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

For i*n vitro* diagnostic use.

For U.S. Export 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 SARS-CoV-2 isolated and purified from nasopharyngeal (NP), nasal, mid-turbinate and oropharyngeal (OP) swab specimens, nasopharyngeal wash/ aspirate or nasal aspirates obtained from individuals meeting COVID-19 clinical and/or epidemiological criteria.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory 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 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 Aptima SARS-CoV-2 assay on the Panther™ and Panther Fusion™ system is intended for use by clinical laboratory personnel specifically instructed and trained in the operation of the Panther and Panther Fusion systems and in vitro diagnostic procedures.

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 began in Wuhan, China, in December 2019.[1]

The most common symptoms of COVID-19 are fever, tiredness, and dry cough. Some patients may have aches and pains, nasal congestion, runny nose, sore throat, new loss of taste or smell, or diarrhea. These symptoms are usually mild and begin gradually. Some people become infected but don't develop any symptoms and don't feel unwell. The disease can spread through respiratory droplets produced when an infected person coughs or sneezes. These droplets can land in the mouths or noses of people who are nearby or possibly be inhaled into the lungs.[2] These droplets also can land on objects and surfaces around the person. Other people may acquire SARS-CoV-2 by touching these objects or surfaces, then touching their eyes, nose, or mouth.

The virus that causes COVID-19 is infecting people and spreading easily from person to person.[3] On March 11, 2020, the COVID-19 outbreak was characterized as a pandemic by the World Health Organization (WHO).[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. Carefully read this entire package insert and the *Panther/Panther Fusion System Operator's Manual*.

B. 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.

C. Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV. https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html.

D. 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]

E. If infection with SARS-CoV-2 is suspected based on current clinical screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.

F. Use only supplied or specified disposable laboratory ware.

G. Use appropriate personal protective equipment when collecting and handling specimens from individuals suspected of being infected with SARS-CoV-2 as outlined in CDC Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019 Novel Coronavirus (2019-nCoV).

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

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

J. Expiration dates listed on the Panther Fusion Specimen Lysis Tubes, Hologic Specimen Lysis Tubes, the Aptima Multitest Collection Kit, the Aptima Unisex Specimen Collection Kit and the Aptima Specimen Transfer 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.

K. 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.

L. 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.

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

N. Store assay components at the recommended storage condition. See *Reagent Storage and Handling Requirements (*page 5), and *Panther System Test Procedure* (page 12) for more information.

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

P. Avoid microbial and ribonuclease contamination of reagents.

Q. 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.

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

Note: Hazard Communication reflects the EU Safety Data Sheets (SDS) classification. For hazard communication information specific to your region, refer to the region specific SDS on the Safety Data Sheet Library at www.hologicsds.com.

Selection Reagent
BORIC ACID 1-5%
WARNING
H315 - Causes skin irritation

Reagent Storage and Handling Requirements

A. The following reagents are stable when stored at 2°C to 8°C (refrigerated):

Aptima SARS-CoV-2 Amplification Reagent

Aptima SARS-CoV-2 Enzyme Reagent

Aptima SARS-CoV-2 Probe Reagent

Aptima SARS-CoV-2 Internal Control

Aptima SARS-CoV-2 Positive Control

Aptima SARS-CoV-2 Negative Control

B. The following reagents are stable when stored at 2°C to 30°C:

Aptima SARS-CoV-2 Amplification Reconstitution Solution

Aptima SARS-CoV-2 Enzyme Reconstitution Solution

Aptima SARS-CoV-2 Probe Reconstitution Solution

Aptima SARS-CoV-2 Selection Reagent

C. The following reagents are stable when stored at 15°C to 30°C (room temperature):

Aptima SARS-CoV-2 Target Capture Reagent

Aptima Wash Solution

Aptima Buffer for Deactivation Fluid

Aptima Oil Reagent

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

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

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

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

H. Reagents stored on-board the Panther System have 72 hours of on-board stability.

I. 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.

J. 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).

K. Do not freeze the reagents.

Specimen Collection and Storage

Specimens - Clinical material collected from patient placed in an appropriate transport system. For the Aptima SARS-CoV-2 assay, this includes NP, nasal, midturbinate and OP swab specimens, or nasopharyngeal wash/aspirate and nasal aspirate specimen collection in viral transport medium (VTM/UTM), saline, Liquid Amies, or specimen transport medium (STM).

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.*

Swab Specimen Collection

Collect NP swab, nasal swab, and OP 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. Swab specimens may alternatively be added to saline, Liquid Amies or

STM. The Aptima Multitest Swab Specimen Collection Kit may be used for the collection of OP and nasal swab samples.

After collection, specimens collected in VTM/UTM can be stored at 2°C to 8°C up to 96 hours before transferring to the Specimen Lysis Tube or transfer tubes as described in the specimen processing section below. Remaining specimen volumes can be stored at ≤-70°C.

After collection, specimens in the Aptima Multitest Tube may be stored at 2°C to 30°C up to 6 days.

Note: *It is recommended that specimens transferred to the Aptima Multitest Tube are stored capped and upright in a rack.*

The following types of VTM/UTM can be used.

- Remel MicroTest M4, M4RT, M5 or M6 formulations
- Copan Universal Transport Medium
- BD Universal Viral Transport Medium

Note: *Do not use medium that may contain Guanidium thiocyanate or any guanidine-containing material.*

Nasopharyngeal Wash/aspirate and Nasal Aspirate Specimen Collection

Collect nasopharyngeal wash/aspirate and nasal aspirate specimens according to standard techniques.

Specimen Processing using the Panther Fusion Specimen Lysis Tube

A. Prior to testing on the Panther system, transfer 500 µL of the collected specimen* to a Panther Fusion Specimen Lysis Tube.

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

Note: *When using the Aptima SARS-CoV-2 uncapped tube assay software, prepare the Panther Fusion Specimen Lysis Tube as described below in Specimen* Processing using the Hologic Specimen Lysis Tube with Solid Cap*.*

Specimen Processing using the Hologic Specimen Lysis Tube with Solid Cap

A. Uncap the Hologic Specimen Lysis Tube and retain the cap.

B. Prior to testing on the Panther system, transfer 500 uL of the specimen to the Hologic Specimen Lysis Tube

C. It is recommended to recap the tube and gently invert three times to ensure viral inactivation and a homogeneous mixture.

D. To avoid contact with the top of the tube, loosen the cap and place the sample tube into the sample rack.

E. Remove and discard the cap. Inspect the sample tube. If bubbles are present, carefully remove from the sample tube (for example, use the tip of a sterile swab or similar method).

F. Place the rack retainer on the sample rack and load the rack into the instrument.

Note: *Specimen processing using the Hologic Specimen Lysis Tube is for use with the Aptima SARS-CoV-2 uncapped tube assay software.*

Specimen Processing using a Custom Specimen Lysis Tube

A. Using a sterile or non-sterile generic tube made of siliconized glass, polypropylene plastic or similar material that is 12 mm to 13 mm in outer diameter and 75 mm to 100 mm in height, aliquot 0.78 mL ± 0.07 mL of bulk STM into the tube using a pipet or repeat pipettor.

   Note: *If tubes are prepared prior to use, recap the tube and store at* 15°C to 30°C until use in specimen processing.

B. Uncap the custom Specimen Lysis Tube containing STM and retain the cap.

C. Prior to testing on the Panther system, transfer 500 uL of the specimen to the custom Specimen Lysis Tube containing STM.

D. It is recommended to recap the sample tube and gently invert three times to ensure viral inactivation and a homogeneous mixture.

E. To avoid contact with the top of the tube, loosen the cap and place the sample tube into the sample rack.

F. Remove and discard the cap. Inspect the sample tube. If bubbles are present, carefully remove from the tube (for example, use the tip of a sterile swab or similar method).

G. Place the rack retainer on the sample rack and load the rack into the instrument.

Note: *Specimen processing using the custom Specimen Lysis Tube is for use with the Aptima SARS-CoV-2 uncapped tube assay software.*

Specimen Processing using the Aptima Specimen Transfer Tube

A. Prior to testing on the Panther system, transfer 1 mL of the collected specimen* to an Aptima Specimen Transfer Tube**.

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

   ****Note:*** *Alternatively, an unused Aptima Multitest Tube or Aptima Unisex Tube can be used.*

B. Recap the Aptima Specimen Transfer Tube tightly.

C. Gently invert the tube 2 to 3 times to ensure complete mixture of the specimen.

   Note: *The Aptima Specimen Transfer Tube cannot be tested on a system using the Aptima SARS-CoV-2 uncapped tube assay software.*

Specimen Processing for Specimen Collected with the Aptima Multitest Collection Kit

A. After placing the collected specimen* into the Aptima Multitest Tube using the Aptima Multitest Collection Kit, no further processing is required.

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

Note: *On a system using the Aptima SARS-CoV-2 uncapped tube assay software, transfer the collected specimen from the Aptima Multitest Tube to a Hologic Specimen Lysis Tube or custom Specimen Lysis Tube as described in the specimen processing sections above.*

Sample Storage

A. Samples on board the Panther system may be archived for additional testing at a later time.

B. Storing samples before or after testing

1. Samples in the Aptima Multitest Tube, Aptima Specimen Tube, or Specimen Lysis Tube should be stored upright in the rack under the following condition:
   - 2°C to 30°C up to 6 days
2. The 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, specimen transport tubes must 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.

   Note: *The Fisherbrand™ VersaClosure™ tube closure should not be used to cover tubes for freezing or shipping.*

Specimen Transport

Maintain specimen storage conditions as described in the *Specimen Collection and Storage section on* page 6.

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-06419

250 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 250 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 250 test kit
AR	**Aptima SARS-CoV-2 Amplification Reconstitution Solution** *Aqueous solution containing preservatives.*	1 x 27.7 mL
ER	**Aptima SARS-CoV-2 Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 x 11.1 mL
PR	**Aptima SARS-CoV-2 Probe Reconstitution Solution** *Succinate buffered solution containing < 5% detergent.*	1 x 35.4 mL
S	**Aptima SARS-CoV-2 Selection Reagent** *600 mM borate buffered solution containing surfactant.*	1 x 108 mL
TCR	**Aptima SARS-CoV-2 Target Capture Reagent** *Buffered salt solution containing solid phase and capture oligomers.*	1 x 54 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
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 conductive, liquid sensing	10612513 (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-06420
Aptima Multitest Swab Specimen Collection Kit	PRD-03546
Aptima Specimen Transfer Kit	301154C
Aptima Specimen Transfer Kit - printable	PRD-05110
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
Hologic Specimen Lysis Tube, 100 each *tube contains 0.71 mL of STM with a solid cap*	PRD-06554
Hologic Specimen Lysis Tube, 1200 each *tube contains 0.71 mL of STM with a solid cap*	PRD-06660
Specimen Transport Medium, 1 bottle, 80 mL	PRD-04423
Specimen Transport Medium, 1 bottle, 120 mL	PRD-06657
Bleach, 5% to 7% (0.7M to 1.0M) sodium hypochlorite solution	—
Disposable gloves	—
Replacement non-penetrable caps	504415
Fisherbrand VersaClosure Tube Closures*, 1000 per pack **a single-use tube cover for the Hologic Specimen Lysis Tube (PRD-06554 only) after testing*	02-707

	Cat. No.
Replacement Caps for the 250-test kits	—
Amplification and Probe reagent reconstitution solutions	*CL0041 (100 caps)*
Enzyme Reagent reconstitution solution	*501616 (100 caps)*
TCR and Selection reagent	*CL0040 (100 caps)*

Optional Materials

	Cat. No.
Hologic Bleach Enhancer for Cleaning *for routine cleaning of surfaces and equipment*	302101
Tube rocker	—

Panther System Test Procedure

Note: *Refer to the Panther/Panther 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. To reconstitute Amplification, Enzyme, and Probe Reagents, combine the bottles of lyophilized reagent with the reconstitution solution. If refrigerated, allow the reconstitution solutions to reach room temperature before use.
   a. Pair each reconstitution solution with its lyophilized reagent. Ensure that the reconstitution solution and reagent have matching label colors before attaching the reconstitution collar.
   b. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired.
   c. Open the lyophilized reagent vial and firmly insert the notched end of the reconstitution collar into the vial opening (Figure 1, Step 1).
   d. Open the matching reconstitution solution, and set the cap on a clean, covered work surface.
   e. 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).

f. Slowly invert the assembled bottles. Allow the solution to drain from the bottle into the glass vial (Figure 1, Step 3).

g. Thoroughly mix the solution in the glass vial by swirling (Figure 1, Step 4).

h. Wait for the lyophilized reagent to go into solution, then invert the assembled bottles again, tilting at a 45° angle to minimize foaming (Figure 1, Step 5). Allow all of the liquid to drain back into the plastic bottle.

i. Remove the reconstitution collar and glass vial (Figure 1, Step 6).

j. Recap the plastic bottle. Record operator initials and reconstitution date on the label (Figure 1, Step 7).

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

Option: Additional mixing of the Amplification, Enzyme, and Probe Reagents using a tube rocker is allowed. The reagents may be mixed by placing the recapped plastic bottle on a tube rocker set to 20 RPM (or equivalent) for a minimum of 5 minutes.

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.*

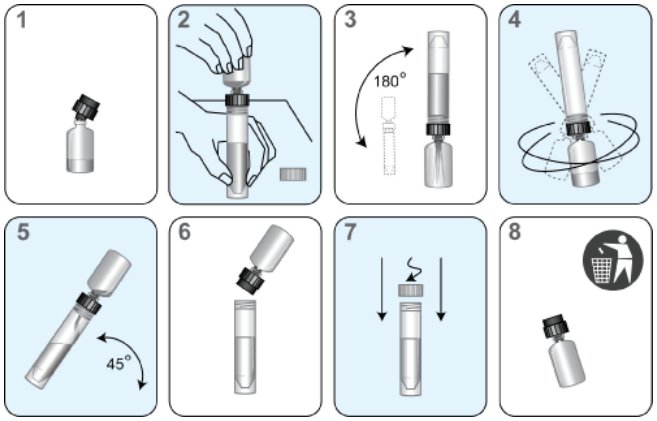

Figure 1. Panther System Reconstitution Process

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 reagents may be brought to room temperature by placing the reconstituted Amplification, Enzyme, and Probe Reagents on a tube rocker set to 20 RPM (or equivalent) for a minimum of 25 minutes.

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 using Panther Fusion Specimen Lysis Tube or Aptima Specimen Transfer Tube.

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 or the Aptima Specimen Transfer 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.*

E. Specimen Handling using Hologic Specimen Lysis Tube or custom Specimen Lysis Tube

1. Prepare specimens per the Specimen Processing instructions in the *Specimen Collection and Storage* section.

Note: *For samples transferred to the Hologic Specimen Lysis Tube or a custom 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 2 nucleic acid extractions*

Note: *When using the Aptima SARS-CoV-2 uncapped tube assay software, remove the cap from the Positive and Negative control before loading onto the Panther system.*

F. 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.

Table1 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. Reliable results are dependent on adequate specimen collection, transport, storage, and processing.

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

D. A positive result indicates the detection of nucleic acid from the relevant virus. Nucleic acid may persist even after the virus is no longer viable.

Panther SARS-CoV-2 Assay Performance

Analytical Sensitivity

The analytical sensitivity (limit of detection or LoD) of the Aptima SARS-CoV-2 assay was determined by testing serial dilutions of pooled negative clinical nasopharyngeal swab specimens spiked with inactivated cultured SARS-CoV-2 virus (USA-WA1/2020; BEI Resources; NR-52281). Ten replicates of each serial dilution were evaluated using each of two assay reagent lots across two Panther systems. The LoD was determined to be 0.01 $TCID_{50}$/mL and verified by testing an additional 20 replicates with one assay reagent lot. The LoD was also confirmed using saline, Liquid Amies and specimen transport medium (STM) swab collection media.

The analytical sensitivity of the Aptima SARS-CoV-2 assay was additionally evaluated using reference material from three commercial vendors. Serial dilutions of the reference material were made in STM and 20 or more replicates at each level were tested using each of two assay reagent lots across two Panther systems. The reference materials and the lowest dilution levels resulting in ≥ 95% detection are listed in Table 2.

Table 2: Analytical Sensitivity Evaluation of Commercial Reference Material

Vendor	Name	Reference #	Lot #	Analytical Sensitivity
ZeptoMetrix	SARS-CoV-2 External Run control	NATSARS(COV2)-ERC	324332	83 Copies/mL
SeraCare	AccuPlex SARS-Cov-2 Reference Material	0505-0126	10483977	83 Copies/mL
Exact Diagnostic	SARS-CoV-2 Standard	COV019	20033001	83 Copies/mL

Analytical Sensitivity with the Aptima Specimen Transfer Tube Workflow

The determined 0.01 $TCID_{50}$/mL analytical sensitivity (limit of detection) of the Aptima SARS-CoV-2 assay was confirmed using the Aptima Specimen Transfer tube specimen preparation workflow. Confirmation was performed using inactivated cultured SARS-CoV-2 virus (USA-QA1/2020; BEI Resources; NR-52281) in negative clinical nasopharyngeal (NP) swab, saline, Liquid Amies and specimen transport medium (STM) swab collection media by testing 20 replicates with one reagent lot (Table 3).

Table 3: LoD Confirmation with the Aptima Specimen Transfer Workflow

Target	Matrix	N Valid	N Positive	% Positive	Avg kRLU	StdDev kRLU	%CV
Inactivated SARS-CoV-2 virus	NP Swab	20	20	100%	1063	61	5.8%
	STM	20	20	100%	1064	116	10.9%
	Saline	20	20	100%	1102	60	5.4%
	Liquid Amies	20	20	100%	1101	51	4.7%

Inclusivity

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 in relation to 9,896 SARS-CoV-2 sequences available in the NCBI and GISAID gene databases. Any sequence with missing or ambiguous sequence information was removed from the analysis, resulting in 9,879 sequences evaluated for the first target region of the assay and 9,880 for the second target region. The *in silico* analysis showed 100% homology to the assay oligos of both target systems for 9,749 (98.5%) of the evaluated sequences and 100% homology to the assay oligos of at least one target system for all 9,896 sequences. There were no evaluated sequences with identified mismatches predicted to impact binding or performance of both target systems.

Analytical Specificity and Microbial Interference

The analytical specificity of the Aptima SARS-CoV-2 assay was evaluated by testing 30 microorganisms representing common respiratory pathogens or closely related species (Table 4). Bacteria were tested at 10^6 CFU/mL and viruses were tested at 10^5 $TCID_{50}$/mL, except where noted. Microorganisms were tested with and without the presence of SARS-CoV-2 inactivated virus at 3x LoD. Analytical specificity of the Aptima SARS-CoV-2 assay was 100% with no evidence of microbial interference.

In addition to microorganism testing, *in silico* analysis was performed to assess the specificity of the assay in relation to the microorganisms listed in Table 4. The *in silico* analysis showed no probable cross reactivity to any of the 112 GenBank sequences evaluated.

Table 4: Aptima SARS-CoV-2 Analytical Specificity and Microbial Interference Microorganisms

Microorganism	Concentration	Microorganism	Concentration
Human coronavirus 229E	1E+5 $TCID_{50}$/mL	Parainfluenza virus 1	1E+5 $TCID_{50}$/mL
Human coronavirus OC43	1E+5 $TCID_{50}$/mL	Parainfluenza virus 2	1E+5 $TCID_{50}$/mL
Human coronavirus HKU1[1]	1E+6 copies/mL	Parainfluenza virus 3	1E+5 $TCID_{50}$/mL
Human coronavirus NL63	1E+4 $TCID_{50}$/mL	Parainfluenza virus 4	1E+3 $TCID_{50}$/mL
SARS-coronavirus[1]	1E+6 copies/mL	Influenza A	1E+5 $TCID_{50}$/mL
MERS-coronavirus	1E+4 $TCID_{50}$/mL	Influenza B	2E+3 $TCID_{50}$/mL
Adenovirus (e.g. C1 Ad. 71)	1E+5 $TCID_{50}$/mL	Enterovirus (e.g. EV68)	1E+5 $TCID_{50}$/mL
Human Metapneumovirus (hMPV)	1E+6 $TCID_{50}$/mL	Rhinovirus	1E+4 $TCID_{50}$/mL
Respiratory syncytial virus	1E+5 $TCID_{50}$/mL	*Legionella pneumophila*	1E+6 CFU/mL
Chlamydia pneumoniae	1E+6 IFU/mL	*Mycobacterium tuberculosis*	1E+6 $TCID_{50}$/mL
Haemophilus influenzae	1E+6 CFU/mL	*Streptococcus pneumoniae*	1E+6 CFU/mL
Bordetella pertussis	1E+6 CFU/mL	*Streptococcus pyogenes*	1E+6 CFU/mL
Pneumocystis jirovecii (PJP)	1E+6 nuc/mL	*Streptococcus salivarius*	1E+6 CFU/mL
Candida albicans	1E+6 CFU/mL	*Mycoplasma pneumoniae*	1E+6 CFU/mL
Staphylococcus epidermidis	1E+6 CFU/mL	*Pseudomonas aeruginosa*	1E+6 CFU/mL
Pooled human nasal wash[2] - *to represent diverse microbial flora in human respiratory tract*	N/A		

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

[2] 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.

Clinical Performance

The clinical performance of the Aptima SARS-CoV-2 assay was evaluated in comparison to the Panther Fusion SARS-CoV-2 assay (Hologic, Inc.) using a panel of remnant clinical specimens. For the study, remnant clinical nasopharyngeal specimens were collected from US patients with signs and symptoms of respiratory infection.

The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) was calculated in relation to the Panther Fusion assay as the reference result, as shown in Table 5. The Aptima SARS-CoV-2 assay showed positive and negative agreements of 100% and 98.2%, respectively.

Nasopharyngeal wash/aspirate, nasal aspirates, nasal swabs and midturbinate nasal swabs are acceptable specimens to test for viral respiratory infections. However, performance with these specimen types has not been specifically evaluated with the Aptima SARS-CoV-2 assay.

Table 5: Aptima SARS-CoV-2 Clinical Agreement

		Panther Fusion SARS-CoV-2 Assay	
		Positive	Negative
Aptima SARS-CoV-2 Assay	Positive	50	1
	Negative	0	54

Positive Percent Agreement: (95% CI): 100% (92.9% – 100%)
Negative Percent Agreement: (95% CI): 98.2% (90.4% – 99.7%)
Overall Agreement: (95% CI): 99.0% (94.8% – 99.8%)

Clinical Performance with Contrived Panel

The clinical performance of the Aptima SARS-CoV-2 assay using the Aptima Specimen Transfer tube specimen preparation workflow was evaluated in comparison to a panel of contrived specimens. For the study, a panel of 115 remnant clinical nasopharyngeal specimens was tested using both the Panther Fusion Specimen Lysis Tube (Specimen Lysis Tube) and Aptima Specimen Transfer tube workflows. All specimens were collected from US patients with signs and symptoms of respiratory infection. The panel consisted of 65 SARS-CoV-2 positive and 50 SARS-CoV-2 negative specimens. Of the 65 positive specimens, 40 were at concentrations 0.5-2x LoD and 25 were at concentrations 3-5x LoD using inactivated cultured SARS-CoV-2 virus (USA-QA1/2020; BEI Resources; NR-52281) as the target.

The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) for both specimen preparation workflows were calculated in relation to the expected result of the contrived specimen panel, as shown in Table 6 for the Aptima Specimen Transfer Tube and Table 7 for the Specimen Lysis Tube. Detection characteristics for the contrived specimens were calculated by target concentration, as shown in Table 8. Both specimen preparation workflows showed 100% agreement for the evaluated panels.

Table 6: Performance of the Aptima Specimen Transfer Tube Workflow Relative to Expected Results

		Expected Result		
		Positive	Negative	Total
Aptima Specimen Transfer Result	Positive	65	0	65
	Negative	0	50	50
	Total	65	50	115

Overall Agreement: 100% (96.8% – 100%)
Positive Agreement: 100% (94.4% – 100%)
Negative Agreement: 100% (92.9% – 100%)

Table 7: Performance of the Specimen Lysis Tube Workflow Relative to Expected Results

		Expected Result		
		Positive	Negative	Total
Specimen Lysis Tube Result	Positive	65	0	65
	Negative	0	50	50
	Total	65	50	115

Overall Agreement: 100% (96.8% – 100%)
Positive Agreement: 100% (94.4% – 100%)
Negative Agreement: 100% (92.9% – 100%)

Table 8: Detection Characteristics for Contrived Nasopharyngeal Swab Specimens

	Aptima Specimen Transfer Sample Workflow						Specimen Lysis Tube Sample Workflow					
Target Conc.	**n Valid**	**n Positive**	**% Positive**	**Average kRLU**	**St Dev kRLU**	**%CV**	**n Valid**	**n Positive**	**% Positive**	**Average kRLU**	**St Dev kRLU**	**%CV**
Neg	50	0	0	299	9.7	3.2	50	0	0	300	9.3	3.1
0.5x LoD	10	10	100	1050	208.5	19.9	10	10	100	1153	113.0	9.8
1.0x LoD	10	10	100	1176	102.1	8.7	10	10	100	1205	24.3	2.0
1.5x LoD	10	10	100	1222	31.6	2.6	10	10	100	1223	21.9	1.8
2.0x LoD	10	10	100	1225	22.6	1.8	10	10	100	1237	26.0	2.1
3.0x LoD	10	10	100	1228	13.6	1.1	10	10	100	1215	25.5	2.1
4.0x LoD	5	5	100	1238	16.7	1.4	5	5	100	1212	12.5	1.0
5.0x LoD	10	10	100	1237	18.2	1.5	10	10	100	1246	28.3	2.3

Clinical Performance with Natural Infected Positive Specimens

The clinical performance of the Aptima SARS-CoV-2 assay using the Aptima Specimen Transfer tube specimen preparation workflow was evaluated in comparison to the Specimen Lysis Tube workflow tested with both the Aptima and Panther Fusion SARS-CoV-2 assays. For the study, three dilutions of 15 unique SARS-CoV-2 positive nasopharyngeal swab specimens were prepared and processed using both workflows. SARS-CoV-2 samples were previously determined to be positive using a non-Hologic molecular assay.

The positive percent agreement between the Aptima SARS-CoV-2 Assay using the Aptima Specimen Transfer Tube and the Specimen Lysis Tube workflows were 97.5% (87.1% – 99.6%) and 100% (91.0% – 100%), respectively, when compared to the Panther Fusion SARS-CoV-2 assay using the Specimen Lysis Tube workflow as reference. The positive percent agreement of the Aptima Specimen Transfer tube workflow was 95.0% (83.5% – 98.6%) when compared to the Specimen Lysis Tube workflow as reference.

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