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

For Emergency Use Authorization (EUA) only

For *in vitro* diagnostic use only

Rx only

CONTENTS

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) and oropharyngeal (OP) swab specimens, nasopharyngeal washes/aspirates or nasal aspirates (collected by a healthcare provider) and anterior nasal (nasal) and mid-turbinate nasal swab specimens (collected under observation of or by a healthcare provider) from individuals who meet COVID-19 clinical and/or epidemiological criteria, as well as NP and OP swab specimens (collected by a healthcare provider) and nasal and mid-turbinate nasal swab specimens (collected under observation of or by a healthcare provider) from any individual, including from individuals without symptoms or other reasons to suspect COVID-19. The Aptima SARS-CoV-2 assay is for use only under Emergency Use Authorization (EUA) in laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

This test is also for the qualitative detection of nucleic acid from SARS-CoV-2 in pooled samples containing up to 5 individual NP or OP swabs (collected by a healthcare provider), nasal, or mid-turbinate nasal swabs (collected under observation of or by a healthcare provider) where each specimen is collected using individual vials containing transport media. Negative results from pooled testing should not be treated as definitive. If a patient's clinical signs and symptoms are inconsistent with a negative result or if results are necessary for patient management, then the patient should be considered for individual testing. Specimens included in pools with a positive or invalid result must be tested individually prior to reporting a result. Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing. For specific patients, whose specimen(s) were the subject of pooling, a notice that pooling was used during testing must be included when reporting the result to the clinician or healthcare provider.

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. The agent detected may not be the definite cause of the disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

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 qualified clinical laboratory personnel specifically instructed and trained in the operation of the Panther and Panther Fusion systems and in vitro diagnostic procedures. The Aptima SARS-CoV-2 assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

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.[3] 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. 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 may be collected in UTM/VTM and then transferred into Hologic Panther Fusion lysis tubes containing specimen transport media (STM). Alternatively, samples can be collected with the Aptima Multitest Kit containing STM or RespDirect Collection Kit containing enhanced specimen transport media (eSTM). STM and eSTM lyse the cells, release target nucleic acid, and protects them from degradation during storage. 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. For use under an Emergency Use Authorization (EUA) only. For prescription use only. Carefully read this entire package insert and the *Panther/Panther Fusion System Operator’s Manual*.

B. The Aptima SARS-CoV-2 assay has not been FDA cleared or approved but has been authorized by FDA under an EUA for use by authorized laboratories.

C. The Aptima SARS-CoV-2 assay is for use only under Emergency Use Authorization (EUA) in laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

D. The emergency use of the Aptima SARS-CoV-2 assay is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

E. The Aptima SARS-CoV-2 assay has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.

F. 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.

G. 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.

H. 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]

I. 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.

J. Use only supplied or specified disposable laboratory ware.

K. 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).

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

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

N. Expiration dates listed on the RespDirect Collection Kit, the Panther Fusion Specimen Lysis Tubes (SLT), the Hologic Specimen Lysis Tubes, the Aptima Multitest Collection Kit, the Aptima Unisex Swab 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.

O. 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.

P. 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.

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

R. Store assay components at the recommended storage condition. See *Reagent Storage and Handling Requirements (*page 6), and *Panther System Test Procedure* (page 19) for more information.

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

T. Avoid microbial and ribonuclease contamination of reagents.

U. Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard quality control procedures.

V. 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.

W. Contamination may occur if carryover of samples is not adequately controlled during sample pool preparation, handling, and processing.

X. Testing of pooled specimens may impact the detection capability of the Aptima SARS-CoV-2 Assay and decrease sensitivity.

Y. A reagent in this kit is labeled with hazard information.

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%*** **WARNING** H315 - Causes skin irritation P264 - Wash face, hands and any exposed skin thoroughly after handling P280 - Wear protective gloves/protective clothing/eye protection/face protection P302 + P352 - IF ON SKIN: Wash with plenty of soap and water P332 + P313 - If skin irritation occurs: Get medical advice/attention P362 - Take off contaminated clothing and wash before reuse

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 120 hours of on-board stability.

Note: *Only Panther Systems with system software version 7.2.5 or higher support 120 hours of reagent on-board stability. Panther Systems with other system software versions support 72 hours of on-board reagent 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, mid-turbinate and OP swab specimens, or nasopharyngeal wash/aspirate and nasal aspirate specimen collection in viral transport medium (VTM/UTM), saline, Liquid Amies, or collected in eSTM with the RespDirect Collection kit, 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, Hologic Specimen Lysis Tube with solid cap, Custom Specimen Lysis Tube, Aptima Specimen Transfer Tube, Aptima Multitest Transport Tube, Hologic Direct Load Tube, Hologic Direct Load Capture Cap 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. The Hologic Direct Load Collection Kit may be used for the collection of OP and nasal swab samples. The Hologic Direct Load Capture Cap Collection Kit - CLASSIQSwab is for the collection of OP and nasal swab samples. The Hologic Direct Load Capture Cap Collection Kit - FLOQSwab is for the collection of mid-turbinate and NP swab samples. The Hologic RespDirect Collection Kit may be used for the collection of NP 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 (i.e., Panther Fusion Specimen Lysis Tube, Hologic Specimen Lysis Tube with solid cap, or Custom Specimen Lysis Tube) or Aptima Specimen Transfer Tube as described in the specimen processing section below. Remaining specimen volumes in VTM/UTM can be stored at ≤-70°C.

After collection, specimens in the Aptima Multitest Tube, the Hologic Direct Load Tube, the Hologic Direct Load Capture Cap Tube, and the Enhanced Direct Load 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, the Hologic Direct Load Tube, and the Hologic Direct Load Capture Cap 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

Nasopharyngeal Wash/aspirate and Nasal Aspirate Specimen Collection

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

Specimen Processing

Capped Workflow using Aptima SARS-CoV-2 Assay Software

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.*

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, Aptima Unisex Tube, or Hologic Direct Load 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.

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:** Allow specimen to reach room temperature prior to processing.*

Specimen Processing for Specimen Collected with the Hologic Direct Load Tube Collection Kit

A. After placing the collected specimen* into the Hologic Direct Load Tube, no further processing is required.

***Note:** Allow specimen to reach room temperature prior to processing.*

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

A. After collecting the specimen into the Enhanced Direct Load Tube (RespDirect Collection Kit), the specimen may be loaded onto the instrument.

***Note:** If CLT or isolated p-flags are observed in specimens, 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.

***Note:** When testing frozen specimen, allow specimen to reach room temperature prior to loading* onto the instrument.

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

Uncapped Workflow using Aptima SARS-CoV-2 Assay Software

Specimen Processing using the Panther Fusion Specimen Lysis Tube

A. Uncap the Panther Fusion Specimen Lysis Tube with penetrable cap. The penetrable cap can be retained or a replacement solid cap can be used in the next step.

B. Prior to testing on the Panther system, transfer 500 µL of the specimen to the Panther Fusion Specimen Lysis Tube, with penetrable cap or replacement solid cap.

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

D. Remove and discard the cap. To avoid contamination, do not pass the cap over any other sample racks or sample tubes. 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).

Note: *Failure to remove bubbles may affect assay processing and cause invalid results.*

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

Specimen Processing using the Hologic Specimen Lysis Tube with Solid Cap

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

B. Prior to testing on the Panther system, transfer 500 µL of the specimen to the Hologic Specimen Lysis Tube with solid cap.

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. To avoid contamination, do not pass the cap over any other sample racks or sample tubes. 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).

Note: *Failure to remove bubbles may affect assay processing and cause invalid results.*

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

Specimen Processing for Specimen Collected with the Hologic Direct Load Capture Cap Collection Kit - CLASSIQSwabs and the Hologic Direct Load Capture Cap Collection Kit - FLOQSwabs

A. After placing the collected specimen* into the Hologic Direct Load Capture Cap Tube, no further processing is required.

Note: *Allow specimen to reach room temperature prior to processing.*

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

C. Remove and discard the cap and swab. To avoid contamination, do not pass the cap over any other sample racks or sample tubes. 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).

Note: *If the swab wasn't captured by the cap, recap the tube to ensure that the swab is captured and removed from the tube. Direct Load Capture Cap tubes containing a swab should not be loaded into the Panther System.*

Note: *Failure to remove bubbles may affect assay processing and cause invalid results.*

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

Specimen Processing using a Custom Specimen Lysis Tube

A. Using a sterile, or non-sterile (unused) generic tube made of polypropylene plastic 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: *This step should be conducted in an area where SARS-CoV-2 specimens are NOT processed.*

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

Note: *When the filled Custom Specimen Lysis Tube is stored closed, if no contaminants were introduced during the filling of the Custom Specimen Lysis Tube, the STM should be stable until the expiration date provided for the STM.*

Note: *There may be an increased risk of contamination when using non-sterile (unused) tubes.*

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

C. Prior to testing on the Panther system, transfer 500 µL 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. To avoid contamination, do not pass the cap over any other sample racks or sample tubes. 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).

Note: *Failure to remove bubbles may affect assay processing and cause invalid results.*

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

Specimen Processing for Specimen Collected with the Aptima Multitest Collection Kit

A. Obtain and follow instructions for Panther Fusion Specimen Lysis Tube (Step A), Hologic Specimen Lysis Tube with Solid Cap (Step A), or Custom Specimen Lysis Tube (Step A-B).

B. Prior to testing on the Panther system, transfer 500 µL of the collected specimen from the Aptima Multitest Tube to a Panther Fusion Specimen Lysis Tube, 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 Transfer Tube, Panther Fusion Specimen Lysis Tube, Hologic Specimen Lysis Tube, Hologic Direct Load Tube, Hologic

Direct Load Capture Cap Tube, or Custom Specimen Lysis Tube should be stored upright in the rack under the following condition:

- 2°C to 30°C up to 6 days

2. For both capped and uncapped workflows, samples should be covered with a new, clean plastic film or foil barrier.
3. If assayed samples need to be frozen or shipped,
   - Capped workflows

     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.

   - Uncapped workflows

     Remove the solid cap and place a new solid 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: *Replacement tube closures and tube plugs should not be used to cover tubes when centrifuging, freezing, or shipping.*

C. Storing Specimens with the Enhanced Direct Load Tube (RespDirect Collection Kit)
   1. Samples can be stored under the following conditions:
      - 2°C to 30°C up to 6 days or
      - 2°C to 8°C, –20°C, and –70°C for up to 1 month. 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.*

Specimen Pooling - Determining Appropriate Strategy for Implementation and Monitoring

When considering specimen pooling, laboratories should evaluate the appropriateness of a pooling strategy based on the positivity rate in the testing population and the efficiency of the

pooling workflow. Refer to Appendix A of these Instructions for Use for additional information ***prior*** to implementation of specimen pooling.

Preparing Samples for Pooling

The following upper respiratory tract specimens authorized for use under the Emergency Use Authorization of the Aptima SARS-CoV-2 assay may be tested with sample pooling: nasopharyngeal, oropharyngeal, mid-turbinate, and nasal swab specimens collected into VTM/UTM, saline, Liquid Amies, and specimen transport media (STM). Only specimens collected into a single type of media may be combined for each sample pool. For example, specimens collected in VTM/UTM should not be combined into a pool with specimens collected in Liquid Amies. Additionally, both neat specimens (those not prepared with STM for testing) and specimens prepared with STM for testing may be included in sample pooling. Each sample pool must be comprised of only neat or only STM prepared specimens. Recommended sample pooling workflow options for different specimen types are provided below.

For Specimens Collected in VTM/UTM, Saline or Liquid Amies

Customers may choose from one of the following two options to perform specimen processing for pooled samples using Specimen Lysis Tubes with the Aptima SARS-CoV-2 assay.

Note: *Hologic testing was performed using pooled samples generated from samples collected in a single collection medium type (i.e., VTM/UTM). Combination of multiple transport media types (e.g., VTM/UTM, saline, and Liquid Amies) in a single pool has not been evaluated.*

Option 1:
Specimen Preparation Instructions for Neat Samples Pooled Directly into a Specimen Lysis Tube (Hologic SLT, Custom SLT, and Panther Fusion SLT)

Perform the following procedure when pooling specimens collected in VTM/UTM, saline, or Liquid Amies by transferring samples directly into a Specimen Lysis Tube (Hologic SLT, Custom SLT, or Panther Fusion SLT).

A. Determine the appropriate volume required from each individual specimen based on the pool size being implemented. The required specimen to STM ratio in the assay test sample must be maintained for sample pooling. For example, if a pool size of 5 specimens is being utilized, 100 µL of each individual specimen (500 µL total) is required.

B. Uncap the Specimen Lysis Tube and retain the cap.

C. Prior to testing on the Panther system, carefully transfer the determined volume of each individual specimen from the specimen collection container to the Specimen Lysis Tube.

D. Ensure homogeneous mixing of each prepared sample pool.

Note: Retain the individual specimens for additional testing, if required.

Option 2:
Specimen Preparation Instructions for Samples Pooled Prior to Transferring to a Specimen Lysis Tube (Hologic SLT, Custom SLT, and Panther Fusion SLT)

Perform the following procedure when pooling specimens collected in VTM/UTM, saline, or Liquid Amies, by pooling the samples prior to transferring into a Specimen Lysis Tube (Hologic SLT, Custom SLT, or Panther Fusion SLT).

A. Obtain a generic empty tube. This tube will not be loaded on the Panther System for testing.

B. Determine the appropriate volume required from each individual specimen based on the pool size being implemented.

   Note: *The total volume of the pooled specimens must be greater than 500* µL to support transfer into a Specimen Lysis Tube.

C. Prior to testing on the Panther system, carefully transfer *500* µL of the pooled specimens from the generic tube into a Specimen Lysis Tube (Hologic SLT, Custom SLT, or Panther Fusion SLT).

D. Ensure homogenous mixing of each prepared sample pool.

   Note: Retain the individual specimens for additional testing, if required.

Option 3:

Specimen Preparation Instructions for Specimens Previously Transferred into Specimen Lysis Tubes and Pooled into an Empty Tube

Perform the following procedure when pooling specimens from Specimen Lysis Tubes (Hologic SLT, Custom SLT, or Panther Fusion SLT) by transferring directly into an empty tube per specifications in the Panther or Panther Fusion System Operators Manual.

A. Obtain a Panther system compatible empty tube.

B. Determine the appropriate volume required from each individual specimen based on the pool size being implemented. Specimens previously transferred into a Specimen Lysis Tube do not require additional dilution with STM prior to testing.

   Note: *The recommended combined volume of each individual specimen is dependent upon the dimensions of tube being utilized. A Hologic representative can provide recommendations on minimum volume requirements for processing on the Panther system.*

C. Prior to testing on the Panther system, carefully transfer the determined volume of each individual specimen from the Specimen Lysis Tubes (Hologic SLT, Custom SLT, or Panther Fusion SLT) to the Panther system compatible empty tube.

D. Ensure homogeneous mixing of each prepared sample pool.

E. Retain the individual specimens for additional testing if required.

For Specimens Collected in Aptima Multitest Transport Tubes

Specimen Preparation Instructions for Samples Pooled Directly into a Generic Tube

Perform the following procedure when pooling specimens collected in Aptima Multitest Transport Tubes by transferring individual specimens directly into an empty tube per specifications in the *Panther or Panther Fusion System Operators Manual*.

A. Obtain a Panther system compatible empty tube.

B. Determine the appropriate volume required from each individual specimen based on the pool size being implemented. Specimens collected in an Aptima Multitest Transport Tube do not require additional dilution with STM prior to testing.

   Note: *The recommended combined volume of each individual specimen is dependent upon the dimensions of tube being utilized. A Hologic representative can provide recommendations on minimum volume requirements for processing on the Panther system.*

C. Prior to testing on the Panther system, carefully transfer the determined volume of each individual specimen from the Aptima Multitest Transport Tubes to the empty tube.

D. Ensure homogeneous mixing of each prepared sample pool.

E. Retain the individual specimens for additional testing if required.

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 filtered, liquid sensing, conductive, and disposable Not all products are available in all regions. Contact your representative for region-specific information.	901121 (10612513 Tecan) 903031 (10612513 Tecan) MME-04134 (30180117 Tecan) MME-04128
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
Hologic Direct Load Tube Collection Kit	PRD-06997
Hologic Direct Load Capture Cap Collection Kit - CLASSIQSwabs	PRD-06951
Hologic Direct Load Capture Cap Collection Kit - FLOQSwabs	PRD-06952
Hologic RespDirect Collection Kit	PRD-07788
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 Tubes, 100 each *tube contains 0.71 mL of STM with a solid cap (for uncapped workflow)*	PRD-06554
Specimen Transport Medium, 1 bottle, 80 mL (for uncapped workflow)	PRD-04423
Bleach, 5% to 8.25% (0.7M to 1.16M) sodium hypochlorite solution	—

	Cat. No.
Disposable powderless gloves	—
Hologic Solid Cap for use with PRD-06951* and PRD-06952*, 100 caps per bag **a single-use cover for the Direct Load Capture Cap (PRD-06951 and PRD-06952) after testing as part of the uncapped workflow*	PRD-07028
Hologic Flange Cap 12/13mm, natural	PRD-06850
Fisherbrand VersaClosure Tube Closures*, 1000 per pack **a single-use tube cover for the Hologic Specimen Lysis Tube (PRD-06554 only) after testing as part of the uncapped workflow*	02-707
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
Generic Sample Tube (for Custom SLT) **Size: 12 x 75 mm to 13 x 100 mm (including 12 x 100 mm, 13 x 75 mm, and 13 x 82 mm)* *Material: Polypropylene plastic* *Non-sterile (unused) or sterile* *Round, flat bottom, or conical (skirted conical)*	—
Multitube Vortex	102160G
Benchtop Vortex	—
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.*

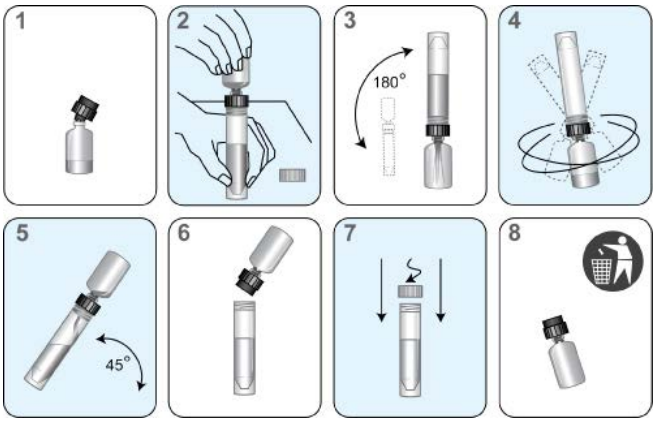

Figure 1. Panther System Reconstitution Process

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.*

Note: *When adequate collected specimen is added to the Hologic Specimen Lysis Tube (PRD-06554) or a custom Specimen Lysis 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.*

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

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.

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.

Interpretation of Results for Pooled Samples

Negative: Negative results from pooled sample testing should not be treated as definitive. If the patient's clinical signs and symptoms are inconsistent with a negative result and results are necessary for patient management, then the patient should be considered for individual testing. The utilization of sample pooling should be indicated for any specimens with reported negative results.

Positive: Specimens with a positive sample pool result must be tested individually prior to reporting a result. Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.

Invalid: Specimens with an invalid result must be tested individually prior to reporting a result. However, in instances of an invalid run, repeat testing of pooled specimens may be appropriate depending on the laboratory workflow and required result reporting time.

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. Negative results do not preclude SARS-CoV-2 infections and should not be used as the sole basis for treatment or other management decisions.

E. 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.

F. Nasopharyngeal wash/aspirate or nasal aspirates and self-collected under supervision of or healthcare provider collected nasal and mid-turbinate nasal swabs are additional acceptable upper respiratory specimens that can be tested with the Aptima SARS-CoV-2 assay; however, performance with these specimen types have not been determined.

G. Nasopharyngeal wash/aspirate and nasal aspirates specimen types should not be pooled.

H. Sample pooling has only been validated using nasopharyngeal swab specimens.

I. Samples should only be pooled when testing demand exceeds laboratory capacity and/or when testing reagents are in short supply.

J. The Aptima SARS-CoV-2 assay may be used to test asymptomatic individuals, although performance has not been demonstrated in an asymptomatic population. This assay has been shown to exhibit high sensitivity when tested with the FDA reference panel.

K. Use of the Aptima SARS-CoV-2 assay in a general, asymptomatic screening population is intended to be used as part of an infection control plan, that may include additional preventative measures, such as a predefined serial testing plan or directed testing of high-risk individuals. Negative results should be considered presumptive and do not preclude current or future infection obtained through community transmission or other exposures. Negative results must be considered in the context of an individual's recent exposures, history, and presence of clinical signs and symptoms consistent with COVID-19.

L. Asymptomatic individuals infected with COVID-19 may not shed enough virus to reach the limit of detection of the test, giving a false negative result.

M. In the absence of symptoms, it is difficult to determine if asymptomatic individuals have been tested too late or too early. Therefore, negative results in asymptomatic individuals may include individuals who were tested too early and may become positive later, individuals who were tested too late and may have serological evidence of infection, or individuals who were never infected.

N. The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent 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.

Conditions of Authorization for Labs

The Aptima SARS-CoV-2 assay Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas.

However, to assist clinical laboratories using the Aptima SARS-CoV-2 assay, the relevant Conditions of Authorization are listed below.

A. Authorized laboratories[1] using the Aptima SARS-CoV-2 assay must include with test result reports of the Aptima SARS-CoV-2 assay, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

B. Authorized laboratories using specimen pooling strategies when testing patient specimens with the authorized test must include with test result reports for specific patients whose specimen(s) were the subject of pooling, a notice that pooling was used during testing and that “Patient specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.”

C. Authorized laboratories using the Aptima SARS-CoV-2 assay must perform the Aptima SARS-CoV-2 assay as outlined in the Aptima SARS-CoV-2 assay Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to perform the Aptima SARS-CoV-2 assay are not permitted.

D. Authorized laboratories implementing pooling strategies for testing patient specimens must use the “Appendix A: Specimen Pooling Implementation and Monitoring Guidelines” available in the authorized labeling to evaluate the appropriateness of continuing to use such strategies based on the recommendations in the protocol.

E. Authorized laboratories that receive the Aptima SARS-CoV-2 assay must notify the relevant public health authorities of their intent to run the test prior to initiating testing.

F. Authorized laboratories using the Aptima SARS-CoV-2 assay must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

G. Authorized laboratories must keep records of specimen pooling strategies implemented including type of strategy, date implemented, and quantities tested, and test result data generated as part of the Protocol for Monitoring of Specimen Pooling Strategies. For the first 12 months from the date of their creation, such records will be made available to FDA within 48 business hours for inspection upon request, and will be made available within a reasonable time after 12 months from the date of their creation.

H. Authorized laboratories must collect information on the performance of the test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Hologic (molecularsupport@hologic.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.

I. All laboratory personnel using the test must be appropriately trained in Transcription Mediated Amplification techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.

J. Hologic, its authorized distributor(s) and authorized laboratories using the Aptima SARS-CoV-2 assay must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1] The letter of authorization refers to "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests" as "authorized laboratories."

Aptima Assay Performance

Analytical Sensitivity

The analytical sensitivity (limit of detection or LoD) of the Aptima SARS-CoV-2 assay using the Panther Fusion Specimen Lysis Tube workflow was determined by testing serial dilutions of processed negative clinical nasopharyngeal swab UTM/VTM specimens 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 using 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 20 replicates with one assay reagent lot. The LoD was also confirmed using saline, Liquid Amies and specimen transport medium (STM) swab collection media. 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 (224 IU/mL in the neat, unprocessed sample). LoD confirmation was also performed with the RespDirect Collection Kit at 24 replicates with a single reagent lot and ≥95% detection was observed at 27.7 IU/mL.

The analytical sensitivity of the Aptima SARS-CoV-2 assay was additionally evaluated using nucleic acid materials from three commercial vendors. Serial dilutions of the nucleic acid materials were made in Aptima Multitest tubes (containing STM and 10,000 copies/mL HeLa cells) and 20 or more replicates at each level were tested using each of two assay reagent lots across two Panther systems. For each material, the lowest dilution resulting in ≥ 95% detection was 83 copies/mL in the test sample. A test sample concentration of 83 copies/mL correlates to 323.7 copies/mL of material added to the Aptima Multitest tube or 212.5 copies/mL of material added to a neat, unprocessed Specimen Lysis Tube (SLT). The nucleic acid materials and the performance of the 83 copies/mL dilution are listed in Table 2.

Table 2: Analytical Sensitivity Evaluation of Commercial Material

Vendor and Name	Reference # and Lot #	N Valid	N Positive	% Positive	Avg kRLU	StdDev kRLU	% CV
ZeptoMetrixSARS-CoV-2 External Run Control	NATSARS(COV2)-ERC 324332	40	39	98%	965	212	21.90%
SeraCare SARS-Cov-2 Reference Material	0505-0126 10483977	40	40	100%	728	38	5.30%
Exact Diagnostics SARS-CoV-2 Standard	COV019 20033001	40	40	100%	1032	152	14.70%

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The study was performed on the fully automated Panther system. The results are summarized in Table 3.

Table 3: Summary of LoD Confirmation Results using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	NP Swabs in VTM/UTM	600 NDU/mL	N/A
MERS-CoV		N/A	ND

NDUmL = RNA NAAT detectable units/mL.
N/A = Not applicable.
ND = Not detected.

Analytical Sensitivity with the Aptima Specimen Transfer Tube Workflow

The determined 0.026 $TCID_{50}$/mL analytical sensitivity (limit of detection) of the Aptima SARS-CoV-2 assay in the neat, unprocessed sample 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.

Precision/Reproducibility

The Aptima SARS-CoV-2 assay precision/reproducibility was evaluated on three Panther systems at a single site using four panel members. Testing was performed using three lots of assay reagents with two operators over six days. 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.

Positive and negative panel members consisted of pooled clinical nasopharyngeal (NP) swab matrix combined with Solution Transport Media (STM) in a ratio of 1:1.56. Positive panel members spiked with inactivated cultured SARS-CoV-2 virus at 0.1x LoD (High Negative), 1x LoD (Low Positive) and 5x LoD (Moderate Positive).

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 gave 68/108 (63%) positive results. Agreement with expected results for all four panels is shown in Table 4.

Table 4: 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	0.1x LoD	0.001	N/A	68	108	627	N/A
Low Positive	1.0x LoD	0.01	Positive	108	108	1131	100% (96.6-100)
Moderate Positive	5.0x LoD	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 5. The High Negative panel member is 10x below the assay LoD and the %CV for this panel is expected to be higher than the others. The highest source of variability for this panel was within-run variability.

Table 5: 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 (%)	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.

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 49,741 SARS-CoV-2 sequences available in the NCBI and GISAID gene databases as of July 16th, 2020. Any sequence with missing or ambiguous sequence information was removed from the analysis for that region, resulting in 49,659 sequences evaluated for the first target region of the assay and 49,514 for the second target region. The *in silico* analysis showed 100% homology to the assay oligos of both target systems for 48,180 (96.9%) of the evaluated sequences and 100% homology to the assay oligos of at least one target system for 49,730 (99.98%) of 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 6). 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 6. The *in silico* analysis showed no probable cross reactivity to any of the 112 GenBank sequences evaluated.

Table 6: 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.

Carryover Contamination

The carryover contamination rate of the Aptima SARS-CoV-2 assay for samples tested with the capped tube and uncapped tube workflows was determined. The evaluation consisted of testing high titer SARS-CoV-2 target panels spiked at over 10,000 times the assay LoD in a checkerboard pattern with negative panels in four runs on three Panther systems. The capped tube workflow had an observed carryover rate of 0%, whereas the uncapped tube workflow carryover rate was 0.67% with 5 of 744 negative samples evaluated giving a false positive result.

Uncapped Workflow Performance

The performance of the Aptima SARS-CoV-2 assay using the uncapped tube workflow was evaluated under variable factors including multiple operators, runs per day, and testing days. The uncapped workflow was evaluated with two tube types prefilled to 0.780 mL with specimen transport medium (STM) as representative of Hologic supplied Specimen Lysis Tubes (SLTs) and with two empty tube types as representative of custom SLTs. A variety of tube sizes covering the recommended tube dimensions and materials were included in the evaluation. The workflow evaluation included both high concentration SARS-CoV-2 positive and negative panels processed using the uncapped tube workflow.

The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) was calculated in relation to the expected result of the panel, as shown in Table 7. The uncapped tube workflow showed positive and negative agreements of 100% (99.4% - 100%) and 99.7% (98.9% - 99.9%) respectively.

Table 7: Aptima SARS-CoV-2 Uncapped Workflow Performance

		Expected SARS-CoV-2 Result		
		Positive	Negative	Total
Aptima SARS-CoV-2 Result	Positive	639	2	641
	Negative	0	638	638
	Total	639	640	1279

Overall Agreement: 99.8% (99.4% - 100%)
Positive Agreement: 100% (99.4% - 100%)
Negative Agreement: 99.7% (98.9% - 99.9%)

Uncapped Tube Workflow Specimen and STM Volume Guardbands

The performance of the Aptima SARS-CoV-2 assay was evaluated under specimen and STM volume variance conditions of up to ±20% of those specified for processing specimens with the custom SLT workflow. Positive SARS-CoV-2 positive panels at the LoD of the assay (i.e.,0.010 $TCID_{50}$/mL) and negative panels were evaluated at each volume guardband variance condition. Agreement to expected result was 100% for the Aptima SARS-CoV-2 assay.

Collection Device Equivalency

Equivalence between NP specimens collected into VTM/UTM and NP and NS specimens collected into RespDirect (eSTM) was evaluated by testing individual negative specimens and contrived positive panels prepared from paired negative clinical NP and NS swab specimens collected from patients with symptoms of respiratory infection. Contrived panels were prepared by spiking individual donor paired NP and NS specimens with WHO International Standard for SARS-CoV-2 at 2X and 5X LoD.

The results of the negative and contrived panels demonstrated similar agreement between the two collection devices and specimen types (Table 8).

Table 8: Results for negative and contrived panels composed of paired individual clinical specimens, collected with each collection device spiked with SARS-CoV-2

Analyte	Sample Concentration	N per Collection Device	VTM/UTM % Agreement	RespDirect-NP % Agreement	RespDirect-NS % Agreement
None (Negative Sample)	0	150	99.3	97.3	100
SARS-CoV-2	2X LoD	50	100	100	100
	5X LoD	50	100	100	100

Clinical Performance

Clinical Performance in Nasopharyngeal Swab Specimens using UTM/VTM

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 9. The Aptima SARS-CoV-2 assay showed positive and negative agreements of 100% and 98.2%, respectively.

Nasopharyngeal wash/aspirate, nasal aspirates, nasal swabs (self collected under supervision of, or healthcare provider-collected) and mid-turbinate 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 9: 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

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

Clinical Performance in Anterior Nasal Swab Specimens Collected Using the RespDirect Collection Kit

The clinical performance of the Aptima SARS-CoV-2 assay in anterior nasal swab (ANS) specimens collected using the RespDirect collection swab in enhanced specimen transport medium (eSTM) from individuals who reported symptoms of respiratory infection consistent with COVID-19 was evaluated in this multicenter study. Two specimens were prospectively collected from each subject, one specimen in viral transport medium (VTM) collected by a qualified healthcare professional (HCP) using a standard flocked swab and one specimen in RespDirect eSTM collected by either the HCP or the patient (under HCP supervision) using the RespDirect collection swab. All the ANS swab specimens included in this study were collected between January 2023 and February 2023.

All ANS specimens in RespDirect eSTM were tested with the Aptima SARS-CoV-2 assay at three US clinical testing sites. All ANS specimens in VTM were tested with two EUA NAATs to establish the SARS-CoV-2 infected status based on a composite comparator algorithm. Any sample positive by either comparator assay yielded a positive SARS-CoV-2 infected status; both comparator assay results had to be negative to yield a negative SARS-CoV-2 infected status. Positive (PPA) and negative (NPA) percent agreement were calculated relative to the SARS-CoV-2 infected status.

The overall PPA and NPA were 96.1% and 97.1%, respectively, for the Aptima SARS-CoV-2 assay in ANS specimens collected in RespDirect eSTM from symptomatic individuals, as shown in Table 10. Ct values for the ANS swab samples with positive SARS-CoV-2 infected status ranged between 18.18 and 35.71 (mean: 27.14) for NAAT 1 and 15.3 and 44.5 (mean: 26.50) for NAAT 2. The five ANS specimens with false positive results were not retested with an alternate NAAT.

Table 10: Clinical Performance in ANS Specimens in RespDirect eSTM

		SARS-CoV-2 Infected Status	
		Positive	**Negative**
Overall	**Positive**	49	5
	Negative	2	169
	PPA: 96.1% (86.8% - 98.9%) NPA: 97.1% (93.5% - 98.8%)		

Clinical Performance with Contrived Panels

The clinical performance of the Aptima SARS-CoV-2 assay using the Aptima Specimen Transfer tube specimen preparation workflow was evaluated by testing 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. Due to limitations in available clinical specimens, reduced specimen/media volumes were utilized, while maintaining the Aptima Specimen Transfer workflow ratio of 1 mL specimen into 2.9 mL STM.

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 11 for the Specimen Lysis Tube and Table 12 for the Aptima Specimen Transfer Tube. Detection characteristics for the contrived specimens were calculated by target concentration, as shown in Table 13, in addition to a breakdown of the positive specimens by concentration in relation to the assay LoD. Both specimen preparation workflows showed 100% agreement for the evaluated panels.

Table 11: 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 12: 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 13: 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 Naturally Infected Positive Specimens

The clinical performance of the Aptima SARS-CoV-2 assay using the Aptima Specimen Transfer (AST) tube specimen preparation workflow was evaluated in comparison to the Specimen Lysis Tube (SLT) 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 AST tube and the SLT 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 SLT workflow as reference (Table 10). The positive percent agreement of the Aptima Specimen Transfer tube workflow was 95.0% (83.5% – 98.6%) when compared to the SLT workflow as reference Table 14.

Table 14: Aptima Specimen Transfer Tube and Specimen Lysis Tube Workflow Comparison with Positive Specimens

	AST workflow tested with the Aptima assay as compared to the SLT workflow tested with the Panther Fusion assay	SLT workflow tested with the Aptima assay as compared to the SLT workflow tested with the Panther Fusion assay	AST workflow tested with the Aptima assay as compared to the SLT workflow tested with the Aptima assay
N Positive	39	39	38
N Negative	1[1]	0	2[2]
N Total	40	39	40
% Agreement	97.5% (87.1% - 99.6%)	100% (91.0% - 100%)	95.0% (83.5% - 98.6%)

[1] Specimen was negative in the AST workflow tested with the Aptima assay and positive in the SLT workflow tested with the Panther Fusion assay. The same specimen was also positive in the SLT workflow tested with the Aptima assay.

[2] Specimens were negative in the AST workflow tested with the Aptima assay and positive with the SLT workflow tested with the Aptima assay. One of these specimens was positive with the SLT workflow tested with the Panther Fusion assay and the other was negative.

Uncapped Workflow Clinical Performance

The clinical performance of the Aptima SARS-CoV-2 assay using the uncapped tube workflow in comparison to the capped tube workflow was evaluated using natural clinical specimens. The evaluation included testing 200 SARS-CoV-2 negative, and 50 SARS-CoV-2 positive specimens. Two test samples were prepared for each specimen, one for the capped tube workflow and one for the uncapped tube workflow.

The observed Positive Percent Agreement (PPA) between specimens tested using the uncapped tube workflow as compared to the same specimens tested using the capped workflow was 98.0% (89.7% - 99.7%). The Negative Percent Agreement (NPA) was 100% (98.1% - 100%). Refer to Table 15.

Table 15: Uncapped Clinical Agreement to Capped Results

		Capped Results		
		Positive	Negative	Total
Uncapped Results	Positive	50	0	50
	Negative	1	199	200
	Total	51	199	250

Overall Agreement: 99.6% (97.8% – 99.9%)
Positive Agreement: 98.0% (89.7% – 99.7%)
Negative Agreement: 100% (98.1% – 100%)

Clinical Performance of Pooling up to 5 Specimens Prior to Testing

The clinical performance of the Aptima SARS-CoV-2 assay was evaluated in pools consisting of up to 5 specimens. For the study, a pool size of 5 specimens was evaluated and included positive and negative specimen pools. Each positive specimen pool consisted of one positive specimen with the remaining specimens being negative, whereas the negative specimen pools consisted only of negative specimens. For the study, 50 positive and 20 negative specimen pools were evaluated. The positive specimens used in the study covered the detectable range of the assay and included low positive specimens. Specimens for inclusion in the clinical performance of pooling study were chosen based on Ct results obtained with the Panther Fusion SARS-CoV-2 assay. The Panther Fusion SARS-CoV-2 assay was used for this purpose because the Panther Fusion SARS-CoV-2 and Aptima SARS-CoV-2 assays have the same LoD when evaluated with the FDA reference panel (i.e., 600 NDU/mL). Low positive specimens included in the study were defined as having a Ct value within 1-2 Ct of the LoD of the Panther Fusion SARS-CoV-2 assay. Both the pooled and individual specimens were evaluated with the Aptima SARS-CoV-2 assay.

The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were calculated in relation to the expected (individual) result, as shown in Table 16. All evaluated positive specimens yielded a positive result in the pool. Since the kRLU values for the Aptima assay do not correspond to target concentration, signal and in silico sensitivity analysis was not performed.

Table 16: Individual and Pooled Specimen Agreement with a Pool Size of 5

		Individual Specimen Result		
		Positive	Negative	Total
Pool of 5 Result	Positive	50	0	50
	Negative	0	20	20
	Total	50	20	70

Overall Agreement: 100% (94.8% – 100.0%)
Positive Agreement: 100% (92.9% – 100.0%)
Negative Agreement: 100% (83.9% – 100.0%)

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Appendix A: Specimen Pooling Implementation and Monitoring Guidelines

Before Implementation of Pooling: Determine Appropriate Pool Size

Before a pooling strategy is implemented, a laboratory should determine the appropriate pool size based on percent positivity rate and desired testing efficiency. The Aptima SARS-CoV-2 assay has been validated for n-sample pool sizes up to five samples per pool.

If historical laboratory data for individual specimens is available:

- If historical data for individual specimens from the previous 7-10* days is available, estimate the percent positivity rate ($P_{individual}$) based on individual results.

  ($P_{individual}$) = (Number of positive specimen over chosen date range ÷ Total number of specimen tested over chosen date range)*100.

- Using the calculated $P_{individual}$ and Table 17, identify the appropriate *n* number of samples to pool.

  - If $P_{individual}$ is less than 5%, the maximum pool size validated, (n=5), should be selected to maximize the efficiency of specimen pooling. Pooling with greater than 5 samples has not been validated and should not be performed.

  - If $P_{individual}$ is greater than 25%, Dorfman pooling of patient specimens is not efficient and should not be implemented.

If historical laboratory data for individual specimens is unavailable:

- If historical data from the previous 7-10* days is unavailable, 5, 4, or 3-specimen pooling may still implemented as the Aptima SARS-CoV-2 assay has been validated for 5-specimen pooling.

- Note: Without calculating $P_{individual}$ the pooling size implemented may not maximize pooling efficiency.

Table 17: Result Interpretation

P, percent of positive subjects in the tested population	$n_{maxefficiency}$ ***(n corresponding to the maximal efficiency)***	***Efficiency of n-sample pooling*** ***(a maximum increase in the number of tested patients when Dorfman n-pooling strategy used)***
5%-6%	5	2.15-2.35
7%-12%	4	1.54-1.99
13%-25%	3	1.10-1.48

Because a positive pool requires individual retesting of each sample in the pool, the efficiency of any pooling strategy depends on the positivity rate. The efficiency (F) of n-sample pooling for positivity rate (P) can be calculated with the following formula $F=1/(1+1/n-(1-P)^n)$. The efficiency (F) indicates how many more patients can be tested with n-sample pools compared to individual testing. For example, a 5-sample pooling strategy increases the number of tested patients by 2.15 times for positivity rate P of 6% (F=2.15). At F=2.15, 1,000 tests can on average cover testing of 2,150 patients.

Implementation of Pooling

See above section titled *Specimen Pooling: Preparing Samples for Pooling* and perform pooling procedure as outlined.

After Implementation of Pooling: Ongoing Monitoring of Pooling Strategy

If historical laboratory data for individual specimens is available:

- After implementing a pooling strategy, evaluate the performance of pooled testing by comparing the percent positivity rate of pooled testing to that of individual testing.
- Calculate the percent positivity rate among patient specimens during specimen pooling (P_{pools}) on a daily basis using a moving average of the data from the previous 7-10* days of testing.

  (P_{pools}) = (Number of patient specimens with a positive result as determined by individual specimen reflex testing of positive pools over chosen date range ÷ Total number of patient specimens tested in pools over chosen date range)*100
- Compare P_{pools} to $P_{individual}$. If P_{pools} is less than 85% of $P_{individual}$. ($P_{pools} < 0.85 \times P_{individual}$), it is recommended that the pool size be reassessed and adjusted to maximize pooling efficiency (if necessary), according to the criteria in Table 17.
- To ensure maximum pooling efficiency, it is recommended that nmaxeffiency be reaccessed periodically while sample pooling is implemented by the laboratory.

If historical laboratory data for individual specimens is unavailable:

- After initiating a pooling strategy, evaluate the performance of pooled testing by calculating the initial percent positivity rate for pooled specimens ($P_{pools\text{-}initial}$). ($P_{pools\text{-}initial}$ is the percent positivity rate for pooled specimens for the first 7-10* days of pooled testing.
- Calculate the initial percent positivity rate for individual specimens from pool testing ($P_{pools\text{-}initial}$) from the first 7-10* days of testing.

  $P_{pools\text{-}initial}$ = (Number of patient specimens with a positive result as determined by individual specimen reflex testing of positive pools in first 7-10* days ÷ Total number of patient specimens tested in pools in the first 7-10* days)*100
  - If $P_{pools\text{-}initial}$ is greater than 25%, pooling of patient specimens is not efficient and should be discontinued until the percent positivity rate decreases.
  - If $P_{pools\text{-}initial}$ is less than or equal to 25%, pooling of patient specimens can be continued.
- Continue to monitor pooling strategy by calculating the percent positivity rate among patient specimens during specimen poling (P_{pools}-x) for subsequent 7-10* day periods. (P_{pools}-x) should be updated daily using a moving average.

- Compare $P_{pools\text{-}x}$ to $P_{pools\text{-}initial}$. If $P_{pools\text{-}x}$ is less than 90% of $P_{pools\text{-}initial}$ ($P_{pools\text{-}x} < 0.90 \times P_{pools\text{-}initial}$), it is recommended that the pool size be reassessed and potentially adjusted to maximize pooling efficiency.

- To ensure maximum pooling efficiency, it is recommended that $n_{maxefficiency}$ be reassessed periodically while sample pooling is implemented by the laboratory.

*7-10 days is recommended for calculating $P_{individual}$, P_{pools}, $P_{pools\text{-}initial}$, and $P_{pools\text{-}x}$. Laboratories should determine if 7-10 days is appropriate by taking into consideration laboratory testing volume and percent positivity. If the number of individual or pooled positive results collected during a given time frame is less than 10, $P_{individual}$, P_{pools}, $P_{pools\text{-}initial}$, and $P_{pools\text{-}x}$ may not be representative of the percent positivity in the testing population. Consider extending the data collection time period to increase the number of positives evaluated.