Aptima® SARS-CoV-2 Assay (Panther® System)

For i*n vitro* diagnostic use

Rx Only

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General Information

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.

Summary and Explanation of the Test

Coronaviruses are a large family of viruses which may cause illness in animals or humans. In humans, several coronaviruses are known to cause respiratory infections ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). The most recently discovered coronavirus, SARS-CoV-2, causes the associated coronavirus disease COVID-19. This new virus and disease were unknown before the outbreak in Wuhan, China, in December 2019.[1] People with COVID-19 have had a wide range of symptoms reported, ranging from mild symptoms to severe illness. Symptoms may appear 2–14 days after exposure to the virus. People with COVID-19 may exhibit fever or chills, cough, shortness of breath or difficulty breathing, fatigue, muscle or body aches, headache, new loss of taste or smell, sore throat, congestion or runny nose, nausea or vomiting, and/or diarrhea.[2] On March 11, 2020, the COVID-19 outbreak was characterized as a pandemic by the World Health Organization (WHO).[3] Over 760 million cases and 6.9 million deaths have been recorded worldwide since December 2019, but the actual number is thought to be higher.[4,5]

Principles of the Procedure

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 amplicon to form stable hybrids. The Selection Reagent differentiates hybridized from unhybridized probe, eliminating the generation of signal from 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 chemiluminescent detection reaction for the IC signal has very rapid kinetics and has the “flasher” kinetic type. The chemiluminescent detection reaction for the SARS-CoV-2 signal is relatively slower and has the “glower” kinetic type. Assay results are determined by a cut-off based on the total RLU and the kinetic curve type.

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

Warnings and Precautions

A. For *in vitro* diagnostic use.

B. Carefully read this entire package insert and the *Panther®/Panther Fusion® System Operator’s Manual*.

C. For professional use.

Laboratory Related

D. Only personnel adequately trained on the use of this assay and in handling potentially infectious materials should perform these procedures. If a spill occurs, immediately disinfect using appropriate site procedures.

E. Handle and process all specimens as if infectious following laboratory practices and procedures that are basic to good microbiological practice and procedures (GMPP). Refer to World Health Organization’s (WHO) Laboratory biosafety guidance related to coronavirus disease (COVID-19): interim guidance. https://www.who.int/teams/health-product-policy-and-standards/assistive-and-medical-technology/medical-devices/ppe/ppe-covid.

F. Specimens may be infectious. Use Universal Precautions when performing this assay. Proper handling and disposal methods should be established by the laboratory director. Only personnel adequately trained in handling infectious materials should be permitted to perform this diagnostic procedure.[6]

G. Use only supplied or specified disposable laboratory ware.

H. Use appropriate personal protective equipment when collecting and handling NP and AN swab specimens. Refer to the CDC Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019 Novel Coronavirus (2019-nCoV) for more information.

I. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and reagents. Wash hands thoroughly after handling specimens and reagents.

J. Dispose of all material that has come into contact with specimens and reagents in accordance with applicable national, international, and regional regulations.

K. Use good standard practices for molecular laboratories including environmental monitoring. See *Lab Contamination Monitoring Protocol for the Panther System* for the Panther System.

L. Viral culture should not be attempted in cases of positive results for SARS-CoV-2 and/or any similar microbial agents unless a facility with an appropriate level of laboratory biosafety (e.g., BSL 3 and BSL 3+, etc.) is available to receive and culture specimens.

Specimen Related

M. Expiration dates listed on the Panther Fusion Specimen Lysis Tubes and the RespDirect™ Collection Kit pertain to the transfer of sample into the tube and not to testing of the sample. Specimens collected/transferred any time prior to these expiration dates are valid for testing provided they are transported and stored in accordance with the appropriate package insert, even if these expiration dates have passed.

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

O. Avoid cross-contamination during the specimen handling steps. Specimens can contain extremely high levels of virus or other organisms. Ensure that specimen containers do not come in contact with one another, and discard used materials without passing them over any open containers. Change gloves if they come in contact with specimens.

Assay Related

P. Do not use the reagents and controls after the expiration date.

Q. Store assay components at the recommended storage condition. See *Reagent Storage and Handling Requirements (*page 6), and *Panther System Test Procedure* (page 12) for more information.

R. Do not combine any assay reagents or fluids. Do not top off reagents or fluids; the Panther system verifies reagent levels.

S. Avoid microbial and ribonuclease contamination of reagents.

T. Do not use material that may contain Guanidinium thiocyanate or any guanidine-containing materials on the instrument. Highly reactive and/or toxic compounds may form if combined with sodium hypochlorite.

U. A reagent in this kit is labeled with risk and safety symbols.

Note: *For information on any hazard and precautionary statements that may be associated with reagents refer to the Safety Data Sheet Library at www.hologicsds.com. For more information on the symbols, refer to the symbol legend on www.hologic.com/package-inserts.*

US Hazard Information
 **Selection Reagent** ***Boric Acid 1-5%*** ***Triton X-100 1-5%*** **Danger** H315 - Causes skin irritation H360FD - May damage fertility. May damage the unborn child. P264 - Wash face, hands and any exposed skin thoroughly after handling. P302 + P352 - IF ON SKIN: Wash with plenty of water and soap. P321 - Specific treatment (see supplemental first aid instructions on this label). P332 + P313 - If skin irritation occurs: Get medical advice/attention. P362 + P364 - Take off contaminated clothing and wash it before reuse. P201 - Obtain special instructions before use. P202 - Do not handle until all safety precautions have been read and understood. P280 - Wear protective gloves/protective clothing/eye protection/face protection P308 + P313 - IF exposed or concerned: Get medical advice/attention. P405 - Store locked up. P501 - Dispose of contents/ container to an approved waste disposal plant

Reagent Storage and Handling Requirements

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

Reagent	Unopened Storage	Open Kit (Reconstituted)	
		Storage	Stability
Aptima SARS-CoV-2 Amplification Reagent	2ºC to 8ºC	N/A	N/A
Aptima SARS-CoV-2 Enzyme Reagent	2ºC to 8ºC	N/A	N/A
Aptima SARS-CoV-2 Probe Reagent	2ºC to 8ºC	N/A	N/A
Aptima SARS-CoV-2 Internal Control	2ºC to 8ºC	N/A	N/A
Aptima SARS-CoV-2 Positive Control	2ºC to 8ºC	N/A	N/A
Aptima SARS-CoV-2 Negative Control	2ºC to 8ºC	N/A	N/A
Aptima SARS-CoV-2 Amplification Reconstitution Solution	2ºC to 30ºC	2ºC to 8ºC	30 days
Aptima SARS-CoV-2 Enzyme Reconstitution Solution	2ºC to 30ºC	2ºC to 8ºC	30 days
Aptima SARS-CoV-2 Probe Reconstitution Solution	2ºC to 30ºC	2ºC to 8ºC	30 days
Aptima SARS-CoV-2 Selection Reagent	2ºC to 30ºC	2ºC to 30ºC	30 days
Aptima SARS-CoV-2 Target Capture Reagent	15ºC to 30ºC	15ºC to 30ºC	30 days

B. If the Selection Reagent is stored refrigerated, let it come to room temperature before placing on the Panther system.

C. Working Target Capture Reagent (wTCR) is stable for 30 days when stored at 15°C to 30°C. Do not refrigerate.

D. After reconstitution, the Enzyme Reagent, Amplification Reagent, and Probe Reagent are stable for 30 days when stored at 2°C to 8°C.

E. Discard any unused reconstituted reagents and wTCR after 30 days or after the Master Lot expiration date, whichever comes first.

F. Controls are stable until the date indicated on the vials.

G. Reagents stored on-board the Panther system have 120 hours of on-board stability.

H. The Probe Reagent and Reconstituted Probe Reagent are photosensitive. Store the reagents protected from light. The specified reconstituted stability is based on 12 hours exposure of the Reconstituted Probe Reagent to two 60W fluorescent bulbs, at a distance of 17 inches (43 cm), and temperature less than 30°C. Light exposure of the Reconstituted Probe Reagent should be limited accordingly.

I. Upon warming to room temperature, some control tubes may appear cloudy or contain precipitates. Cloudiness or precipitation associated with controls does not affect control performance. The controls may be used whether they are clear or cloudy/precipitated. If clear controls are desired, solubilization may be expedited by incubating them at the upper end of the room temperature range (15°C to 30°C).

J. Do not freeze the reagents.

Specimen Collection and Storage

Specimens - NP or AN swabs collected from patients and placed in an appropriate transport system. For the Aptima SARS-CoV-2 Assay, place NP or AN swab specimens in viral transport medium (VTM/UTM) or enhanced specimen transport medium (eSTM), if collected with the RespDirect Collection Kit.

Samples - Represents a more generic term to describe any material for testing on the Panther system including specimens, specimens transferred into a Panther Fusion Specimen Lysis Tube and controls.

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

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

Specimen Collection

Collect NP or AN swab specimens according to standard technique using a polyester-, rayon-, or nylon-tipped swab. Immediately place the swab specimen into 3mL of VTM or UTM. NP and AN swab specimens can also be collected with the RespDirect Collection Kit.

The following types of VTM/UTM were verified for use with the Aptima SARS-CoV-2 Assay:

- Remel MicroTest M4RT, M5 or M6 formulations
- Copan Universal Transport Medium
- BD Universal Viral Transport Medium
- Hardy Diagnostics Viral Transport Medium

Specimen Processing

Specimen Processing with the Panther Fusion Specimen Lysis Tube

1. Prior to testing on the Panther system, transfer 500 µL of the specimen collected in UTM or VTM into a Panther Fusion Specimen Lysis Tube.

Note: *When testing frozen specimen, allow specimen to reach room temperature prior to processing.*

Specimen Processing with the Enhanced Direct Load Tube (RespDirect Collection Kit)

1. After collecting the specimen into the Enhanced Direct Load Tube (RespDirect Collection Kit), the specimen may be loaded on the Panther system.

   Note: *If clots are observed, samples may be vortexed for 5–10 minutes at 1,800 rpm on a multi-tube vortex (or setting 5 on Cat. No. 102160G).*

   Alternatively, individual tubes may be vortexed by hand for 15 seconds on max. speed on a standard bench top vortex.

   If previously pierced, recap tubes with a new penetrable cap before vortexing.

   If a CLT result is obtained upon retesting, collect a new sample.

   Note: *When testing frozen specimen, allow specimen to reach room temperature prior to loading on the Panther system.*

Note: *If the lab receives an Enhanced Direct Load Tube (RespDirect Collection Kit) with no swab or two swabs, the specimen must be rejected.*

Specimen Storage

Storing Specimens with the Panther Specimen Lysis Tube

1. After collection, NP and AN swab specimens in VTM/UTM can be stored at 2°C to 8°C up to 96 hours before transfer to the Panther Fusion Specimen Lysis Tube. Remaining specimen volumes can be stored at ≤–70°C. Freeze/thaw cycles should be minimized due to potential for sample degradation.
2. Samples in the Panther Fusion Specimen Lysis Tube can be stored under the following conditions:
    - 15°C to 30°C up to 6 days or
    - 2°C to 8°C, –20°C, and –70°C for up to 3 months. Freeze/thaw cycles should be minimized due to potential for sample degradation.
3. Previously tested samples should be covered with a new, clean plastic film or foil barrier.
4. If assayed samples need to be frozen or shipped, remove the penetrable cap and place a new non-penetrable cap on the specimen tubes. If samples need to be shipped for testing at another facility, recommended temperatures must be maintained. Prior to uncapping previously tested and recapped samples, specimen tubes may be centrifuged for 5 minutes at 420 Relative Centrifugal Force (RCF) to bring all of the liquid down to the bottom of the tube. Avoid splashing and cross-contamination.

Storing Specimens with the Enhanced Direct Load Tube (RespDirect Collection Kit)

1. NP and AN swab samples can be stored under the following conditions:
    - 15°C to 30°C up to 6 days or
    - 2°C to 8°C, –20°C, and –70°C for up to 3 months. Freeze/thaw cycles should be minimized due to potential for sample degradation.
2. Previously tested samples should be covered with a new, clean plastic film or foil barrier.
3. If assayed samples need to be frozen or shipped, remove the penetrable cap and place a new non-penetrable cap on the specimen tubes. If samples need to be shipped for testing at another facility, recommended temperatures must be maintained. Prior to uncapping previously tested and recapped samples, specimen tubes may be centrifuged for 5 minutes at 420 RCF to bring all of the liquid down to the bottom of the tube. Avoid splashing and cross-contamination.

Specimen Transport

Maintain specimen storage conditions as described in the *Specimen Collection and Storage section on* page 7.

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

Panther System

Reagents for the Aptima SARS-CoV-2 Assay are listed below for the Panther system. Reagent Identification Symbols are also listed next to the reagent name.

Reagents and Materials Provided

Aptima SARS-CoV-2 Assay Kit PRD-07881

100 tests (2 boxes)

Aptima SARS-CoV-2 Refrigerated Box (Box 1 of 2)
(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity 100 test kit
A	**Aptima SARS-CoV-2 Amplification Reagent** *Non-infectious nucleic acids dried in buffered solution containing < 5% bulking agent.*	1 vial
E	**Aptima SARS-CoV-2 Enzyme Reagent** *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing < 10% bulking reagent.*	1 vial
P	**Aptima SARS-CoV-2 Probe Reagent** *Non-infectious chemiluminescent DNA probes dried in succinate buffered solution containing < 5% detergent.*	1 vial
IC	**Aptima SARS-CoV-2 Internal Control**	1 vial

Aptima SARS-CoV-2 Room Temperature Box (Box 2 of 2)
(store at 15°C to 30°C upon receipt)

Symbol	Component	Quantity 100 test kit
AR	**Aptima SARS-CoV-2 Amplification Reconstitution Solution** *Aqueous solution containing preservatives.*	1 x 12.2 mL
ER	**Aptima SARS-CoV-2 Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 x 6.6 mL
PR	**Aptima SARS-CoV-2 Probe Reconstitution Solution** *Succinate buffered solution containing < 5% detergent.*	1 x 15.7 mL
S	**Aptima SARS-CoV-2 Selection Reagent** *600 mM borate buffered solution containing surfactant.*	1 x 45.0 mL
TCR	**Aptima SARS-CoV-2 Target Capture Reagent** *Buffered salt solution containing solid phase and capture oligomers.*	1 x 27.0 mL
	Reconstitution Collars	3
	Master Lot Barcode Sheet	1 sheet

Materials Required and Available Separately

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

	Cat. No.
Panther System	303095
Panther System Continuous Fluid and Waste (Panther Plus)	PRD-06067
Aptima Assay Fluids Kit *(Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent)*	303014 (1000 tests)
Aptima Auto Detect Kit	303013 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther Waste Bag Kit	902731
Panther Waste Bin Cover	504405
Or Panther Run Kit *contains MTUs, waste bags, waste bin covers, assay fluids, and auto detects*	303096 (5000 tests)
Tips, 1000 µL filtered, conductive, liquid sensing, and disposable *Not all products are available in all regions. Contact your representative for region-specific information*	901121 (10612513 Tecan) 903031 (10612513 Tecan) MME-04128 MME-04134 (30180117 Tecan)
Aptima SARS-CoV-2 Controls Kit *PC - Aptima SARS-CoV-2 Positive Control. Non-infectious nucleic acid in a buffered solution containing < 5% detergent. Quantity 5 x 1.7 mL* *NC - Aptima SARS-CoV-2 Negative Control. A buffered solution containing <5% detergent. Quantity 5 x 1.7 mL*	PRD-07882
RespDirect Collection Kit, 50 per box	PRD-07403
Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens	301041
Panther Fusion Specimen Lysis Tubes, 100 per bag *tube contains 0.71 mL of STM with a penetrable cap*	PRD-04339
Bleach, 5% to 8.25% (0.7M to 1.16M) sodium hypochlorite solution	—
Disposable gloves	—
Replacement non-penetrable caps	504415
Replacement Caps for the 100-test kits *Amplification, Enzyme, and Probe reagent reconstitution solutions CL0041 (100 caps)* *TCR and Selection reagent 501604 (100 caps)*	—

Optional Materials

	Cat. No.
Hologic Bleach Enhancer for Cleaning *for routine cleaning of surfaces and equipment*	302101
Tube rocker	—
Multitube Vortex	102160G
Benchtop Vortex	—
Panther Fusion Module Upgrade	PRD-04173
Panther Fusion System	PRD-04172

Panther System Test Procedure

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

A. Work Area Preparation

Clean work surfaces where reagents and samples will be prepared. Wipe down work surfaces with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and then follow with a water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface on which the reagents and samples will be prepared with clean, plastic-backed absorbent laboratory bench covers.

B. Reagent Reconstitution/Preparation of a New Kit

Note: *Reagent reconstitution should be performed prior to beginning any work on the Panther system.*

1. Prior to testing, Amplification, Enzyme, and Probe Reagents, must be reconstituted by combining contents of the bottles of lyophilized reagent with the appropriate reconstitution solution.
   a. Allow the lyophilized reagents to reach room temperature (15°C to 30°C) before use.
   b. Pair each reconstitution solution with its lyophilized reagent. Before attaching the reconstitution collar, ensure that the reconstitution solution and reagent have matching label symbols.
   c. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired. Label caps of reconstitution solution bottles.
   d. Open the lyophilized reagent glass vial and firmly insert the notched end of the reconstitution collar into the glass vial opening (Figure 1, Step 1).
   e. Open the matching reconstitution solution, and set the cap on a clean, covered work surface.
   f. While holding the reconstitution solution bottle on the bench, firmly insert the other end of the reconstitution collar into the bottle opening (Figure 1, Step 2).
   g. Slowly invert the assembled bottles. Allow the solution to drain from the bottle into the glass vial (Figure 1, Step 3).
   h. Pick up the assembled bottles and swirl the assembled bottles for at least 10 seconds. Avoid creating foam while swirling the bottle. (Figure 1, Step 4)
   i. To ensure the lyophilized reagent goes completely into solution. Swirl the bottles again for at least 10 seconds, and then slightly rock the solution within the glass vial back and forth to mix thoroughly.
   j. Visually check to see if reagent is completely in solution with no powder, clumps or wavy lines.
   k. Slowly tilt the assembled bottles again to allow all of the solution to drain back into the reconstitution solution bottle. (Figure 1, Step 5).
   l. Remove the reconstitution collar and glass vial (Figure 1, Step 6).

m. Recap the plastic bottle with either the saved labeled cap that corresponds to the reagent or a new cap. Do not mismatch caps. Record operator initials and reconstitution date on the label (Figure 1, Step 7).

n. Discard the reconstitution collar and glass vial (Figure 1, Step 8).

o. Thoroughly mix each reagent by gently inverting prior to loading onto the Panther system.

Option: Additional mixing of the Amplification, Enzyme, and Probe Reagents is allowed by placing recapped plastic bottles on a tube rocker set at a moderate speed and tilt for a minimum of 5 minutes. Ensure reagents are thoroughly mixed.

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

Warning: *Adequate mixing of the reagents is necessary to achieve expected assay results.*

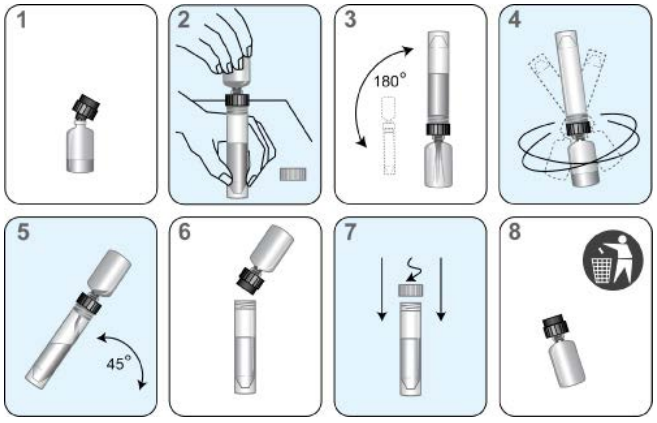

Figure 1. Panther System Reconstitution Process

2. Prepare Working Target Capture Reagent (wTCR)
   a. Pair the appropriate bottles of TCR and IC.
   b. Check the reagent lot numbers on the Master Lot Barcode Sheet to make sure that the appropriate reagents in the kit are paired.
   c. Open the bottle of TCR, and set the cap on a clean, covered work surface.
   d. Open the IC bottle and pour the entire contents into the bottle of TCR. Expect a small amount of liquid to remain in the IC bottle.
   e. Cap the bottle of TCR and gently swirl the solution to mix the contents. Avoid creating foam during this step.
   f. Record operator initials and the current date on the label.
   g. Discard the IC bottle and cap.
3. Prepare Selection Reagent
   a. Check the lot number on the reagent bottle to make sure it matches the lot number on the Master Lot Barcode Sheet.
   b. Record operator initials and the current date on the label.

Note: *Thoroughly mix by gently inverting all reagents prior to loading on the system. Avoid creating foam during inversion of reagents.*

C. Reagent Preparation for Previously Reconstituted Reagents

1. Previously reconstituted Amplification, Enzyme, and Probe Reagents must reach room temperature (15°C to 30°C) prior to the start of the assay.

   Option: The reconstituted Amplification, Enzyme, and Probe reagents capped plastic bottles may be placed on a tube rocker set at a moderate speed and tilt for a minimum of 25 minutes to ensure reagents reach room temperature and are thoroughly mixed.

2. If reconstituted Probe Reagent contains precipitate that does not return to solution at room temperature, heat the capped bottle at a temperature that does not exceed 62°C for 1 to 2 minutes. After this heat step, the Probe Reagent may be used even if residual precipitate remains. Mix Probe Reagent by inversion, being careful not to induce foam, prior to loading onto the system.
3. Thoroughly mix each reagent by gently inverting prior to loading on the system. Avoid creating foam during inversion of reagents. This step is not required if reagents are loaded onto the system directly after mixing on the tube rocker.
4. Do not top off reagent bottles. The Panther system will recognize and reject bottles that have been topped off.
5. *Adequate mixing of the reagents is necessary to achieve expected assay results.*

D. Specimen Handling

Note: *Prepare specimens per the Specimen Processing instructions in the Specimen Collection and Storage section before loading specimens onto the Panther system.*

1. Inspect sample tubes before loading into the rack. If a sample tube contains bubbles or has a lower volume than is typically observed, gently tap the bottom of the tube to bring contents to the bottom.

Note: *For samples transferred to the Panther Fusion Specimen Lysis Tube to avoid a processing error, ensure adequate specimen volume is added to the tube. When adequate collected specimen is added to the tube, there is sufficient volume to perform 3 nucleic acid extractions.*

Note: *For the Enhanced Direct Load Tube (RespDirect Collection Kit), there is sufficient volume to perform 4 nucleic acid extractions.*

E. System Preparation

1. Set up the system according to the instructions in the *Panther/Panther Fusion System Operator's Manual* and *Procedural Notes*. Make sure that the appropriately sized reagent racks and TCR adapters are used.
2. Load samples.

Procedural Notes

A. Controls

1. To work properly with the Aptima Assay software for the Panther system, one pair of controls is required. The Aptima SARS-CoV-2 positive and negative controls can be

loaded in any rack position or in any Sample Bay Lane on the Panther system. Patient specimen pipetting will begin when one of the following two conditions has been met:

a. A pair of controls is currently being processed by the system.

b. Valid results for the controls are registered on the system.

2. Once the control tubes have been pipetted and are processing for a specific reagent kit, patient specimens can be run with the associated kit up to 24 hours unless:
   a. Controls results are invalid.
   b. The associated assay reagent kit is removed from the system.
   c. The associated assay reagent kit has exceeded stability limits.
3. Each Aptima control tube can be tested once. Attempts to pipette more than once from the tube can lead to processing errors.
4. Patient specimen pipetting begins when one of the following two conditions is met:
   a. Valid results for the controls are registered on the system.
   b. A pair of controls is currently in process on the system.

B. Temperature

Room temperature is defined as 15°C to 30°C.

C. Glove Powder

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

D. Lab Contamination Monitoring Protocol for the Panther System

There are many laboratory-specific factors that may contribute to contamination, including testing volume, workflow, disease prevalence and various other laboratory activities. These factors should be taken into consideration when contamination monitoring frequency is being established. Intervals for contamination monitoring should be established based on each laboratory's practices and procedures.

To monitor for laboratory contamination, the following procedure may be performed using the Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens:

1. Label swab transport tubes with numbers corresponding to the areas to be tested.
2. Remove the specimen collection swab (blue shaft swab with green printing) from its packaging, wet the swab in the specimen transport medium (STM), and swab the designated area using a circular motion.
3. Immediately insert the swab into transport tube.
4. Carefully break the swab shaft at the score line; use care to avoid splashing of the contents.
5. Recap the swab transport tube tightly.
6. Repeat Steps 2 to 5 for each area to be swabbed.

E. If the results are positive, see *Interpretation of Results*. For additional Panther system-specific contamination monitoring information, contact Hologic Technical Support.

Quality Control

A run or specimen result may be invalidated by the Panther system if problems occur while performing the assay. Specimens with invalid results must be retested.

Negative and Positive Controls

To generate valid results, a set of assay controls must be tested. One replicate of the negative assay control and positive assay control must be tested each time a new kit is loaded on the Panther system or when the current set of valid controls have expired.

The Panther system is configured to require assay controls run at an administrator-specified interval of up to 24 hours. Software on the Panther system alerts the operator when assay controls are required and does not start new tests until the assay controls are loaded and have started processing.

During processing, criteria for acceptance of the assay controls are automatically verified by the Panther system. To generate valid results, the assay controls must pass a series of validity checks performed by the Panther system.

If the assay controls pass all validity checks, they are considered valid for the administrator-specified time interval. When the time interval has passed, the assay controls are expired by the Panther system which requires a new set of assay controls be tested prior to starting any new samples.

If any one of the assay controls fails the validity checks, the Panther system automatically invalidates the affected samples and requires a new set of assay controls be tested prior to starting any new samples.

Internal Control

An internal control is added to each sample with the wTCR. During processing, the internal control acceptance criteria are automatically verified by the Panther system software. Detection of the internal control is not required for samples that are positive for SARS-CoV-2. The internal control must be detected in all samples that are negative for SARS-CoV-2 targets; samples that fail to meet that criteria will be reported as Invalid. Each sample with an Invalid result must be retested.

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

Interpretation of Results

The Panther system automatically determines the test results for samples and controls. A test result may be negative, positive, or invalid.

The first valid result is the result that should be reported. Samples with invalid results should be retested. If the result is invalid upon retest, a new specimen should be collected.

Table 1 shows the possible results reported in a valid run with result interpretations.

Table 1: Result Interpretation

SARS-CoV-2 Result	IC Result	Interpretation
Neg	Valid	SARS-CoV-2 not detected.
POS	Valid	SARS-CoV-2 detected.
Invalid	Invalid	Invalid. There was an error in the generation of the result; retest sample.

Note: Detection of internal control is not required for samples that are positive for SARS-CoV-2.

Limitations

A. Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.

B. Accurate results are dependent on adequate specimen collection, transport, storage, and processing. Failure to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of false positive or false negative values resulting from improperly collected, transported, or handled specimens.

C. Avoid contamination by adhering to good laboratory practices and to the procedures specified in this package insert.

D. The Aptima SARS-CoV-2 Assay has not been validated for the testing of pooled specimens or the screening of specimens from asymptomatic individuals that do not have signs and symptoms of respiratory infection.

E. The performance of the Aptima SARS-CoV-2 Assay has not been specifically evaluated for NP and anterior nasal swab specimens from immunocompromised individuals.

F. Results should be interpreted by a trained professional in conjunction with the patient's history and clinical signs and symptoms, epidemiological risk factors, and other clinical and laboratory data available to the clinician.

G. A positive result indicates the detection of nucleic acid from SARS-CoV-2. Nucleic acid may persist *in vivo* even after SARS-CoV-2 is no longer viable. Detection of SARS-CoV-2 does not imply that the detected organisms are infectious or are the causative agent for clinical symptoms.

H. A negative test result does not preclude the possibility of infection should not be the sole basis of a patient treatment/management or public health decision.

I. Performance characteristics of the Aptima SARS-CoV-2 Assay have only been determined in the NP and AN swab specimens. Other specimen types have not been evaluated and should not be used with this assay. The performance of this device has not been specifically assessed in individuals without signs or symptoms of respiratory infection.

J. Detection of SARS-CoV-2 RNA may be affected by sample collection, patient factors (e.g. presence of symptoms), and/or stage of infection.

K. The substances tested in Table 8 have been evaluated for potential interference on assay performance. The impact of other substances has not been evaluated.

L. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the common variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2, and their prevalence, which change over time.

M. Positive and negative predictive values are highly dependent on prevalence. The likelihood of a negative result being false is higher during peak activity when prevalence of disease is high. The likelihood of a positive result being false is higher during periods when prevalence is moderate to low.

N. Viral culture should not be attempted in cases of positive results for SARS-CoV-2 and/or any similar microbial agents unless a facility with an appropriate level of laboratory biosafety (e.g, BSL 3 and BSL 3+, etc.) is available to receive and culture specimens.

Analytical Performance

Analytical Sensitivity

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 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 2 shows the lowest concentration of each strain in which 100% positivity was observed.

Table 2: 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

Table 2: Analytical Reactivity Summary for SARS-CoV-2 (continued)

Description	Concentration
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 (Table 3) 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 3: 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*	$1x10^6$ CFU/mL
		Streptococcus salivaris	$1x10^6$ 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.

Interference

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 4.

No impact on the performance of the Aptima SARS-CoV-2 Assay was seen for any of the substances at the concentration tested.

Table 4: 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 Rhinacort. 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 was assessed by testing high titer panels consisting of SARS-CoV-2 virus in negative clinical NP swab VTM/UTM matrix, spiked at 100 TCID50/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 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 5.

Table 5: 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 6. The High Negative panel member is 10x below the assay LoD and the %CV for this panel is expected to be higher thanthe others. The highest source of variability for this panel was within-run variability.

Table 6: 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 between the two collection devices (Table 7).

Table 7: 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 SARS-CoV-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 8).

Table 8: 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.

Clinical Performance

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 9.

Table 9: 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 10. 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 10: 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 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 anterior nasal swab specimens in eSTM.

Demographic information for the 2177 evaluable individuals is provided in Table 11.

Table 11: Summary of Subject Demographics for Prospectively Collected Anterior 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 12. PPA and NPA percent agreement were calculated as described for Clinical Study 1.

Table 12: 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.

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