms of respiratory infection consistent with COVID-19. Four participating US pediatric/adolescent, private and/or university hospitals prospectively provided remnant NP swab specimens stored in viral transport medium (VTM). These specimens were tested at three US sites with the Aptima SARS-CoV-2 Assay. The Aptima SARS-CoV-2 Assay was evaluated for SARS-CoV-2 performance by comparing its results from NP swab specimens in UTM/VTM to a composite comparator algorithm (CCA) consisting of two highly sensitive US FDA EUA SARS-

CoV-2 molecular tests and a validated PCR followed by bi-directional sequencing (PCR/BDS) assay. A final CCA result was assigned when two of the three comparator assay results were in concordance. Of the 1646 specimens enrolled during the study, 300 were collected between June 2020 and July 2020, while the remaining 1346 were collected between January 2023 and April 2023. A total of 1646 NP swab specimens were tested in valid Aptima SARS-CoV-2 Assay runs, including 9 (0.5%) with initial invalid results. Upon retest, all 1646 specimens yielded final valid results. The final data set consisted of 1495 evaluable NP swab specimens, including 1195 (79.9%) tested fresh and 300 (20.1%) tested after freezing; 149 NP swab specimens were excluded from analysis due to mishandling at the sites. Demographic information for the 1495 evaluable individuals is provided in Table 11.

Table 11: Summary of Subject Demographics for Evaluable Prospectively Collected NP Swab Specimens

Total		1495
Sex	Female	842 (56.3%)
	Male	651 (43.5%)
	Unknown	2 (0.1%)
Age (years)	Mean	33.3
	Median	29.0
	Range	0 – 98
	<5	270 (18.1%)
	5-21	373 (24.9%)
	22-59	499 (33.4%)
	≥60	353 (23.6%)

The performance of the Aptima SARS-CoV-2 Assay with prospective NP swab specimens is summarized in Table 12. Positive Percent Agreement (PPA) was calculated as 100% × (TP / (TP + FN)). True positive (TP) indicates that both the Aptima SARS-CoV-2 assay and the CCA had a positive result for SARS-CoV-2, and false negative (FN) indicates that the Aptima SARS-CoV-2 Assay result was negative while the CCA was positive. Negative Percent Agreement

(NPA) was calculated as 100% × (TN / (TN + FP)). True negative (TN) indicates that both the Aptima SARS-CoV-2 assay and the CCA had negative results, and false positive (FP) indicates that the Aptima SARS-CoV-2 Assay result was positive while the CCA was negative. NP specimens that obtained discordant results underwent additional testing with a US FDA EUA SARS-CoV-2 molecular test, volume permitting.

Table 12: Aptima SARS-CoV-2 Assay Performance with NP Swab Specimens

NP Swab Specimen Type	Positive Percent Agreement			Negative Percent Agreement		
	TP/ (TP+FN)	%	95% CI[1]	TN/ (FP+TN)	%	95% CI[1]
Fresh[2]	80/82	97.6	91.5-99.3	1107/1113	99.5	98.8-99.8
Frozen[2]	44/48	91.7	80.4-96.7	251/252	99.6	97.8-99.9
Overall	124/130[3]	95.4	90.3-97.9	1358/1365[4]	99.5	98.9-99.8

CI = confidence interval, FN = false negative, FP = false positive, TN = true negative, TP = true positive.

[1] Score CI.

[2] All fresh samples were collected in 2023. All frozen samples were collected in 2020.

[3] One (1) specimen with a false negative result tested negative for SARS-CoV-2 with a US FDA EUA SARS-CoV-2 molecular test, while 4 tested positive and 1 had an inconclusive result using the same assay.

[4] One (1) specimen with a false positive result tested positive for SARS-CoV-2 with a US FDA EUA SARS-CoV-2 molecular test, while 5 tested negative and 1 had no result using the same assay.

Clinical Study 2: Prospective Clinical Study – Anterior Nasal Swab Specimens in UTM/VTM and eSTM (RespDirect Collection Kit)

This study was performed to demonstrate clinical performance characteristics for the Aptima SARS-CoV-2 Assay in anterior nasal swab specimens. The clinical performance of the Aptima SARS-CoV-2- 2 Assay was evaluated using anterior nasal swab specimens collected in a prospective, multicenter clinical study. Male and female individuals of all ages exhibiting signs and/or symptoms of respiratory infection consistent with COVID-19 were enrolled at nine geographically and ethnically diverse US sites during the 2022-2023 respiratory season. Two anterior nasal swab specimens were prospectively collected from each individual (in a clinical setting): one specimen collected using a synthetic flocked swab by a healthcare professional

(HCP) and stored in UTM/VTM; one specimen collected by the patient (under HCP supervision) or the HCP using either a synthetic flocked swab and stored in UTM/VTM or using the RespDirect flocked swab and stored in a Direct Capture Tube containing eSTM (RespDirect Collection Kit). The Aptima SARS-CoV-2 Assay was evaluated for SARS-CoV-2 performance by comparing its results from anterior nasal swab specimens in UTM/VTM or in eSTM to a composite comparator algorithm (CCA) consisting of two highly sensitive US FDA EUA SARS-CoV-2 molecular tests and a validated PCR/BDS assay. A final CCA result was assigned when two of the three comparator assay results were in concordance. Of the 2301 enrolled subjects, six did not meet eligibility criteria and were withdrawn. A total of 2241 specimens in UTM/VTM and eSTM from 2295 non-withdrawn subjects were tested in valid Aptima SARS-CoV-2 Assay runs, including 23 (1.0%) with initial invalid results. Upon retest, 13 specimens yielded valid results and 10 yielded final invalid results, for a total of 2231 (99.6%) specimens with final valid results. An additional 118 subjects were not evaluable due to specimen withdrawal, missing/invalid Aptima results, or an unknown CCA result, leaving 2177 individuals evaluable for the performance analyses, including 1159 with evaluable anterior nasal swab specimens in UTM/VTM, and 1018 with evaluable nasal swab specimens in eSTM. Demographic information for the 2177 evaluable individuals is provided in Table 13.

Table 13: Summary of Subject Demographics for Prospectively Collected Nasal Swab Specimens

Total		2177
Sex	Female	1287 (59.1%)
	Male	890 (40.9%)
Age (years)	Mean	40.7
	Median	40.0
	Range	0 – 90
COVID-19 vaccination status	Fully vaccinated	1451 (66.7%)
	Partially vaccinated	106 (4.9%)
	Unvaccinated	601 (27.6%)
	Unknown	19 (0.9%)

The performance of the Aptima SARS-CoV-2 Assay with prospective anterior nasal swab specimens is summarized in Table 14. PPA and NPA percent agreement were calculated as described for Clinical Study 1.

Table 14: Aptima SARS-CoV-2 Assay Performance with Anterior Nasal Swab Specimens

Nasal Swab Specimen Type	Positive Percent Agreement			Negative Percent Agreement		
	TP/ (TP+FN)	%	95% CI[1]	TN/ (FP+TN)	%	95% CI[1]
UTM/VTM	138/143	96.5	92.1-98.5	992/1016	97.6	96.5-98.4
RespDirect eSTM	108/108	100	96.6-100	892/910	98.0	96.9-98.7

CI = confidence interval, eSTM = enhanced specimen transport medium, FN = false negative, FP = false positive, TN = true negative, TP = true positive, UTM/VTM = universal/viral transport medium.

[1] Score CI.

VIII. CONCLUSIONS

The analytical and clinical study results demonstrate that the Aptima SARS-CoV-2 Assay on the Panther/Panther Fusion system performs comparably to the predicate device that is currently marketed for the same intended use.