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

For Emergency Use Authorization (EUA) only

For *in vitro* diagnostic use only

Rx Only

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General Information

Intended Use

The Aptima SARS-CoV-2/Flu assay is an automated multiplexed target nucleic acid amplification test intended for the simultaneous *in vitro* qualitative detection and differentiation of RNA from SARS-CoV-2 virus, influenza A virus (Flu A) and/or influenza B virus (Flu B) isolated and purified from clinician-collected nasopharyngeal (NP) and anterior nasal (nasal) swab specimens and patient-collected nasal swab specimens in a health care setting, obtained from individuals suspected of respiratory viral infection consistent with COVID-19 by their healthcare provider.

Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2 and influenza can be similar. The Aptima SARS-CoV-2/Flu 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.

Results are for the identification of SARS-CoV-2, Flu A and/or Flu B RNA. The Aptima SARS-CoV-2/Flu assay is not intended to detect influenza C. SARS-CoV-2, Flu A and Flu B RNA are generally detectable in nasopharyngeal (NP), and anterior nasal (nasal) swab specimens during the acute phase of infection. Positive results are indicative of active infection but do not rule out bacterial infection or co-infection with other pathogens not detected by the test. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all SARS-CoV-2 results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2, Flu A, or Flu B 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/Flu assay on the Panther® and Panther Fusion® system is intended for use by clinical laboratory personnel specifically instructed and trained in the operation of the Panther and Panther Fusion systems and *in vitro* diagnostic procedures. The Aptima SARS-CoV-2/Flu assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

Summary and Explanation of the Test

Influenza (flu) and COVID-19 are both contagious respiratory illnesses, but they are caused by different viruses. COVID-19 is caused by infection with a new coronavirus (called SARS-CoV-2) and flu is caused by infection with influenza viruses. Because some of the symptoms of flu and COVID-19 are similar, it may be hard to tell the difference between them based on symptoms alone.[1]

Flu is a contagious respiratory illness caused by influenza viruses. It can cause mild to severe illness. Serious outcomes of flu infection can result in hospitalization or death. Some people, such as older people, young children, and people with certain health conditions, are at high risk of serious flu complications. There are two main types of flu virus: types A and B. Flu A and B viruses that routinely spread in people (human influenza viruses) are responsible for seasonal flu epidemics each year.[2]

Flu signs and symptoms usually come on suddenly. People who are sick with flu may experience fever or feeling feverish/chills, cough, sore throat, runny or stuffy nose, muscle or body aches, headaches, fatigue, and some people may have vomiting and diarrhea, though this is more common in children than adults.[3]

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 outbreak in Wuhan, China, in December 2019.[3]

People with COVID-19 have had a wide range of symptoms reported, ranging from mild symptoms to severe illness. Symptoms may appear 2-14 days after exposure to the virus. People with COVID-19 may exhibit fever or chills, cough, shortness of breath or difficulty breathing, fatigue, muscle or body aches, headache, new loss of taste or smell, sore throat, congestion or runny nose, nausea or vomiting, and/or diarrhea.[5]

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).[3,5]

Principles of the Procedure

The Aptima SARS-CoV-2/Flu assay combines the technologies of target capture, Real-Time Transcription Mediated Amplification (RT-TMA), and real time detection of amplicons using fluorescently labeled torches.

Specimens are collected and transferred into their respective specimen transport tubes. The transport solutions in these tubes release the RNA target and protect them from degradation during storage. When the Aptima SARS-CoV-2/Flu assay is performed in the laboratory on the Panther system, an Internal Control (IC) nucleic acid is added to each specimen reaction, and the IC along with 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 and IC nucleic acid strands. The Aptima SARS-CoV-2/Flu assay replicates specific regions of the RNA from SARS-CoV-2, Flu A and Flu B via DNA intermediates. Detection is achieved using single-stranded nucleic acid torches that are present during the amplification of the target and that hybridize specifically to

the amplicon in real-time. Each torch has a fluorophore and a quencher. When the torch is not hybridized to the amplicon, the quencher is in close proximity of the fluorophore and suppresses the fluorescence. When the torch binds to the amplicon, the quencher is moved farther away from the fluorophore and it will emit a signal at a specific wavelength when excited by a light source. As more torch hybridize to amplicon, a higher fluorescent signal is generated. The fluorophores associated with the viral targets and IC targets emit light at different wavelengths, thus allowing these targets to be distinguished from one another. The fluorescent signals generated by the amplification are measured by fluorometers then used by the system to generate qualitative results.

The Aptima SARS-CoV-2/Flu assay amplifies and detects two conserved regions of the ORF1ab gene in the same reaction for SARS-CoV-2, one region of the Matrix gene for Flu A, and one region of the Matrix gene for Flu B. For detection, both SARS-CoV-2 gene targets are reported into the FAM fluorescent channel, the Flu A target is reported into the ROX fluorescent channel, and the Flu B target is reported into the HEX fluorescent channel of the Panther system. The two regions of the SARS-CoV-2 target are not differentiated, and amplification of either or both regions leads to RFU signal. The assay results for all targets are determined by fluorescence and emergence cut-offs.

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. This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.

C. This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, Flu A, and/or Flu B, not for any other viruses or pathogens.

D. The emergency use of this product 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/Flu 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.

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, Flu A, and/or Flu B 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, Flu A, and/or Flu B 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 Panther Fusion Specimen Lysis Tubes, Hologic Specimen Lysis Tubes, the Aptima Multitest Swab Specimen Collection Kit, the Hologic Direct Load Capture Cap Collection Kit - CLASSIQSwabs, the Hologic Direct Load Capture Cap Collection Kit - FLOQSwabs, and the Hologic Direct Load Tube Collection Kit, pertain to the transfer of sample into the tube and not to testing of the sample. Specimens collected/transferred any time prior to these expiration dates are valid for testing provided they are transported and stored in accordance with the appropriate package insert, even if these expiration dates have passed.

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 14) 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.

Reagent Storage and Handling Requirements

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

Aptima SARS-CoV-2/Flu Amplification Reagent

Aptima SARS-CoV-2/Flu Enzyme Reagent

Aptima SARS-CoV-2/Flu Promoter Reagent

Aptima SARS-CoV-2/Flu Internal Control

Aptima SARS-CoV-2/Flu Positive Control

Aptima SARS-CoV-2/Flu Negative Control

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

Aptima SARS-CoV-2/Flu Amplification Reconstitution Solution

Aptima SARS-CoV-2/Flu Enzyme Reconstitution Solution

Aptima SARS-CoV-2/Flu Promoter Reconstitution Solution

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

Aptima SARS-CoV-2/Flu 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 Promoter Reagent are stable for 30 days when stored at 2°C to 8°C.

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

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

H. Reagents stored on-board the Panther system have 72 hours of on-board stability. Reagents can be loaded onto the Panther system up to 5 times. The Panther system logs each time the reagents are loaded.

I. The Promoter Reagent and Reconstituted Promoter Reagent are photosensitive. Store the reagents protected from light. The specified reconstituted stability is based on 12 hours exposure of the Reconstituted Promoter 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 Promoter 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/Flu assay, this includes NP and anterior nasal swab specimen collection in viral transport medium (VTM/UTM), saline, 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 Multitest Transport Tubes, Hologic Direct Load Tube Collection Kit, the Hologic Direct Load Capture Cap Collection Kit - CLASSIQSwabs, the Hologic Direct Load Capture Cap Collection Kit - FLOQSwabs, 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 and anterior nasal 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 or STM. The Aptima Multitest Swab Specimen Collection Kit may be used for the collection of anterior nasal swab samples. The Hologic Direct Load Tube Collection Kit may be used for the collection of anterior nasal swab samples. The Hologic Direct Load Capture Cap Collection Kit - CLASSIQSwabs is for the collection of anterior nasal swab samples. The Hologic Direct Load Capture Cap Collection Kit - FLOQSwabs is for the collection of NP swab samples.

After collection, specimens collected in VTM/UTM or saline 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) as described in the specimen processing section below. Remaining specimen volumes in VTM/UTM or saline 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 Collection Kit - CLASSIQSwabs, and the Hologic Direct Load Capture Cap Collection Kit - FLOQSwabs, may be stored at 2°C to 30°C up to 6 days.

Note: *It is recommended that specimens collected in the Aptima Multitest Tube,* the Hologic Direct Load Tube, the Hologic Direct Load Capture Cap Collection Kit -

CLASSIQSwabs, and the Hologic Direct Load Capture Cap Collection Kit - FLOQSwabs, *are stored capped and upright in a rack.*

The following types of VTM/UTM can be used.

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

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

Specimen Processing

Capped Workflow using Aptima SARS-CoV-2/Flu 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 for Specimen Collected with the Aptima Multitest Collection Kit

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

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

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

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

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

Uncapped Workflow using Aptima SARS-CoV-2/Flu Uncapped Tube 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. 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 tube. 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 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 tube. 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, Flu A, and Flu B 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 tube. 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 Specimens 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, Panther Fusion Specimen Lysis Tube, Hologic Specimen Lysis Tube, Custom Specimen Lysis Tube, Hologic Direct Load Tube, Hologic Direct Load Capture Cap Collection Kit - CLASSIQSwabs, or the Hologic Direct Load Capture Cap Collection Kit - FLOQSwabs 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

      If samples need to be shipped for testing at another facility, place a new solid cap on the Specimen Lysis Tube, and the 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.*

Specimen Transport

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

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

Panther System

Reagents for the Aptima SARS-CoV-2/Flu 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/Flu Assay PRD-06815

250 tests (2 boxes)

Aptima SARS-CoV-2/Flu 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/Flu Amplification Reagent** *Non-infectious nucleic acids dried in buffered solution.*	1 vial
E	**Aptima SARS-CoV-2/Flu Enzyme Reagent** *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution.*	1 vial
PRO	**Aptima SARS-CoV-2/Flu Promoter Reagent** *Non-infectious nucleic acids dried in buffered solution.*	1 vial
IC	**Aptima SARS-CoV-2/Flu Internal Control** *Non-infectious RNA nucleic acids in buffered solution.*	1 vial

Aptima SARS-CoV-2/Flu 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/Flu Amplification Reconstitution Solution** *Aqueous solution containing preservatives.*	1 x 27.7 mL
ER	**Aptima SARS-CoV-2/Flu Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 x 11.1 mL
PROR	**Aptima SARS-CoV-2/Flu Promoter Reconstitution Solution** *Aqueous solution containing preservatives.*	1 x 35.4 mL
TCR	**Aptima SARS-CoV-2/Flu Target Capture Reagent** *Buffered salt solution containing solid phase and nucleic acids.*	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)
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, liquid handling (LiHa), 1000 µL filtered, conductive, and disposable	901121 (10612513 Tecan) 903031 (10612513 Tecan) MME-04134 (30180117 Tecan) MME-04128 MME-04110
Aptima SARS-CoV-2/Flu Controls Kit *PC - Aptima SARS-CoV-2/Flu Positive Control. Non-infectious nucleic acid in a buffered solution containing < 5% detergent. Quantity 5 x 1.7 mL* *NC - Aptima SARS-CoV-2/Flu Negative Control. A buffered solution containing < 5% detergent. Quantity 5 x 1.7 mL*	PRD-06816
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
Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens* **used for lab contamination monitoring*	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
Hologic Specimen Lysis Tubes, 1200 each *tube contains 0.71 mL of STM with a solid cap (for uncapped workflow)*	PRD-06660
Hologic Solid Cap for use with PRD-06554*, 100 caps per bag **a single-use cover for the Hologic Specimen Lysis Tube (PRD-06554 only) after testing as part of the uncapped workflow*	PRD-06744

	Cat. No.
Hologic Solid Cap for use with PRD-06660*, 1000 caps per bag **a single-use cover for the Hologic Specimen Lysis Tube (PRD-06660 only) after testing as part of the uncapped workflow*	PRD-06723
Specimen Transport Medium, 1 bottle, 80 mL (for uncapped workflow)	PRD-04423
Bleach, 5% to 7% (0.7M to 1.0M) sodium hypochlorite solution	—
Disposable 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 Promoter reagent reconstitution solutions* *CL0041 (100 caps)* *Enzyme Reagent reconstitution solution* *501616 (100 caps)* *TCR 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 Specimen Lysis Tube) *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)*	—
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 Promoter 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 Promoter 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.*

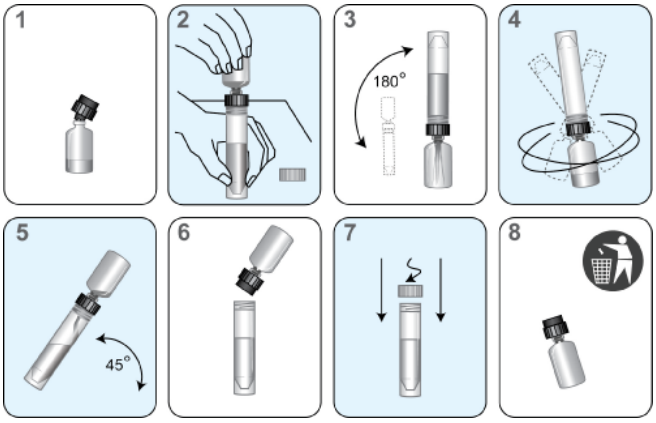

Figure 1. Panther System Reconstitution Process

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.

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 Promoter 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 Promoter Reagents on a tube rocker set to 20 RPM (or equivalent) for a minimum of 25 minutes.

2. If reconstituted Promoter 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 Promoter Reagent may be used even if residual precipitate remains. Mix Promoter 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

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

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

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

E. Specimen Handling using Hologic Specimen Lysis Tube with Solid Cap 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 with solid cap 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 adequate collected specimen is added to the Hologic Specimen Lysis tube (PRD-06660), there is sufficient volume to perform 1 nucleic acid extraction. If an additional extraction is desired, a second Hologic Specimen Lysis Tube (PRD-06660) should be prepared using specimen from the primary collection tube (i.e. UTM, VTM, saline, etc.).*

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

F. System Preparation

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

Procedural Notes

A. Controls

1. To work properly with the Aptima Assay software for the Panther system, one pair of controls is required. The Aptima SARS-CoV-2/Flu 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 and/or flu. The internal control must be detected in all samples that are negative for SARS-CoV-2 and flu 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, No Test, or Invalid.

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

Table 1: Aptima SARS-CoV-2/Flu Results Interpretation

SARS-CoV-2 Result	Flu A Result	Flu B Result	IC Result	Interpretation
Negative	Negative	Negative	Valid	SARS-CoV-2, Flu A, and Flu B not detected.
Positive	Negative	Negative	Valid	SARS-CoV-2 detected. Flu A and Flu B not detected.
Negative	Positive	Negative	Valid	Flu A detected. SARS-CoV-2 and Flu B not detected.
Negative	Negative	Positive	Valid	Flu B detected. SARS-CoV-2 and Flu A not detected.
Positive	Positive	Negative	Valid	SARS-CoV-2 and Flu A detected. Flu B not detected
Negative	Positive	Positive	Valid	Flu A and Flu B detected. SARS-CoV-2 not detected.
Positive	Negative	Positive	Valid	SARS-CoV-2 and Flu B detected. Flu A not detected.
Positive	Positive	Positive	Valid	SARS-CoV-2, Flu A and Flu B detected.
Invalid	Invalid	Invalid	Invalid	Invalid. There was an error in the generation of the result; retest sample.

Note: Positive result will be accompanied by TTime values.
Note: Detection of IC is not required for samples that are positive for SARS-CoV-2, Flu A, and/or Flu B.
Note: Users can only mask Flu A and/or Flu B results but not SARS-CoV-2 results. The result is shown as No Test if the analyte is masked in the software.
Note: If an invalid result due to an assay processing error (p flag) is observed with a sample collected directly into Specimen Transport Medium, consider vortexing the sample for a minimum of 5 minutes before repeating the test.

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. Self-collected under supervision of a healthcare provider or healthcare provider-collected anterior nasal swabs are additional acceptable upper respiratory specimens that can be tested with the Aptima SARS-CoV-2/Flu assay; however, performance with these specimen types have not been validated.

G. Influenza positive specimens were validated in the clinical study by testing archived, selected specimens only, as no influenza positive anterior nasal swabs or nasopharyngeal swab specimens were procured during the prospective clinical study. If an influenza result is inconsistent with clinical presentation and/or other clinical and epidemiological information, FDA-cleared Influenza NAATs are available for confirmation if clinically indicated.

H. Interference was observed for Flu A when evaluated with high concentrations of Flu B or high concentrations of SARS-CoV-2 and observed for Flu B when evaluated with high concentrations of Flu A. There is a risk of false negative Flu A or Flu B results under certain co-infection circumstances.

I. Evaporation of STM in the Hologic Specimen Lysis Tube, Solid Cap (1200 Tubes), PRD-06660, has been observed when stored at the upper end of the recommended temperature range (sustained storage at 28°C) near the end of the product's shelf-life (i.e., 9-months). Due to the observed evaporation, the section "Panther System Test Procedure, E" was revised to indicate that only a single extraction should be performed after an aliquot of the collected specimen has been transferred into the Hologic Specimen Lysis Tube, Solid Cap (1200 Tubes), PRD-06660.If an additional extraction is desired, a second Hologic Specimen Lysis Tube, Solid Cap should be prepared using specimen from the primary collection tube (i.e. UTM, VTM, saline, etc.).

J. 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/Flu 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/Flu assay, the relevant Conditions of Authorization are listed below.

A. Authorized laboratories[1] using the Aptima SARS-CoV-2/Flu assay must include with test result reports 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 the Aptima SARS-CoV-2/Flu assay must use your product as outlined in the authorized labeling. 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/Flu assay are not permitted.

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

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

E. 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.

F. 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.

G. Hologic, its authorized distributor(s) and authorized laboratories using the Aptima SARS-CoV-2/Flu 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.”

Panther SARS-CoV-2/Flu Assay Performance

Analytical Sensitivity

The analytical sensitivity (limit of detection or LoD) of the Aptima SARS-CoV-2/Flu assay was determined by testing serial dilutions of pooled negative clinical nasopharyngeal swab VTM/UTM specimens spiked with the following virus cultures: 1 SARS-CoV-2 strain, 2 Flu A strains, and 2 Flu B strains. VTM/UTM specimens were transferred into a Specimen Lysis Tube prior to testing. Ten replicates of each serial dilution for each strain were evaluated using each of two assay reagent lots to estimate the LoD. The LoD is defined as the lowest concentration at which ≥95% of all replicates tested positive, as summarized in Table 2. Each target specific LoD was confirmed by testing an additional 20 replicates in negative clinical NP swab VTM/UTM matrix with one reagent lot. The LoD was also confirmed in negative clinical Multitest matrix and negative clinical saline matrix. The LoD for each strain was identical, regardless of the matrix/ media type evaluated.

Table 2: Analytical Sensitivity in Clinical VTM/UTM Matrix

Viral Strain	LoD Concentration
SARS-CoV-2 (USA-WA1/2020)	0.001 $TCID_{50}$/mL
Influenza A/California/07/2009 (H1N1)	0.03 $TCID_{50}$/mL
Influenza A/Switzerland/9715293/2015 (H3N2)	0.003 $TCID_{50}$/mL
Influenza B/Brisbane/33/08 (Victoria lineage)	0.01 $TCID_{50}$/mL
Influenza B/Massachusetts/02/2012 (Yamagata lineage)	0.3 $TCID_{50}$/mL

The analytical sensitivity of the Aptima SARS-CoV-2/Flu assay was additionally confirmed using the Hologic Direct Load Capture Cap Collection Kit with both the CLASSIQSwab and the FLOQSwab. Virus cultures for 1 strain of SARS-CoV-2, 2 strains of Flu A, and 2 strains of Flu B were spiked into simulated nasal matrix which consisted of Specimen Transport Medium, mucin, and HeLa cells at $2e^4$ HeLa cells/mL. Each virus was spiked at the target-specific LoD for VTM/ UTM matrix and tested in 20 replicates. All targets demonstrated ≥95% positivity, confirming the LoD determined in VTM/UTM.

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 corroborate 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	$1.8x10^2$ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL.
N/A = Not Applicable.
ND = Not Detected.

Reactivity

The reactivity of the Aptima SARS-CoV-2/Flu assay was evaluated against multiple strains of Flu A (H1N1 & H3N2) and multiple strains of Flu B (Victoria and Yamagata lineages). Viral strains were tested in triplicate with one reagent lot. Table 4 shows the lowest concentration of each strain in which 100% positivity was observed. Additionally, the 2020 CDC Human Influenza Panel was evaluated with the assay. Five-fold dilutions of each panel member were evaluated with a minimum of five replicates according to the CDC protocol. Table 5 shows the lowest concentration of each panel member in which at least one replicate yielded a positive result.

Table 4: Analytical Reactivity Summary for Flu A and Flu B Strains

Strain	Subtype	Concentration ($TCID_{50}$/mL)	Concentration Relative to LoD	SARS-CoV-2	Flu A	Flu B
Influenza A/Massachusetts/15/13	Flu A (H1N1)	0.09	3x LOD	-	+	-
A/Taiwan/42/2006	Flu A (H1N1)	0.09	3x LOD	-	+	-
A/Henan/8/05	Flu A (H1N1)	0.09	3x LOD	-	+	-
A/Kentucky/2/06	Flu A (H1N1)	0.3	10x LOD	-	+	-
A/Hawaii/15/01	Flu A (H1N1)	3	100x LOD	-	+	-
A/Brisbane/59/2007	Flu A (H1N1)	0.09	3x LOD	-	+	-
A/Solomon Islands/03/06	Flu A (H1N1)	0.09	3x LOD	-	+	-
A1/Mal/302/54	Flu A (H1N1)	0.09	3x LOD	-	+	-
A1/Denver/1/57	Flu A (H1N1)	0.9	30x LOD	-	+	-
Ohio/09SW1477/2009	Flu A (H1N2)	0.3	10x LOD	-	+	-
Michigan/45/2015	Flu A (H1N1)	0.09	3x LOD	-	+	-
A/Hiroshima/52/05	Flu A(H3N2)	0.009	3x LOD	-	+	-
A/Victoria/3/75	Flu A(H3N2)	9	3000x LOD	-	+	-
A/Brazil/1137/99	Flu A (H3N2)	0.09	30x LOD	-	+	-
A/Hong Kong/8/68	Flu A (H3N2)	0.9	300x LOD	-	+	-
A/Aichi/2/68	Flu A (H3N2)	0.3	100x LOD	-	+	-
Indiana/08/2011	Flu A (H3N2)	0.03	10x LOD	-	+	-

Table 4: Analytical Reactivity Summary for Flu A and Flu B Strains

Strain	Subtype	Concentration ($TCID_{50}$/mL)	Concentration Relative to LoD	SARS-CoV-2	Flu A	Flu B
Perth/16/2009	Flu A (H3N2)	0.009	3x LOD	-	+	-
A/Costa Rica/07/99	Flu A (H3N2)	3	1000x LOD	-	+	-
Port Chalmers/1/73	Flu A (H3N2)	0.3	100x LOD	-	+	-
HongKong/4801/2014	Flu A (H3N2)	0.009	3x LOD	-	+	-
Texas/50/2012	Flu A (H3N2)	0.009	3x LOD	-	+	-
B/Ohio/1/2005	Flu B (Victoria)	0.03	3x LOD	-	-	+
Alabama/2/17	Flu B (Victoria)	0.03	3x LOD	-	-	+
Florida/78/2015	Flu B (Victoria)	0.03	3x LOD	-	-	+
Colorado/06/2017	Flu B (Victoria)	0.03	3x LOD	-	-	+
B/St. Petersburg/14/06	Flu B (Yamagata)	0.9	3x LOD	-	-	+
Utah/9/14	Flu B (Yamagata)	0.9	3x LOD	-	-	+
Wisconsin/1/2010	Flu B (Yamagata)	0.9	3x LOD	-	-	+
Phuket/3073/2013	Flu B (Yamagata)	0.9	3x LOD	-	-	+
B/Lee/40	Flu B	3	N/A	-	-	+

Table 5: 2020 CDC Human Influenza Panel

Virus	Strain	Minimum Reactive Concentration (EID_{50}/mL)
Influenza A	A/Perth/16/2009 (H3N2)	1.02E+01
	A/Hong Kong/2671/2019 (H3N2)	8.10E-01
	A/Christ Church/16/2010 (H1N1 pdm)	1.62E+01
	A/Guangdong-maonan/1536/2019 pdm)	1.29E+00
Influenza B	B/Michigan/09/2011	8.13E-03
	B/Washington/02/2019	1.62E+00
	B/Texas/81/2016	2.04E-01
	B/Phuket/3073/2013	8.13E+00

Inclusivity

The inclusivity of the Aptima SARS-CoV-2/Flu assay was evaluated using *in silico* analysis of the assay target capture oligos, amplification primers, and detection torches for the SARS-CoV-2, Flu A and Flu B target systems in relation to sequences available in the NCBI and GISAID gene databases as of September 30, 2020. Any sequence with missing or ambiguous sequence information was removed from the analysis for that target region.

For SARS-CoV-2, there were 111,055 sequences evaluated for the first target region, 110,932 sequences evaluated for the second target region, and 110,784 sequences with complete information for both regions. The *in silico* analysis showed 100% homology to the assay oligos of both target systems for 96,883 (87.5%) of the evaluated sequences and 100% homology to the assay oligos of at least one target system for 110,743 (99.96%) of the sequences. There were no evaluated sequences with identified mismatches predicted to impact binding or assay performance.

For Flu A and Flu B, there were 79,898 and 28,146 sequences, respectively, since January 01, 2015 with information corresponding to the oligos for the target regions of the assay. Of the available sequences for Flu A, 38,700 (48.4%) showed 100% homology to all oligos of the target region. Of the remaining 41,198 sequences, oligo binding is predicted for all but 687 for an overall inclusivity of 99.1% for the evaluated sequences. Of the available sequences for Flu B, 5,867 (20.8%) showed 100% homology to all oligos of the target region. Of the remaining 22,279 sequences, oligo binding is predicted for all but 22 for an overall inclusivity of 99.9% for the evaluated sequences.

For the SARS-CoV-2 target, a second round of *in silico* inclusivity analysis was conducted with strains uploaded to the NCBI and GISAID gene databases between July 11, 2021, and August 12, 2021. There were 120,707 sequences evaluated for the first target region, 120,206 sequences evaluated for the second target region, and 120,062 sequences with complete information for both regions. The *in silico* analysis showed 100% homology to the assay oligos of both target systems for 115,349 (96.1%) of the evaluated sequences and 100% homology to the assay oligos of at least one target system for 120,019 (99.96%) of the sequences. There were no evaluated sequences with identified mismatches predicted to impact binding or assay performance.

Analytical Specificity and Microbial Interference

The analytical specificity of the Aptima SARS-CoV-2/Flu assay was evaluated by testing 37 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, Flu A (H1N1) and Flu B (Victoria lineage) cultured virus at 3x LoD concentrations. Analytical specificity of the Aptima SARS-CoV-2/Flu assay was 100% with no evidence of microbial interference from non-target microorganisms. In addition to microorganism testing, *in silico* BLAST 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 202 GenBank sequences evaluated.

Table 6: Analytical Specificity and Microbial Interference Microorganisms

Microorganism	Concentration	Microorganism	Concentration
Adenovirus	1.0E+06 $TCID_{50}$/mL	*Legionella pneumophila*	1.0E+06 CFU/mL
Enterovirus (e.g. EV68)	1.0E+04 $TCID_{50}$/mL	*Mycobacterium tuberculosis*	1.0E+08 $TCID_{50}$/mL
Rhinovirus	1.0E+04 $TCID_{50}$/mL	*Mycoplasma pneumoniae*	1.0E+05 CFU/mL
Human coronavirus 229E	1.0E+06 $TCID_{50}$/mL	*Pneumocystis jirovecii* (PJP)	1.0E+06 nuc/mL
Human coronavirus HKU1	1.0E+06 c/mL	*Pseudomonas aeruginosa*	1.0E+06 CFU/mL

Table 6: Analytical Specificity and Microbial Interference Microorganisms

Microorganism	Concentration	Microorganism	Concentration
Human coronavirus[1] NL63	1.0E+03 $TCID_{50}$/mL	*Staphylococcus epidermidis*	1.0E+06 CFU/mL
Human coronavirus OC43	1.0E+04 $TCID_{50}$/mL	*Streptococcus pneumonia*	1.0E+04 CFU/mL
MERS-coronavirus	1.0E+03 $TCID_{50}$/mL	*Streptococcus pyogenes*	1.0E+06 CFU/mL
SARS-coronavirus[1]	1.0E+06 c/mL	*Streptococcus salivarius*	1.0E+06 CFU/mL
Parainfluenza virus 1	1.0E+05 $TCID_{50}$/mL	Influenza A[3]	1.0E+05 $TCID_{50}$/mL
Parainfluenza virus 2	1.0E+03 $TCID_{50}$/mL	Influenza B[3]	1.0E+04 $TCID_{50}$/mL
Parainfluenza virus 3	1.0E+05 $TCID_{50}$/mL	*Neisseria meningitides*	1.0E+06 CFU/mL
Parainfluenza virus 4a	1.0E+05 $TCID_{50}$/mL	*Neisseria gonorrhea*	1.0E+06 CFU/mL
Human Metapneumovirus (hMPV)	1.0E+05 $TCID_{50}$/mL	*Moraxella catarrhalis*	1.0E+06 CFU/mL
Respiratory syncytial virus	1.0E+04 $TCID_{50}$/mL	*Lactobacillus plantarum*	1.0E+06 CFU/mL
Bordetella pertussis	1.0E+06 CFU/mL	*Corynebacterium diphtheria*	1.0E+06 CFU/mL
Candida albicans	1.0E+06 CFU/mL	*Escherichia coli*	1.0E+06 CFU/mL
Chlamydia pneumonia	1.0E+05 CFU/mL	SARS-CoV-2[3]	1.0E+05 $TCID_{50}$/mL
Haemophilus influenzae	1.0E+06 CFU/mL	30 Individual negative clinical NP swab VTM/UTM specimens[2]	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, 30 individual negative clinical NP swab specimens were tested in triplicate to represent diverse microbial flora in the human respiratory tract.

[3] SARS-CoV-2, Influenza A, and Influenza B are targets of the assay. Analysis of cross-reactivity was only performed for the other targets.

Competitive Interference

Competitive interference of the Aptima SARS-CoV-2/Flu assay was evaluated using pairs of target viruses at low/high concentrations in negative clinical NP swab VTM/UTM matrix. The low concentration virus was tested at 3x LoD, while the high concentration virus was tested at the maximum allowable concentration based on the stock titer. Testing was performed using one SARS-CoV-2, one Flu A (H1N1), and one Flu B (Victoria lineage) virus strain. Results of the study are shown in Table 7. Competitive interference impacting the detection of SARS-CoV-2 was not observed when high concentrations of Flu A (≤ 3.16E+04 $TCID_{50}$/mL) or high concentrations of Flu B (≤ 1.17E+04 $TCID_{50}$/mL) were evaluated. Competitive interference impacting the detection of Flu A was observed when SARS-CoV-2 was evaluated at concentrations ≥ 1.40E+02 $TCID_{50}$/mL or Flu B was evaluated at concentrations ≥ 1.17E+01 $TCID_{50}$/mL. Competitive interference impacting the detection of Flu B was not observed when

high concentrations of SARS-CoV-2 (≤ 1.40E+04 $TCID_{50}$/mL) were evaluated but was observed when Flu A was evaluated at concentrations ≥ 3.16 E+04 $TCID_{50}$/mL.

Table 7: Competitive Interference

Condition	Target 1		Target 2		SARS-CoV-2 % Positive (n detected/ n tested)	Flu A % Positive (n detected/ n tested)	Flu B % Positive (n detected/ n tested)
	Virus	3x LoD Concentration ($TCID_{50}$/mL)	Virus	High Concentration ($TCID_{50}$/mL)			
1	SARS-CoV-2	0.003	Flu A	**3.16E+04**	**100% (3/3)**	100% (3/3)	0% (0/3)
				3.16E+03	100% (3/3)	100% (3/3)	0% (0/3)
				3.16E+02	100% (3/3)	100% (3/3)	0% (0/3)
2	SARS-CoV-2	0.003	Flu B	**1.17E+04**	**100% (3/3)**	0% (0/3)	100% (3/3)
				1.17E+03	100% (3/3)	0% (0/3)	100% (3/3)
				1.17E+02	100% (3/3)	0% (0/3)	100% (3/3)
3	Flu A	0.09	SARS-CoV-2	1.40E+04	100% (2/2)+	0% (0/3)	0% (0/3)
				1.40E+03	100% (3/3)	0% (0/3)	0% (0/3)
				1.40E+02	100% (3/3)	33% (1/3)	0% (0/3)
				1.40E+01	100% (3/3)	**100% (3/3)**	0% (0/3)
				1.40E+00	100% (3/3)	100% (3/3)	0% (0/3)
				1.40E-01	100% (3/3)	100% (3/3)	0% (0/3)
4	Flu A	0.09	Flu B	1.17E+04	0% (0/3)	0% (0/3)	100% (3/3)
				1.17E+03	0% (0/3)	0% (0/3)	100% (3/3)
				1.17E+02	0% (0/3)	0% (0/3)	100% (3/3)
				1.17E+01	0% (0/3)	**100% (3/3)**	100% (3/3)
				1.17E+00	0% (0/3)	100% (3/3)	100% (3/3)
				1.17E-01	0% (0/3)	100% (3/3)	100% (3/3)
5	Flu B	0.03	SARS-CoV-2	**1.40E+04**	100% (3/3)	0% (0/3)	**100% (3/3)**
				1.40E+03	100% (3/3)	0% (0/3)	100% (3/3)
				1.40E+02	100% (3/3)	0% (0/3)	100% (3/3)
6	Flu B	0.03	Flu A	3.16E+04	0% (0/3)	100% (3/3)	0% (0/3)
				3.16E+03	0% (0/3)	100% (3/3)	**100% (3/3)**
				3.16E+02	0% (0/3)	100% (3/3)	100% (3/3)

Bolded font indicates the highest concentration tested for each condition that yielded 100% positivity for the low target (Target 1).

Clinical Performance

The clinical performance of the Aptima SARS-CoV-2/Flu assay was evaluated in comparison to an FDA EUA authorized high sensitivity nucleic acid amplification test (NAAT) and an FDA-cleared Flu assay using a panel of remnant clinical nasopharyngeal specimens in VTM/UTM collected from patients with signs and symptoms of respiratory infection. For the evaluation, a combination of negative, SARS-CoV-2 positive, Flu A positive, and Flu B positive specimens were tested with each assay.

The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) for SARS-CoV-2 was calculated in relation to the FDA EUA authorized NAAT assay as the reference result, as shown in Table 8. The assay showed positive and negative percent agreements of 96.1% and 99.6%, respectively for SARS-CoV-2.

For Flu A and Flu B, the PPA and NPA were calculated in relation to the FDA-cleared Flu assay as the reference result, as shown in Table 9 for Flu A and Table 10 for Flu B. The assay showed positive and negative percent agreements of 100% and 99.2%, respectively, for Flu A and of 100% and 100%, respectively, for Flu B.

Table 8: Clinical Performance Results for SARS-CoV-2

SARS-CoV-2		FDA EUA Authorized NAAT Result		
		Positive	**Negative**	**Total**
Aptima SARS/Flu Result	**Positive**	49	1	50
	Negative	2	247	249
	Total	51	248	299
Positive Agreement		96.1%	(86.8% - 98.9%)	
Negative Agreement		99.6%	(97.8% - 99.9%)	

Table 9: Clinical Performance Results for Flu A

Flu A		FDA-Cleared Flu Assay		
		Positive	**Negative**	**Total**
Aptima SARS/Flu Result	**Positive**	48	2	50
	Negative	0	249	249
	Total	48	251	299
Positive Agreement		100%	(92.6% - 100%)	
Negative Agreement		99.2%	(97.1% - 99.8%)	

Table 10: Clinical Performance Results for Flu B

Flu B		FDA-Cleared Flu Assay		
		Positive	**Negative**	**Total**
Aptima SARS/Flu Result	**Positive**	49	0	49
	Negative	0	250	250
	Total	49	250	299
Positive Agreement		100%	(92.7% - 100%)	
Negative Agreement		100%	(98.5% - 100%)	

The clinical performance of the Aptima SARS-CoV-2/Flu assay was further evaluated for Flu A and Flu B in a prospective clinical study compared with an FDA-cleared assay with fresh and frozen NP and anterior nasal swab clinical specimens collected into UTM/VTM or saline. The specimens were collected from individuals with signs and symptoms of respiratory viral infection consistent with COVID-19. A total of 405 specimens with valid comparator results were evaluated. Assay negative percent agreement (NPA) was 100% (95% CI: 99.6% to 100%) for both Flu A and Flu B in NP specimens, and 100% (95% CI: 96.3% to 100%) for both Flu A and Flu B in nasal specimens. Assay positive percent agreement (PPA) was not calculable for Flu A and Flu B, as there were no NP or anterior nasal specimens with comparator positive results.

Since no prospectively collected clinical specimens were positive for Flu A or Flu B by the comparator, the performance of the Aptima SARS-CoV-2/Flu assay for the detection of Flu A and Flu B was further evaluated using 50 Flu A known positive (46 NP and 4 nasal) and 40 Flu B known positive (36 NP and 4 nasal) archived specimens stored in VTM. PPA was assessed versus the known positive status (i.e., previous testing results from FDA-cleared molecular assays). The PPA was 100% (95% CI: 92.9% to 100%) for Flu A and 97.5% (95% CI: 87.1% to 99.6%) for Flu B. There was 1 false negative Flu B result for an NP specimen. The specimen underwent additional testing with an alternate FDA-cleared NAAT and was confirmed negative.

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