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

For Emergency Use Authorization (EUA) only

For *in vitro* diagnostic use only

Rx only

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General Information

Intended Use

The Panther Fusion® SARS-CoV-2 assay is a real-time RT-PCR *in vitro* diagnostic test intended for the qualitative detection of RNA from SARS-CoV-2 isolated and purified from upper respiratory specimens (such as nasopharyngeal (NP), nasal, mid-turbinate, and oropharyngeal (OP) swab specimens, nasopharyngeal wash/aspirates or nasal wash), and lower respiratory tract (LRT) specimens (such as bronchoalveolar lavage) obtained from individuals who meet COVID-19 clinical and/or epidemiological criteria, as well as upper respiratory specimens (such as nasopharyngeal, nasal, mid-turbinate or oropharyngeal swab specimens) collected from an individual, including from individuals without symptoms or other reasons to suspect COVID-19 infection. The Panther Fusion SARS-CoV-2 assay is for use only under Emergency Use Authorization (EUA) in the 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 the SARS-CoV-2 in pooled samples containing up to 5 individual upper respiratory swab specimens (nasopharyngeal, nasal, mid-turbinate, or oropharyngeal swabs) where each specimen is collected under observation or by a healthcare provider 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 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 and lower 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 disease. Laboratories within the United States and its territories are required to report all positive 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 other clinical observations, patient history, and epidemiological information.

The Panther Fusion SARS-CoV-2 assay on the Panther Fusion system is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the operation of the Panther Fusion system and *in vitro* diagnostic procedures. The Panther Fusion 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 Panther Fusion SARS-CoV-2 assay involves the following steps: sample lysis, nucleic acid capture, elution transfer, and multiplex RT-PCR when analytes are simultaneously amplified and detected. Nucleic acid capture and elution takes place in a single tube on the Panther Fusion system. The eluate is transferred to the Panther Fusion system reaction tube containing the assay reagents. Multiplex RT-PCR is then performed for the eluted nucleic acid on the Panther Fusion system.

Nucleic acid capture and elution: Prior to processing and testing on the Panther Fusion system, specimens need to be transferred to a Specimen Lysis Tube containing specimen transport media (STM) that lyses the cells, releases target nucleic acid and protects them from degradation during storage.

The Internal Control-S (IC-S) is added to each test specimen and controls via the working Panther Fusion Capture Reagent-S (wFCR-S). The IC-S in the reagent monitors specimen processing, amplification and detection.

Capture oligonucleotides hybridize to nucleic acid in the test specimen. Hybridized nucleic acid is then separated from the specimen in a magnetic field.

Wash steps remove extraneous components from the reaction tube. The elution step elutes purified nucleic acid. During the nucleic acid capture and elution step, total nucleic acid is isolated from specimens.

Elution transfer and RT-PCR: During the elution transfer step, eluted nucleic acid is transferred to a Panther Fusion reaction tube already containing oil and reconstituted mastermix.

Target amplification occurs via RT-PCR. A reverse transcriptase generates a DNA copy of the target sequences. Target specific forward and reverse primers and probes then amplify targets while simultaneously detecting and discriminating multiple target types via multiplex RT-PCR. To safeguard against potential mutational drift in the SARS-CoV-2 genome, the Panther Fusion SARS-CoV-2 assay amplifies and detects two conserved regions of the ORF1ab gene in the

same fluorescence channel. The two regions are not differentiated and amplification of either or both regions leads to a fluorescence signal.

The Panther Fusion system compares the fluorescence signal to a predetermined cut-off to produce a qualitative result for the presence or absence of the analyte.

The analytes and the channel used for their detection on the Panther/Panther Fusion system are summarized in the table below.

Analyte	Gene Targeted	Instrument Channel
SARS-CoV-2	ORF1ab Region 1 ORF1ab Region 2	ROX
Internal Control	Not applicable	RED677

Warnings and Precautions

A. For *in vitro* diagnostic use. For use under an Emergency Use Authorization (EUA) only.

B. This test has not been FDA cleared or approved; the test has been authorized by the FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

C. This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.

D. This test 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 authorization is terminated or revoked sooner.

E. Carefully read this entire package insert and the *Panther/Panther Fusion System Operator's Manual*.

F. The Panther Fusion Enhancer Reagent-S (FER-S) is corrosive, harmful if swallowed and causes severe skin burns and eye damage.

G. 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.

H. 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.

I. 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]

J. If infection with 2019-nCoV is suspected based on current clinical screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.

K. Use only supplied or specified disposable laboratory ware.

L. 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).

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

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

O. Expiration dates listed on the Panther Fusion Specimen Lysis Tubes 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.

P. 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.

Q. 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.

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

S. Store assay components at the recommended storage condition. See *Reagent Storage and Handling Requirements (*page 7), and *Panther Fusion System Test Procedure* (page 15) for more information.

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

U. Avoid microbial and ribonuclease contamination of reagents.

V. 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.

W. Do not use the assay cartridge if the storage pouch has lost its seal or if the assay cartridge foil is not intact. Contact Hologic if either occurs.

X. Do not use the fluid packs if the foil seal is leaking. Contact Hologic if this occurs.

Y. Handle the assay cartridges with care. Do not drop or invert assay cartridges. Avoid prolonged exposure to ambient light.

Z. 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.

AA. Some reagents in this kit are labeled with risk and safety symbols.

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

AC. Testing of pooled specimens may impact the detection capability of the Panther Fusion SARS-CoV-2 assay and impact sensitivity.

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.*

Panther Fusion Oil / Aptima Oil

Polydimethylsiloxane 95-100%

WARNING

H315 - Causes skin irritation
H319 - Causes serious eye irritation
P264 - Wash face, hands and any exposed skin thoroughly after handling
P280 - Wear protective gloves/protective clothing/eye protection/face protection
P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing
P337 + P313 - If eye irritation persists: Get medical advice/attention
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

Panther Fusion Enhancer Reagent-S

Lithium Hydroxide, Monohydrate 5-10%

DANGER

H302 - Harmful if swallowed
H314 - Causes severe skin burns and eye damage
P260 - Do not breathe dust/fume/gas/mist/vapors/spray
P264 - Wash face, hands and any exposed skin thoroughly after handling
P270 - Do not eat, drink or smoke when using this product
P280 - Wear protective gloves/protective clothing/eye protection/face protection
P301 + P312 - IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell
P301 + P330 + P331 - IF SWALLOWED: rinse mouth. Do NOT induce vomiting
P303 + P361 + P353 - IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower
P304 + P340 - IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing
P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P310 - Immediately call a POISON CENTER or doctor/physician
P330 - Rinse mouth
P363 - Wash contaminated clothing before reuse

Reagent Storage and Handling Requirements

A. The following table provides storage and handling requirements for this assay.

Reagent	Unopened Storage	On Board/ Open Stability[1]	Opened Storage
Panther Fusion Open Access RNA/DNA Enzyme Cartridge	2°C to 8°C	60 days	2°C to 8°C
Panther Fusion Capture Reagent-S (FCR-S)	15°C to 30°C	30 days	15°C to 30°C
Panther Fusion SARS-CoV-2 Assay PPR Solution	-15°C to -85°C	3 days	On-board instrument
Panther Fusion Enhancer Reagent-S (FER-S)	15°C to 30°C	30 days	15°C to 30°C
Panther Fusion Internal Control-S (IC-S)	2°C to 8°C	(In wFCR-S)	Not applicable
Panther Fusion Elution Buffer	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion Oil	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion SARS-CoV-2 Positive Control	2°C to 8°C	Single use vial	Not applicable-single use
Panther Fusion SARS-CoV-2 Negative Control	2°C to 8°C	Single use vial	Not applicable-single use

When reagents are removed from the Panther Fusion system, return them immediately to their appropriate storage temperatures.

[1] On board stability starts at the time the reagent is placed on the Panther Fusion system for the Panther Fusion Open Access RNA/DNA Enzyme cartridge, SARS-CoV-2 Assay PPR Solution, FCR-S, FER-S, and IC-S. On board stability starts for the Panther Fusion Reconstitution Buffer I, Panther Fusion Elution Buffer and Panther Fusion Oil when the reagent pack is first used.

B. Working Panther Fusion Capture Reagent-S and Panther Fusion Enhancer Reagent-S are stable for 60 days when capped and stored at 15ºC to 30ºC. Do not refrigerate.

C. Discard any unused reagents that have surpassed their on board stability.

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

E. Avoid cross-contamination during reagent handling and storage.

F. **Do not freeze reagents. Once thawed, do not re-freeze the Panther Fusion SARS-CoV-2 assay PPR Solution.**

Specimen Collection and Storage

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

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

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

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

A. 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 3 mL 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 following types of VTM/UTM were verified for use.

- 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 guanidinecontaining material.*

B. Nasopharyngeal Wash/aspirate and Nasal Aspirate Specimen Collection

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

C. LRT specimen collection

Collect bronchoalveolar lavage fluid and bronchial wash specimens according to standard techniques.

D. Specimen processing using the Panther Fusion Specimen Lysis Tube

1. Prior to testing on the Panther Fusion system, transfer swab or LRT specimen* to a Panther Fusion Specimen Lysis Tube.
   - For swab specimens, transfer 500 µL of the collected specimen to a Panther Fusion Specimen Lysis Tube. Affix the provided penetrable cap.
   - For LRT specimens, transfer 250 uL of the LRT specimen (avoid transferring mucus) and 250 uL of VTM/UTM to a Panther Fusion Specimen Lysis Tube. Affix the provided penetrable cap.

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

2. Storing specimens before testing
   a. After collection, specimens can be stored at 2°C to 8°C up to 96 hours before transferred to the Panther Fusion Specimen Lysis Tube. Remaining specimen volumes can be stored at ≤-70°C.
   b. Specimens in the Panther Fusion Specimen Lysis Tube may be stored under one of the following conditions:
      - 15°C to 30°C up to 6 days or
      - 2°C to 8°C up to 3 months.

      Note: *It is recommended that specimens transferred to the Panther Fusion Specimen Lysis Tube are stored capped and upright in a rack.*

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

F. Storing samples after testing
   1. Samples that have been assayed should be stored upright in the rack under one of the following conditions:
      - 15°C to 30°C up to 6 days or
      - 2°C to 8°C up to 3 months.
   2. The samples should be covered with a new, clean plastic film or foil barrier.
   3. If assayed samples need to be frozen or shipped, remove the penetrable cap and place a new non-penetrable cap on the specimen tubes. If samples need to be shipped for testing at another facility, recommended temperatures must be maintained. Prior to uncapping previously tested and recapped samples, 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.

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 under the Emergency Use Authorization of the Panther Fusion SARS-CoV-2 assay may be tested with sample pooling. This includes 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.

For Specimens Collected in VTM/UTM, Saline, 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 Panther Fusion 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 Panther Fusion Specimen Lysis Tube (Panther Fusion SLT)

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

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

D. Ensure homogenous 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 Panther Fusion Specimen Lysis Tube (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 Panther Fusion Specimen Lysis Tube (Panther Fusion SLT).

A. Obtain a generic empty tube.

B. 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. The minimum combined volume of individual specimens pooled prior to transferring to an SLT is 500 µL.The same volume of each specimen included in the pool needs to be used.

Note: *it is recommended to prepare > 500 µL of the specimen pool to account for the potential volume loss associated during transfer to the Panther Fusion SLT.*

C. Carefully transfer the determined volume of each individual specimen from the specimen collection container to the generic empty tube.

D. Uncap the Panther Fusion Specimen Lysis Tube and retain the cap.

E. Prior to testing on the Panther Fusion system, carefully transfer 500 µL of the combined specimen mixture from the generic tube to the Panther Fusion Specimen Lysis Tube.

F. Ensure homogenous mixing of each prepared sample pool.

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

For Specimens Collected in Aptima Multitest Transport Tubes

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

Option 1:
Specimen Preparation Instructions for Samples Pooled Directly into a Panther Fusion Specimen Lysis Tube

Perform the following procedure when pooling specimens collected in Aptima Multitest Transport Tubes by transferring individual specimens directly into a Panther Fusion Specimen Lysis Tube.

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 Panther Fusion Specimen Lysis Tube and retain the cap

C. Prior to testing on the Panther Fusion system, carefully transfer the determined volume of each individual specimen from the Aptima Multitest Transport Tubes to the Panther Fusion Specimen Lysis Tube.

D. Ensure homogenous mixing of each prepared sample pool and apply the penetrable cap.

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

Perform the following procedure when pooling specimens collected in Aptima Multitest Transport Tubes by pooling the samples prior to transferring into a Specimen Lysis Tube.

A. Obtain a generic empty tube.

B. 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. The minimum combined volume of individual specimens pooled prior to transferring to an SLT is 500 µL. The same volume of each specimen included in the pool needs to be used.

Note: *it is recommended to prepare > 500 µL of the specimen pool to account for the potential volume loss associated during transfer to the SLT.*

C. Carefully transfer the determined volume of each individual specimen from the specimen collection container to the generic empty tube.

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

E. Prior to testing on the Panther Fusion system, carefully transfer 500 µL of the combined specimen mixture from the generic tube to the Specimen Lysis Tube.

F. Ensure homogenous mixing of each prepared sample pool.

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

Specimen Transport

Maintain specimen storage conditions as described in the *Specimen Collection and Storage section on* page 8.

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

Panther Fusion System

The Panther Fusion System is an integrated nucleic acid testing system that fully automates all steps necessary to perform various Panther Fusion assays from sample processing through amplification, detection, and data reduction.

Reagents and Materials Provided for the Panther Fusion SARS-CoV-2 Assay

Assay Packaging

Components[1]	Part No.	Storage
Panther Fusion Open Access RNA/DNA Enzyme Cartridges 96 Tests Panther Fusion Open Access RNA/DNA cartridge, 12 tests, 8 per box	PRD-04303	2°C to 8°C
Panther Fusion Internal Control-S 960 Tests Panther Fusion Internal Control-S tube, 4 per box	PRD-04332	2°C to 8°C
Panther Fusion SARS-CoV-2 Assay Controls Panther Fusion SARS-CoV-2 Positive Control tube, 5 per box Panther Fusion Negative Control tube, 5 per box	PRD-06404	2°C to 8°C
Panther Fusion Extraction Reagent-S 960 Tests Panther Fusion Capture Reagent-S bottle, 240 tests, 4 per box Panther Fusion Enhancer Reagent-S bottle, 240 tests, 4 per box	PRD-04331	15°C to 30°C
Panther Fusion Elution Buffer 2400 Tests Panther Fusion Elution Buffer pack, 1200 tests, 2 per box	PRD-04334	15°C to 30°C
Panther Fusion SARS-CoV-2 Assay PPR Solution Panther Fusion SARS-CoV-2 Assay PPR Solution tube, 40 tests, 4 per bag	PRD-06391	-15°C to -85°C
Panther Fusion Oil 1920 Tests Panther Fusion Oil pack, 960 tests, 2 per box	PRD-04335	15°C to 30°C
Aptima Oil Reagent	PRD-04304	15°C to 30°C

[1] Components can also be ordered in the following bundles:
Panther Fusion Universal Fluids Kit, PRD-04430, contains 1 each Panther Fusion Oil and Panther Fusion Elution buffer.
Panther Fusion Assay Fluids I-S, PRD-04431, contains 2 Panther Fusion Extraction Reagents-S, 2 Panther Fusion Internal Control-S, and 1 Panther Fusion Reconstitution Buffer I.

Individually Packaged Items

Items	Part No.
Panther Fusion Specimen Lysis Tubes, 100 per bag	PRD-04339

Materials Required and Available Separately

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

Material	Cat. No.
Panther System	303095
Panther Fusion Module	ASY-09600
Panther Fusion Open Access Pack	PRD-04305
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 System Run Kit for Real Time Assays contains MTUs, waste bags, waste bin covers, and assay fluids	PRD-03455 (5000 tests)
Or Panther System Run Kit (when running TMA assays in parallel with real time-TMA assays) contains MTUs, waste bags, waste bin covers, auto detect*, and assay fluids	303096 (5000 tests)
Aptima Multitest Swab Specimen Collection Kit	PRD-03546
Panther Fusion Tube Trays, 1008 tests, 18 trays per box	PRD-04000
Liquid Handling (LiHa) Disposable Tips, 1000 µL	10612513 (Tecan)
Aptima penetrable caps (optional)	105668
Replacement non-penetrable caps (optional)	103036A
Replacement extraction reagent bottle caps	CL0040
P1000 pipettor and tips with hydrophobic plugs	-
Bleach, 5% to 7% (0.7 M to 1.0 M) sodium hypochlorite solution **Note**: Mix one part bleach with one part deionized water to make diluted working bleach solution 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution.	-
Disposable powderless gloves	-

*Needed only for Panther Aptima TMA assays.

Panther Fusion System Test Procedure

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

A. Work Area Preparation

1. Wipe down work surfaces with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and follow with a deionized (DI) water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface with clean, plastic-backed absorbent laboratory bench covers.
2. Clean a separate work surface where samples will be prepared using the procedure described in step A.1.

B. Reagent Preparation

1. Remove the bottles of IC-S, FCR-S and FER-S from storage.
2. Open the bottles of IC-S, FCR-S and FER-S, and discard the caps. Open the TCR door on the upper bay of the Panther Fusion system.
3. Place the IC-S, FCR-S and FER-S bottles in the appropriate positions on the TCR carousel.
4. Close the TCR door.

Note: *The Panther Fusion system adds the IC-S to the FCR-S. After the IC-S is added to the FCR-S, it is referred to as wFCR-S (working FCR-S). If the FCR-S and FER-S are removed from the system, use new caps and immediately store according to the proper storage conditions.*

C. PPR Solution Preparation

1. Thaw the Panther Fusion SARS-CoV-2 assay PPR Solution to room temperature, protect from light.
2. Mix the solution by vortexing and perform a quick centrifugation to allow contents to settle to the bottom of the tube.
3. Uncap the tube and add 400 µL Aptima Oil Reagent on top of the Panther Fusion SARS-CoV-2 PPR Solution.
4. Recap the tube and perform a quick centrifugation to allow contents to settle to the bottom of the tube and the oil to create an environmental barrier at the top of the tube.
5. **Uncap PPR tube.**
6. Load 1-4 Panther Fusion SARS-CoV-2 assay PPR Solution tubes with the oil overlay into each Open Access Pack.

   Note: *Do not mix or cool the Panther Fusion SARS-CoV-2 PPR Solution once the oil overlay has been added.*

D. PPR Loading onto Panther Fusion

1. Open the Fusion Universal Fluids Drawer from the Load Universal Fluids option on the Tasks screen or the icon on the bottom of the screen.
2. Place the Open Access Pack with loaded PPR tubes into any open Reconstitution Buffer position.
3. Select "Loaded" for the position(s) in the pack with loaded PPR tubes.

4. Select “set” to select the LDT-SARS-CoV-2 assay from the menu.
5. Confirm that 40 tests have been assigned to the LDT-SARS-CoV-2 tube.
6. Repeat for each PPR tube loaded in the Open Access Pack.
7. Repeat for each additional Open Access Pack containing Panther Fusion SARS-CoV-2 assay PPR Solution tubes until the desired number of tests are loaded.
8. After all tubes are assigned, click Save to complete PPR loading.
9. Gently close the Fusion Universal Fluids Drawer.

E. Specimen Handling

Note: *Prepare specimens per the Specimen Processing instructions in the Specimen Collection and Storage section before loading specimens onto the Panther Fusion 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: *To avoid a processing error, ensure adequate specimen volume is added to the Panther Fusion Specimen Lysis Tube. When 500 µL of collected specimen is added to the Panther Fusion Specimen Lysis Tube, there is sufficient volume to perform 3 nucleic acid extractions.*

F. System Preparation

For instructions on setting up the Panther Fusion system including loading samples, reagents, assay cartridges and universal fluids, refer to the *Panther/Panther Fusion System Operator’s Manual.*

G. Load Panther Fusion SARS-CoV-2 Controls onto the Panther Fusion

1. Load the Sample Rack with the Panther Fusion SARS-CoV-2 Negative Control and the Panther Fusion SARS-CoV-2 Positive Control.
2. From the Sample Rack Bay screen, select the rack containing the controls and then Rack Details from the bottom of the screen.
3. Select the tube position in which the Panther Fusion SARS-CoV-2 Negative Control is loaded. The Sample Details screen opens.
4. Select Assign Open Access Control from the bottom of the screen.
5. Select the LDT-SARS-CoV-2 assay under the Assays column and the Negative Control under Control Types.
6. Select Assign.
7. Select the tube position in which the Panther Fusion SARS-CoV-2 Positive Control is loaded. The Sample Details screen opens.
8. Select Assign Open Access Control from the bottom of the screen.
9. Select the LDT-SARS-CoV-2 assay under the Assays column and the SARS-CoV-2 Positive Control under Control Types.
10. Select Assign.

Procedural Notes

A. Controls

1. The Panther Fusion SARS-CoV-2 Positive Control and Panther Fusion SARS-CoV-2 Negative Control tubes can be loaded in any rack position, in any Sample Bay lane on the Panther Fusion system.
2. Once the control tubes are pipetted and are processed for the Panther Fusion SARS-CoV-2 assay, they are active for up to 30 days (control frequency configured by an administrator) unless control results are invalid or a new assay cartridge lot is loaded.
3. Each control tube can be tested once.
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.

Quality Control

A run or specimen result may be invalidated by the Panther Fusion 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 lot of assay cartridges or Panther SARS-CoV-2 assay PPR Solution lot is loaded on the Panther Fusion system or when the current set of valid controls have expired.

The Panther Fusion system is configured to require assay controls run at an administrator-specified interval of up to 30 days. Software on the Panther Fusion 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 Fusion system. To generate valid results, the assay controls must pass a series of validity checks performed by the Panther Fusion 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 Fusion 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 Fusion 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 during the extraction process. During processing, the internal control acceptance criteria are automatically verified by the Panther Fusion 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 Fusion 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 Fusion system automatically determines the test results for samples and controls. A test result may be negative, positive, or invalid. The Panther Fusion SARS-CoV-2 assay is not a laboratory developed test. The LDT flag associated with reported results does not apply to this test. This assay has been authorized by the FDA under an Emergency Use Authorization.

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: POS result will be accompanied by cycle threshold (Ct) values.
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 if 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 pool 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 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. Nasal and mid-turbinate nasal swab specimens self-collected under the supervision of or collected by a health care provider and nasopharyngeal wash/aspirate or nasal aspirates are additional acceptable upper respiratory specimens that can be tested with the Panther Fusion 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 Panther Fusion 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 Panther Fusion 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.

Conditions of Authorization for Labs

The Panther Fusion 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/vitro-diagnostics-euas.

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

A. Authorized laboratories[a] using the Panther Fusion SARS-CoV-2 assay will include with result reports of the Panther Fusion 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 will 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 Panther Fusion SARS-CoV-2 assay as outlined in the Panther Fusion 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 Panther Fusion SARS-CoV-2 assay are not permitted.

D. Authorized laboratories implementing pooling strategies for testing patient specimens must use the “Specimen Pooling Implementation and Monitoring Guidelines” provided in the authorized tests’ Instructions for Use/Package Insert to evaluate the appropriateness of continuing to use such strategies based on the recommendations in the protocol.

E. Authorized laboratories that receive the Panther Fusion 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 Panther Fusion SARS-CoV-2 assay will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

G. Authorized laboratories will 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.

H. All laboratory personnel using the test must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use the test in accordance with the authorized labeling.

I. Hologic, its authorized distributor(s) and authorized laboratories using the Panther Fusion SARS-CoV- 2 assay will 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.

J. Authorized laboratories will 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.

[a] 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 Fusion SARS-CoV-2 Assay Performance

Analytical Sensitivity

The analytical sensitivity (limit of detection or LoD) of the Panther Fusion SARS-CoV-2 assay was determined by testing serial dilutions of pooled negative clinical nasopharyngeal swab specimens spiked with inactivated cultured SARS-CoV-2 virus (USA-WA1/2020; BEI Resources; NR-52281). Ten replicates of each serial dilution were evaluated using two assay reagent lots across two Panther Fusion systems. The LoD was determined to be $1x10^{-2}$ $TCID_{50}$/mL and verified by testing an additional 20 replicates with one assay reagent lot. The LoD of $1x10^{-2}$ $TCID_{50}$/mL was also confirmed using saline, Liquid Amies, and specimen transport medium (STM) swab collection media.

A similar analytical sensitivity study was performed using pooled negative clinical bronchoalveolar lavage fluid lower respiratory tract specimens. The LoD was determined and verified to be $1x10^{-2}$ $TCID_{50}$/mL in the Panther Fusion test sample.

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 Fusion system. The results are summarized in Table 2.

Table 2: 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.

Inclusivity

The inclusivity of the Panther Fusion SARS-CoV-2 assay was evaluated using *in silico* analysis of the assay primers and probes in relation to 341 SARS-CoV-2 sequences available in the NCBI and GISAID gene databases. Of the 341 sequences, 339 contained information corresponding to both target systems of the assay and 2 contained information corresponding to only one of the two target systems. The *in silico* analysis showed 100% homology to the primers and probes of both target systems for 335 of the evaluated sequences and 100% homology to the primers and probes of at least one target system for the 6 of the evaluated sequences. Five out of the 6 had only one nucleotide mismatch in one oligonucleotide of the second amplification system and were predicted to be reactive.

Analytical Specificity and Microbial Interference

The analytical specificity of the Panther Fusion SARS-CoV-2 assay was evaluated by testing 26 microorganisms representing common respiratory pathogens or closely related species (Table 3). Bacteria were tested at 10^6 CFU/mL and viruses were tested at 10^5 TCID50/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 Panther Fusion SARS-CoV-2 assay was 100% with no evidence of microbial interference.

In addition to microorganism testing, *in silico* analysis was performed to assess the specificity of the assay in relation to the microorganisms listed in Table 4. The *in silico* analysis showed no probable cross activity to any of the 183 GenBank sequences evaluated.

Table 3: Panther Fusion SARS-CoV-2 Analytical Specificity and Microbial Interference Microorganisms

Microorganism	Concentration	Microorganism	Concentration
Human coronavirus 229E	1E+5 $TCID_{50}$/mL	Parainfluenza virus 1	8.6E+4 $TCID_{50}$/mL
Human coronavirus OC43	1E+5 $TCID_{50}$/mL	Parainfluenza virus 2	1.5E+4 $TCID_{50}$/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+4 $TCID_{50}$/mL
SARS-coronavirus[1]	1E+6 copies/mL	Influenza A	1E+4 $TCID_{50}$/mL
MERS-coronavirus	2.5E+4 $TCID_{50}$/mL	Influenza B	6E+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	9.9E+4 $TCID_{50}$/mL
Respiratory syncytial virus	1E+5 $TCID_{50}$/mL	*Legionella pneumophila*	1E+6 CFU/mL
Chlamydia pneumoniae	5E+6 IFU/mL	*Mycobacterium tuberculosis*	9.9E+5 $TCID_{50}$/mL
Haemophilus influenzae	1E+6 CFU/mL	*Streptococcus pneumoniae*	1E+6 CFU/mL
Bordetella pertussis	1E+6 CFU/mL	*Streptococcus pyrogenes*	1E+6 CFU/mL
Pneumocystis jirovecii (PJP)	1E+6 nuc/mL	*Mycoplasma pneumoniae*	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.

Table 4: In Silico Analysis Microorganisms

Microorganism	Number of Strains Evaluated	Microorganism	Number of Strains Evaluated
Human coronavirus 229E	3	*Streptococcus pneumoniae*	3
Human coronavirus OC43	3	*Streptococcus pyrogenes*	2
Human coronavirus HKU1	3	*Bordetella pertussis*	3
Human coronavirus NL63	3	*Mycoplasma pneumoniae*	2
SARS-coronavirus	2	*Pneumocystis jirovecii* (PJP)	2
MERS-coronavirus	3	Influenza C	1
Adenovirus (e.g. C1 Ad. 71)	17	Parechovirus	24
Human Metapneumovirus (hMPV)	3	*Candida albicans*	1
Parainfluenza virus 1-4	15	*Corynebacterium diphtheriae*	7
Influenza A	2	*Bacillus anthracis (Anthrax)*	2
Influenza B	1	*Moraxella catarrhalis*	1
Enterovirus (e.g. EV68)	8	*Neisseria elongata and meningitidis*	4
Respiratory syncytial virus	3	*Pseudomonas aeruginosa*	2
Rhinovirus	3	*Staphylococcus epidermis*	2
Chlamydia pneumoniae	3	*Streptococcus salivarius*	4
Haemophilus influenzae	3	*Leptospirosis*	10
Legionella pneumophila	4	*Chlamydia psittaci*	1
Legionella non-pneumophila	24	*Coxiella burneti (Q-Fever)*	3
Mycobacterium tuberculosis	3	*Staphylococcus aureus*	3

Clinical Performance

The clinical performance of the Panther Fusion SARS-CoV-2 assay was evaluated in comparison to a panel of contrived specimens. For the study, a panel of 178 remnant clinical nasopharyngeal specimens was tested using two Panther Fusion SARS-CoV-2 assay reagent lots. All specimens were collected from US patients with signs and symptoms of respiratory infection. The panel consisted of 69 SARS-CoV-2 positive and 109 SARS-CoV-2 negative specimens. Of the 69 positive specimens, 45 were at concentrations 1-2x LoD and 24 were at concentrations 3-5x LoD using inactivated cultured SARS-CoV-2 virus (USA-WA1/2020; BEI Resources; NR-52281) as the target.

The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) was calculated in relation to the expected result of the contrived specimen panel, as shown in Table 5. Detection characteristics for positive contrived specimens were calculated by target concentration, as shown in Table 6.

A similar study was performed using a contrived panel of 178 remnant clinical bronchoalveolar lavage fluid lower respiratory tract specimens. Testing was performed using one assay reagent lot and two Panther Fusion systems. The PPA and NPA was calculated in relation to the expected result of the contrived specimen panel, as shown in Table 7. Detection characteristics for positive contrived specimens were calculated by target concentrations, as shown in Table 8.

Table 5: Panther Fusion SARS-CoV-2 Performance Relative to Expected Results for Swab Specimens

		Contrived Specimen Expected Result	
		Positive	Negative
Panther Fusion SARS-CoV-2 Assay	Positive	69	0
	Negative	0	109

Positive Percent Agreement: 100% (94.7% – 100%)
Negative Percent Agreement: 100% (96.6% – 100%)
Overall Agreement: 100% (96.6% – 100%)

Table 6: Panther Fusion SARS-CoV-2 Detection Characteristics for Positive Contrived Swab Specimens

Target Concentration	% Detected	Average SARS-CoV-2 Ct	SARS-CoV-2 Ct % CV
1xLoD	100% (10/10)	35.6	1.9%
1.5xLoD	100% (10/10)	35.0	1.7%
2xLoD	100% (25/25)	34.4	1.3%
3xLoD	100% (9/9)	33.7	0.7%
4xLoD	100% (5/5)	33.4	1.6%
5xLoD	100% (10/10)	33.0	0.6%

Table 7: Panther Fusion SARS-CoV-2 Performance Relative to Expected Results for LRT Specimens

		Contrived Specimen Expected Result	
		Positive	Negative
Panther Fusion SARS-CoV-2 Assay	Positive	70	0
	Negative	0	108

Positive Percent Agreement: 100% (94.8% – 100%)
Negative Percent Agreement: 100% (96.6% – 100%)
Overall Agreement: 100% (97.9% – 100%)

Table 8: Panther Fusion SARS-CoV-2 Detection Characteristics for Positive Contrived LRT Specimens

Target Concentration	% Detected	Average SARS-CoV-2 Ct	SARS-CoV-2 Ct % CV
1xLoD	100% (10/10)	35.6	2.4%
1.5xLoD	100% (10/10)	35.0	1.6%
2xLoD	100% (25/25)	34.5	2.4%
3xLoD	100% (10/10)	33.9	1.5%
4xLoD	100% (5/5)	33.5	1.9%
5xLoD	100% (10/10)	33.1	0.9%

Clinical Performance of Pooling up to 5 Specimens

The clinical performance of the Panther Fusion SARS-CoV-2 assay was evaluated in pools consisting of up to 5 specimens. For the study, a pool size of 3 specimens and a pool size of 5 specimens was evaluated. Testing for both pool sizes 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 pool size of 3 study, 32 positive and 20 negative specimen pools were evaluated. For the pool size of 5 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 (defined as within 1-2 Ct of the assay LoD). Both the pooled and individual specimens were evaluated with the Panther Fusion SARS-CoV-2 assay.

The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were calculated in relation to the expected (individual) result for each evaluated pool size, as shown in Table 9 and Table 10. With a pool size of 5, two of the ten specimens evaluated with target concentrations at or near the LoD of the assay yielded an individual positive result but were not detected as part of a specimen pool.

Scatter plots for the observed individual specimen and pooled specimen Ct values for a pool size of 3 and pool size of 5 are shown in Figure 1 and Figure 2, respectively. With a pool size of 3, a linear relationship was observed between most individual and pooled specimen Ct values, and the observed average shift in Ct of 1.5 between the individual specimens and pooled specimens aligns to the expected shift. With a pool size of 5, a linear relationship was observed between most individual and pooled specimen Ct values for specimens with an individual result, and the observed average shift in Ct of 2.3 between the individual specimens and pooled specimens aligns to the expected shift. With a pool size of 5, individual specimens at or near the LoD of the assay with a Ct value ≥ 34.1 did not show consistent linearity between the individual and pooled specimen Ct values.

An *in silico* analysis of historical data for individual specimens from four geographically distinct customer sites was performed to estimate the expected PPA for a pool size of 3 or a pool size of 5. The *in silico* analysis was performed using the pooling interpretative criteria determined in the pooling clinical study. These criteria are illustrated as line graphs in Figure 3 and Figure 4 respectively. The applied pooling interpretative criteria are as follows: for a pool size of 3, specimens with an individual test Ct value < 40.5 are not expected to be missed, and 100% of specimens with an individual test Ct ≥ 40.5 are expected to be missed. For a pool size of 5, specimens with an individual test Ct value < 34.1 are not expected to be missed, 20% of specimens with an individual test Ct value ≥ 34.1 and < 39.7 are expected to be missed, and 100% of specimens with an individual test Ct ≥ 39.7 are expected to be missed. The results of this analysis are shown in Table 11. Overall, ≥ 99.7% and ≥ 94.5% PPA was observed across all sites for a pool size of 3 and pool size of 5, respectively. The results of the *in silico* analysis support 3- and 5-specimen pooling on the Panther Fusion SARS-CoV-2 analysis.

Table 9: Individual and Pooled Specimen Agreement with a Pool Size of 3

		Individual Specimen Result		
		Positive	Negative	Total
Pool of 3 Result	Positive	32	0	32
	Negative	0	20	20
	Total	32	20	52

Positive Percent Agreement: 100.0% (89.3% - 100.0%)
Negative Percent Agreement: 100.0% (83.9% - 100.0%)
Overall Agreement: 100.0% (93.1% - 100.0%)

Table 10: Individual and Pooled Specimen Agreement with a Pool Size of 5

		Individual Specimen Result		
		Positive	Negative	Total
Pool of 5 Result	Positive	47	0	47
	Negative	2	20	22
	Total	49*	20	69

*One specimen with an invalid individual result was removed from analysis.

Positive Percent Agreement: 95.9% (86.3% - 98.9%)
Negative Percent Agreement: 100.0% (83.9% - 100.0%)
Overall Agreement: 97.1% (90.0% - 99.2%)

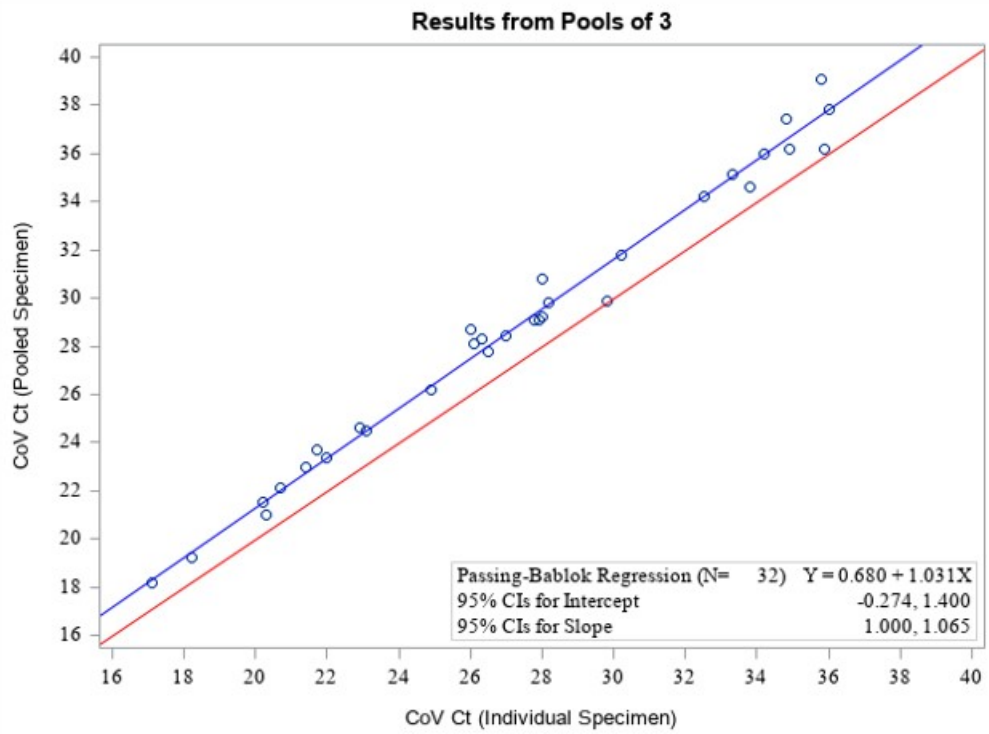

Figure 1. Individual Specimen and Pooled Specimen Ct Scatter Plot for Pool Size of 3

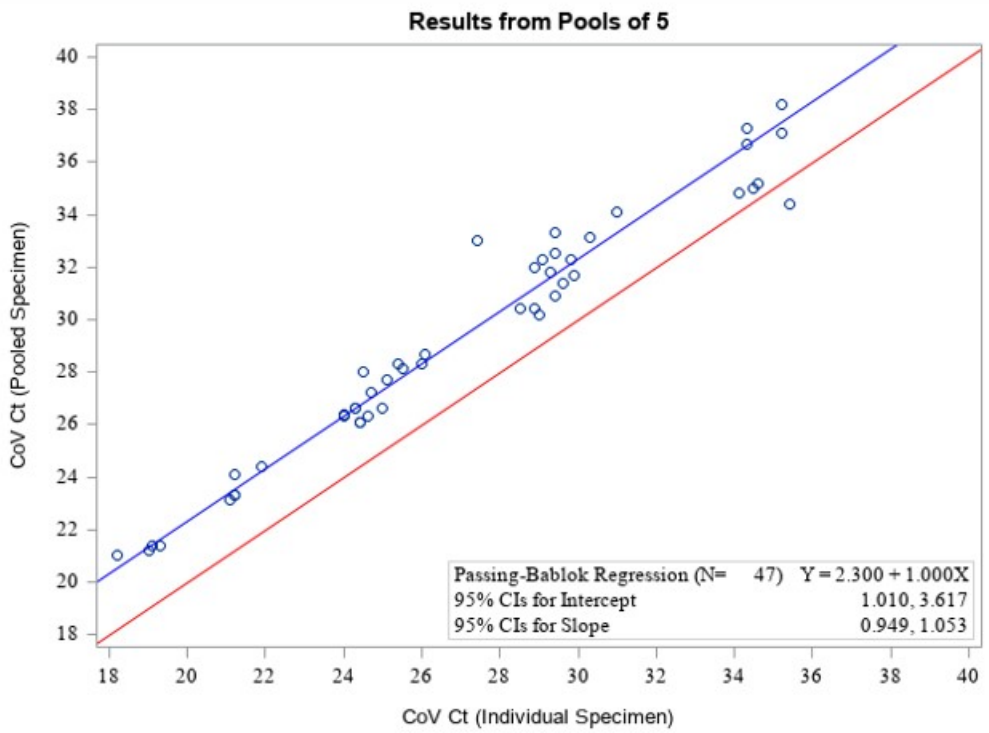

Figure 2. Individual Specimen and Pooled Specimen Ct Scatter Plot for Pooled Size of 5

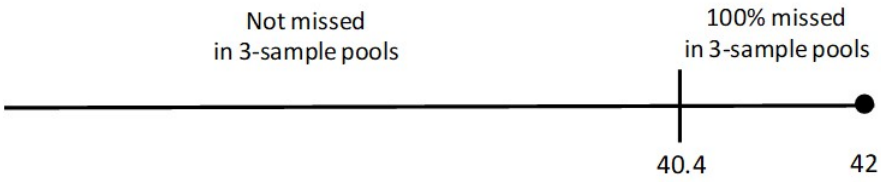

Figure 3. Performance Line Graph for Pool Size of 3

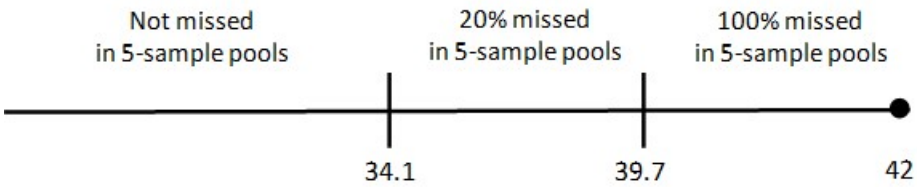

Figure 4. Performance Line Graph for Pool Size of 5

Table 11: In-silico Sensitivity Analysis of Customer Data Set

	Pool Size of 3			Pool Size of 5		
Site Location	**N Total**	**N Positive**	**PPA (95% C.I.)**	**N Total**	**N Positive**	**PPA (95% C.I.)**
New York	28,801	28,780	99.9% (99.9% - 100%)	28,801	27,349.2	95.0% (94.7% - 95.2%)
Utah	1,906	1,900	99.7% (99.3% - 99.9%)	1,906	1811.0	95.0% (93.9% - 95.9%)
California	288	288	100% (98.7% - 100%)	288	272.2	94.5% (91.3% - 96.6%)
Wisconsin	60	60	100% (94.0% - 100%)	60	58.2	97.0% (89.1% - 99.2%)
All Sites	31,055	31,028	99.9% (99.9% - 99.9%)	31,055	29,490.6	95.0% (94.7% - 95.2%)

Bibliography

1. **World Health Organization.** Q&A on coronaviruses (COVID-19). March 9, 2020. World Health Organization Web site https://www.who.int/news-room/q-a-detail/q-a-coronaviruses. Accessed March 10, 2020.
2. **Centers for Disease Control and Prevention.** https://www.cdc.gov/coronavirus/2019-ncov/prevent-getting-sick/how-covid-spreads.html. Accessed June 17,2020.
3. **Centers for Disease Control and Prevention.** Coronavirus Disease 2019-(COVID-19) in the U.S. Updated March 10, 2020. Centers for Disease Control and Prevention Web site https://www.cdc.gov/coronavirus/2019-ncov/cases-in-us.html. Accessed March 10, 2020.
4. **Centers for Disease Control and Prevention.** Coronavirus Disease 2019 Information for Travel. Page last reviewed March 8, 2020. Centers for Disease Control and Prevention Web site https://www.cdc.gov/coronavirus/2019-ncov/travelers/index.html. Accessed March 10, 2020.
5. **Centers for Disease Control and Prevention.** Coronavirus Disease 2019-(COVID-19) Situation Summary. Updated March 9, 2020. Centers for Disease Control and Prevention Web site https://www.cdc.gov/coronavirus/2019-ncov/summary.html. Accessed March 10, 2020.
6. **Clinical & Laboratory Standards Institute.** Document M29 Protection of Laboratory Workers from Occupationally Acquired Infections. CLSI Web site https://clsi.org/standards/products/microbiology/documents/m29/. Accessed September 2017.

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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 Panther Fusion 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 12, 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 Panther Fusion SARS-CoV-2 assay has been validated for 5-specimen pooling.

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

Table 12: 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 12.
- 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.