Rx Only

cobas® SARS-CoV-2

Qualitative assay for use on the cobas® 6800/8800 Systems

For use under the Emergency Use Authorization (EUA) only

For in vitro diagnostic use

cobas® SARS-CoV-2	P/N: 09175431190
cobas® SARS-CoV-2 Control Kit	P/N: 09175440190
cobas® 6800/8800 Buffer Negative Control Kit	P/N: 07002238190

Table of Contents

Intended use

cobas® SARS-CoV-2 for use on the **cobas**® 6800/8800 Systems is a real-time RT-PCR test intended for the qualitative detection of nucleic acids from SARS-CoV-2 in clinician-instructed self-collected nasal swab specimens (collected on site), and clinician-collected nasal, nasopharyngeal, and oropharyngeal swab specimens from individuals who meet COVID-19 clinical and/or epidemiological criteria. **cobas**® SARS-CoV-2 is for use only under Emergency Use Authorization (EUA) in U.S. laboratories certified under CLIA to perform high or moderate complexity tests.

Results are for the detection of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in 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. 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 clinical observations, patient history, and epidemiological information.

cobas® SARS-CoV-2 is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and on the use of the **cobas**® 6800/8800 Systems. **cobas**® SARS-CoV-2 is only for use under the Food and Drug Administration's Emergency Use Authorization.

Summary and explanation of the test

Explanation of the test

cobas® SARS-CoV-2 is a qualitative test for use on the **cobas**® 6800 System and **cobas**® 8800 System for the detection of the 2019 novel coronavirus (SARS-CoV-2) RNA in nasal, nasopharyngeal, and oropharyngeal swab samples collected in Copan Universal Transport Medium System (UTM-RT), BD™ Universal Viral Transport System (UVT), **cobas**® PCR Media, or 0.9% physiological saline. The RNA Internal Control, used to monitor the entire sample preparation and PCR amplification process, is introduced into each specimen during sample processing. In addition, the test utilizes external controls (low titer positive control and a negative control).

Principles of the procedure

cobas® SARS-CoV-2 is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The **cobas**® 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the **cobas**® 6800/8800 software, which assigns test results for all tests. Results can be reviewed directly on the system screen, and printed as a report.

Nucleic acid from patient samples and added internal control RNA (RNA IC) molecules are simultaneously extracted. Nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris and potential PCR inhibitors, are removed with subsequent wash steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature. External controls (positive and negative) are processed in the same way with each **cobas**® SARS-CoV-2 run.

Selective amplification of target nucleic acid from the sample is achieved by the use of target-specific forward and reverse primers for ORF1 a/b non-structural region that is unique to SARS-CoV-2. Additionally, a conserved region in the structural protein envelope E-gene were chosen for pan-Sarbecovirus detection. The pan-Sarbecovirus detection sets will also detect SARS-CoV-2 virus.

Selective amplification of RNA Internal Control is achieved by the use of non-competitive sequence specific forward and reverse primers which have no homology with the coronavirus genome. A thermostable DNA polymerase enzyme is used for amplification.

The **cobas**® SARS-CoV-2 master mix contains detection probes which are specific for the coronavirus type SARS-CoV-2, members of the Sarbecovirus subgenus, and the RNA Internal Control nucleic acid. The coronavirus and RNA Internal Control detection probes are each labeled with unique fluorescent dyes that act as a reporter. Each probe also has a second dye which acts as a quencher. When not bound to the target sequence, the fluorescent signals of the intact probes are suppressed by the quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5' to 3' exonuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly. Each reporter dye is measured at defined wavelengths, which enables simultaneous detection and discrimination of the amplified coronavirus target and the RNA Internal Control. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon). Any contaminating amplicons from previous PCR runs are destroyed by the AmpErase enzyme [uracil-N-glycosylase], which is included in the PCR mix, when heated in the first thermal cycling step. However, newly formed amplicons are not destroyed since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

Reagents and materials

The materials provided for **cobas**® SARS-CoV-2 can be found in Table 1. Materials required, but not provided can be found in Table 2, Table 3, Table 4, Table 7, Table 8, and Table 9.

Refer to the **Reagents and materials** section and **Precautions and handling requirements** section for the hazard information for the product.

cobas® SARS-CoV-2 reagents and controls

All unopened reagents and controls shall be stored as recommended in Table 1 to Table 4.

Table 1 cobas® SARS-CoV-2

cobas® SARS-CoV-2 Store at 2-8°C 192 test cassette (P/N 09175431190)		
Kit components	**Reagent ingredients**	**Quantity per kit 192 tests**
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% proteinase EUH210: Safety data sheet available on request. EUH208: Contains Subtilisin. May produce an allergic reaction.	22.3 mL
RNA Internal Control (RNA IC)	Tris buffer, <0.05% EDTA, <0.001% non-Sarbecovirus related armored RNA construct containing primer and probe specific primer sequence regions (non-infectious RNA in MS2 bacteriophage), <0.1% sodium azide	21.2 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxybenzoate	21.2 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	7.5 mL
SARS-CoV-2 Master Mix Reagent 2 (SARS-CoV-2 MMX-R2)	Tricine buffer, potassium acetate, < 18% dimethyl sulfoxide, glycerol, < 0.1% Tween 20, EDTA, < 0.12% dATP, dCTP, dGTP, dUTPs, < 0.01% upstream and downstream SARS-CoV-2 and Sarbecovirus primers, < 0.01% Internal Control forward and reverse primers, < 0.01% fluorescent-labeled oligonucleotide probes specific for SARS-CoV-2, Sarbecovirus, and the RNA Internal Control, < 0.01% oligonucleotide aptamer, < 0.1% Z05D DNA polymerase, < 0.10% AmpErase (uracil-N-glycosylase) enzyme (microbial), < 0.1% sodium azide	9.7 mL

Table 2 cobas® SARS-CoV-2 Control Kit

cobas® SARS-CoV-2 Control Kit Store at 2–8°C (P/N 09175440190)		
Kit components	**Reagent ingredients**	**Quantity per kit**
SARS-CoV-2 Positive Control (SARS-CoV-2 (+)C)	Tris buffer, < 0.05% Sodium azide, < 0.005% EDTA, < 0.003% Poly rA, < 0.01% Non-infectious plasmid DNA (microbial) containing SARS-CoV-2 sequence, < 0.01% Non-infectious plasmid DNA (microbial) containing pan-Sarbecovirus sequence	16 mL (16 x 1 mL)

Table 3 cobas® Buffer Negative Control Kit

cobas® Buffer Negative Control Kit Store at 2-8°C (P/N 07002238190)		
Kit components	**Reagent ingredients**	**Quantity per kit**
cobas® Buffer Negative Control (BUF (-) C)	Tris buffer, < 0.1% sodium azide, EDTA, < 0.002% Poly rA RNA (synthetic)	16 mL (16 x 1 mL)

cobas omni reagents for sample preparation

Table 4 **cobas omni** reagents for sample preparation*

Reagents	Reagent ingredients	Quantity per kit	Safety symbol and warning**
cobas omni MGP Reagent (MGP) Store at 2–8°C (P/N 06997546190)	Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	480 tests	Not applicable
cobas omni Specimen Diluent (SPEC DIL) Store at 2–8°C (P/N 06997511190)	Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	4 x 875 mL	Not applicable
cobas omni Lysis Reagent (LYS) Store at 2–8°C (P/N 06997538190)	43% (w/w) guanidine thiocyanate***, 5% (w/v) polydocanol***, 2% (w/v) dithiothreitol***, dihydro sodium citrate	4 x 875 mL	  **DANGER** H302 + H332: Harmful if swallowed or if inhaled. H314: Causes severe burns and eye damage. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P261: Avoid breathing dust/fume/gas/mist/vapours/ spray. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol
cobas omni Wash Reagent (WASH) Store at 15–30°C (P/N 06997503190)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2 L	Not applicable

* These reagents are not included in the **cobas**® SARS-CoV-2 test kit. See listing of additional materials required (Table 7).
** Product safety labeling primarily follows EU GHS guidance
***Hazardous substance

Reagent storage and handling requirements

Reagents shall be stored and will be handled as specified in Table 5 and Table 6.

When reagents are not loaded on the **cobas**® 6800/8800 Systems, store them at the corresponding temperature specified in Table 5.

Table 5 Reagent storage (when reagent is not on the system)

Reagent	Storage temperature
cobas® SARS-CoV-2 -192	2–8°C
cobas® SARS-CoV-2 Control Kit	2–8°C
cobas® Buffer Negative Control Kit	2–8°C
cobas omni Lysis Reagent	2–8°C
cobas omni MGP Reagent	2–8°C
cobas omni Specimen Diluent	2–8°C
cobas omni Wash Reagent	15–30°C

Reagents loaded onto the **cobas**® 6800/8800 Systems are stored at appropriate temperatures and their expiration is monitored by the system. The **cobas**® 6800/8800 Systems allow reagents to be used only if all of the conditions shown in Table 6 are met. The system automatically prevents use of expired reagents. Table 6 allows the user to understand the reagent handling conditions enforced by the **cobas**® 6800/8800 Systems.

Table 6 Reagent expiry conditions enforced by the **cobas**® 6800/8800 Systems

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	On-board stability (cumulative time on board outside refrigerator)
cobas® SARS-CoV-2 – 192	Date not passed†	90 days from first usage*,†	Max 40 runs†	Max 40 hours†
cobas® SARS-CoV-2 Control Kit	Date not passed†	Not applicable[a]	Not applicable	Max 8 hours†
cobas® Buffer Negative Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 10 hours
cobas omni Lysis Reagent	Date not passed	30 days from loading*	Not applicable	Not applicable
cobas omni MGP Reagent	Date not passed	30 days from loading*	Not applicable	Not applicable
cobas omni Specimen Diluent	Date not passed	30 days from loading*	Not applicable	Not applicable
cobas omni Wash Reagent	Date not passed	30 days from loading*	Not applicable	Not applicable

[a]Single use reagents

*Time is measured from the first time that reagent is loaded onto the **cobas**® 6800/8800 Systems.

†The performance has not been established for suggested use cycles and time, but is based on similar reagents used on the same system.

Additional materials required

Table 7 Materials and consumables for use on **cobas**® 6800/8800 Systems

Material	P/N
cobas omni Processing Plate	05534917001
cobas omni Amplification Plate	05534941001
cobas omni Pipette Tips	05534925001
cobas omni Liquid Waste Container	07094388001
cobas omni Lysis Reagent	06997538190
cobas omni MGP Reagent	06997546190
cobas omni Specimen Diluent	06997511190
cobas omni Wash Reagent	06997503190
Solid Waste Bag	07435967001
Solid Waste Bag and Solid Waste Container or Solid Waste Bag With Insert and Kit Drawer	07435967001 and 07094361001 or 08030073001 and 08387281001
Solid Waste Container	07094361001
cobas omni Secondary Tubes 13x75 (optional)	06438776001
cobas® PCR Media Tube Replacement Cap Kit	07958056190
cobas® PCR Media Disposable Tube Stand (Optional)	07958064190
MPA RACK 16 MM LIGHT GREEN 7001-7050*,**	03143449001
RD5 RACK – RD Standard rack 0001-0050 *LR*,**	11902997001

* MPA 16mm and RD5 racks are required to use **cobas**® SARS-COV-2. Contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack trays accepted on the instruments.

MPA 16mm rack is the preferred rack for use with samples collected in **cobas® PCR Media tubes. If RD5 racks are used, make sure to fill in the sample tubes with not less than the recommended minimum sample input. The tubes sit higher in an RD5 rack because of the rubber gasket at the bottom of each tube position. Therefore, it is possible that when using RD5 racks, the system could accept tubes that are below the minimum sample input volume and cause pipetting errors later in the run.

Table 8 Specimen collection kits used with **cobas**® SARS-CoV-2

Collection Kit	P/N
cobas® PCR Media Uni Swab Sample Kit	07958030190
cobas® PCR Media Dual Swab Sample Kit	07958021190
cobas® PCR Media 100 tube kit	06466281190

Instrumentation and software required

The **cobas**® 6800/8800 software and **cobas**® SARS-CoV-2 analysis package must be installed on the instrument(s). The Instrument Gateway (IG) server will be provided with the system.

Table 9 Instrumentation

Equipment	P/N
cobas® 6800 System (Moveable Platform)	05524245001
cobas® 6800 System (Fixed Platform)	06379664001
cobas® 8800 System	05412722001
Sample Supply Module	06301037001
Instrument Gateway	06349595001

For additional information, please refer to the **cobas**® 6800/8800 Systems – User Assistance and/or User Guide.

Note: Contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack trays accepted on the instruments.

Precautions and handling requirements

Warnings and precautions

As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

- For *in vitro* diagnostic use under Emergency Use Authorization only.
- Positive results are indicative of the presence of SARS-CoV-2 RNA.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.[1,2] Only personnel proficient in handling infectious materials and the use of **cobas**® SARS-CoV-2 and **cobas**® 6800/8800 Systems should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures.
- The use of sterile disposable pipettes and nuclease-free pipette tips is recommended. Use only supplied or specified required consumables to ensure optimal test performance.
- Safety Data Sheets (SDS) are available on request from your local Roche representative.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.

Reagent handling

- Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples or controls.
- Before use, visually inspect each reagent cassette, diluent, lysis reagent, and wash reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- **cobas omni** Lysis Reagent contains guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.
- **cobas**® SARS-CoV-2 test kit, **cobas**® SARS-CoV-2 Control kit, **cobas**® Buffer Negative Control kit, **cobas omni** MGP Reagent, and **cobas omni** Specimen Diluent contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
- Do not allow **cobas omni** Lysis Reagent, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.
- Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink, or smoke in designated work areas.
- Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples and **cobas**® SARS-CoV-2 kits, **cobas**® SARS-CoV-2 Control kit, **cobas**® Buffer Negative Control kit and **cobas omni** reagents to prevent contamination. Avoid contaminating gloves when handling samples and controls.
- Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas**® 6800/8800 instrument, follow the instructions in the **cobas**® 6800/8800 Systems – User Assistance and/or User Guide to properly clean and decontaminate the surface of instrument(s).

Sample collection, transport, and storage

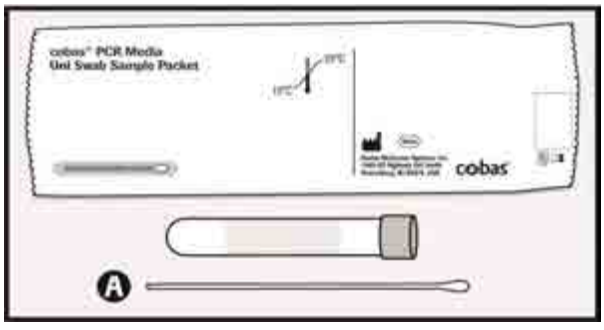
Note: Handle all samples and controls as if they are capable of transmitting infectious agents.

Sample collection

- Collect nasal, nasopharyngeal and oropharyngeal specimens according to standard collection technique using flocked or polyester-tipped swabs and immediately place in 3 mL of Copan Universal Transport Medium (UTM-RT) or BD™ Universal Viral Transport (UVT).
- Collect nasal specimens according to standard collection technique using flocked or polyester-tipped swabs and immediately place into **cobas**® PCR Media tube from **cobas**® PCR Media Kit (P/N 06466281190).
- Collect nasal specimens using the **cobas**® PCR Media Uni Swab Sample Kit (P/N 07958030190) or **cobas**® PCR Media Dual Swab Sample Kit (P/N 07958021190) according to instructions below.

Nasal (anterior nares) swab specimen collection – clinician or self-collected on site

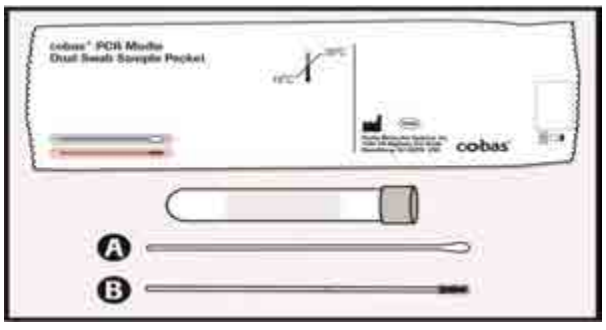
WARNING: DO NOT PRE-WET SWAB IN cobas® PCR MEDIA BEFORE COLLECTION!



The **cobas**® PCR Media Uni Swab Sample kit contains:

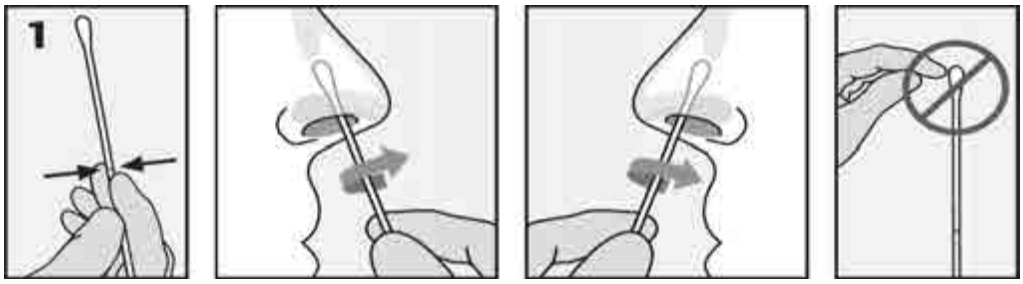
cobas® PCR Media tube
Woven Swab: A

OR



The **cobas**® PCR Media Dual Swab Sample kit contains:

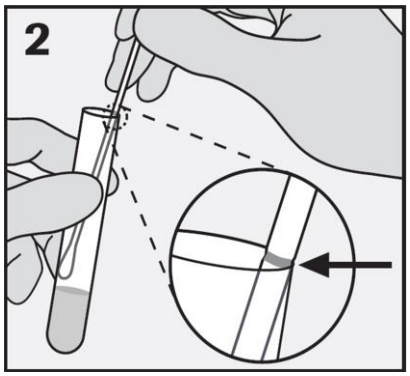
cobas® PCR Media Tube
Woven Swab: A
Flocked Swab: B



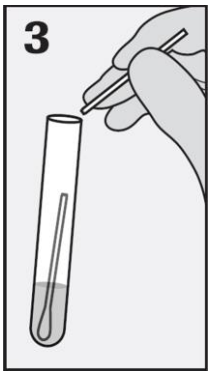
DO NOT PRE-WET SWAB IN cobas® PCR MEDIA BEFORE COLLECTION!

1. **COLLECT:** Hold the woven swab (Swab A) or the flocked swab (Swab B) with the scoreline above your hand. Insert the swab 1–2 cm into one of the anterior nares. Rotate the swab against the nasal mucosa for about 3 seconds and withdraw. Repeat with the other anterior nare using the same swab.

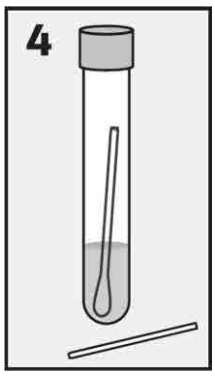
   Do not let the swab touch any surface before placing it into the collection tube.



2. **ALIGN:** Remove the cap from the **cobas**® PCR Media Tube and lower the swab specimen into the tube until the visible scoreline on the swab is aligned with the tube rim.



3. **BREAK:** Carefully leverage the swab against the tube rim to break the swab shaft at the scoreline.



4. **CLOSE: Tightly** re-cap the **cobas**® PCR Media Tube. The specimen is now ready for transport. Discard the top portion of the swab.

- Collect nasal specimens according to standard collection technique using flocked or polyester-tipped swabs and immediately place in 3 mL of 0.9% physiological saline.

Transport and storage

- Transportation of collected specimens must comply with all applicable regulations for the transport of etiologic agents.
- Transport and store samples collected in **cobas**® PCR Media or 0.9% physiological saline as follows:
  - After collection, specimens in **cobas**® PCR Media or 0.9% physiological saline should be stored at 2-8°C and processed within 48 hours.
- Sample stability when using **cobas**® SARS-CoV-2 has not been established for suggested temperatures and time, but is based on viability data from testing similar viruses in the UTM-RT or UVT Systems as stated in Copan UTM-RT System Instructions For Use and shown below:
  - After collection, the specimen should be stored at 2-25°C and processed within 48 hours.
  - If delivery and processing exceed 48 hours, specimens should be transported in dry ice and once in laboratory frozen at -70°C or colder.

Instructions for use

Procedural notes

- Do not use **cobas**® SARS-CoV-2 reagents, **cobas**® SARS-CoV-2 Control Kit, **cobas**® Buffer Negative Control Kit, or **cobas omni** reagents after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- Refer to the **cobas**® 6800/8800 Systems – User Assistance and/or User Guide for proper maintenance of instruments.

Running cobas® SARS-CoV-2

cobas® SARS-CoV-2 can be run with a minimum required sample volume of 0.6 mL in the **cobas omni** secondary tube for specimens collected in Copan Universal Transport Medium (UTM-RT), BD™ Universal Viral Transport (UVT), **cobas**® PCR Media or 0.9% physiological saline. Specimens collected using **cobas**® PCR Media Uni Swab Sample Kit or **cobas**® PCR Media Dual Swab Sample Kit can be run in their primary collection tube with a minimum required sample volume of 1.0 mL.

Specimens collected in cobas® PCR Media, 0.9% physiological saline, UTM-RT or UVT

Specimens collected in Copan Universal Transport Medium (UTM-RT), BD™ Universal Viral Transport (UVT), **cobas**® PCR Media or 0.9% physiological saline must be transferred into a cobas omni Secondary tube prior to processing on the **cobas**® 6800/8800 Systems. Samples transferred to **cobas omni** Secondary tubes should be processed using the 'Swab' sample type selection in the user interface (UI) of the **cobas**® SARS-CoV-2 as described in Table 10.

Always use caution when transferring specimens from a primary collection tube to a secondary tube.

Use pipettes with aerosol-barrier or positive-displacement tips to handle specimens.

Always use a new pipette tip for each specimen.

Ensure samples are equilibrated to room temperature prior to transfer into a cobas omni Secondary Tube.

Follow the steps below to transfer patient sample from a primary collection tube into a **cobas omni** Secondary Tube:

- Unscrew the primary sample tube cap.
- Lift the cap and any attached swab to allow a pipette to be inserted into the sample tube.
- Transfer 0.6 mL into the prepared barcoded secondary tube.
- Transfer secondary tube to a rack. Close the primary sample tube cap.

Specimens collected using cobas® PCR Media Uni or Dual Swab Sample Kit

Samples collected using **cobas**® PCR Media Uni Swab Sample Kit or **cobas**® PCR Media Dual Swab Sample Kit must be uncapped and can be loaded directly onto racks for processing on the **cobas**® 6800/8800 Systems. Transfer into a secondary tube is not neccessary. **cobas**® PCR Media tubes fit on to the MPA RACK 16 MM LIGHT GREEN 7001-7050 (P/N 03143449001) and can be processed with the swab remaining in the tube. Samples collected using **cobas**® PCR Media Uni Swab Sample Kit or **cobas**® PCR Media Dual Swab Sample Kits should be processed using the '**cobas**® PCR Media swab' sample type selection in the user interface (UI) of the cobas® SARS-CoV-2 as described inTable 10.

A properly collected swab specimen should have a single swab with the shaft broken at the scoreline. Swab shafts which are broken above the score line will appear longer than normal and may also be bent over to fit into the cobas® PCR Media tube. This may create an obstruction to the pipetting system which might cause the loss of sample, test results and/or mechanical damage to the instrument. In the event that a swab specimen has an improperly broken shaft, remove the swab prior to sample processing on the **cobas**® 6800/8800 Systems. Use caution when disposing of specimen swabs; avoid splashing or touching swabs to other surfaces during disposal to prevent contamination.

Incoming **cobas**® PCR Media primary swab specimen tubes with no swabs or with two swabs have not been collected according to the instructions in their respective collection kit IFU and should not be tested. If the sample containing two swabs in the **cobas**® PCR Media primary tubes must be tested, transfer 0.6 mL into the prepared barcoded secondary tube.

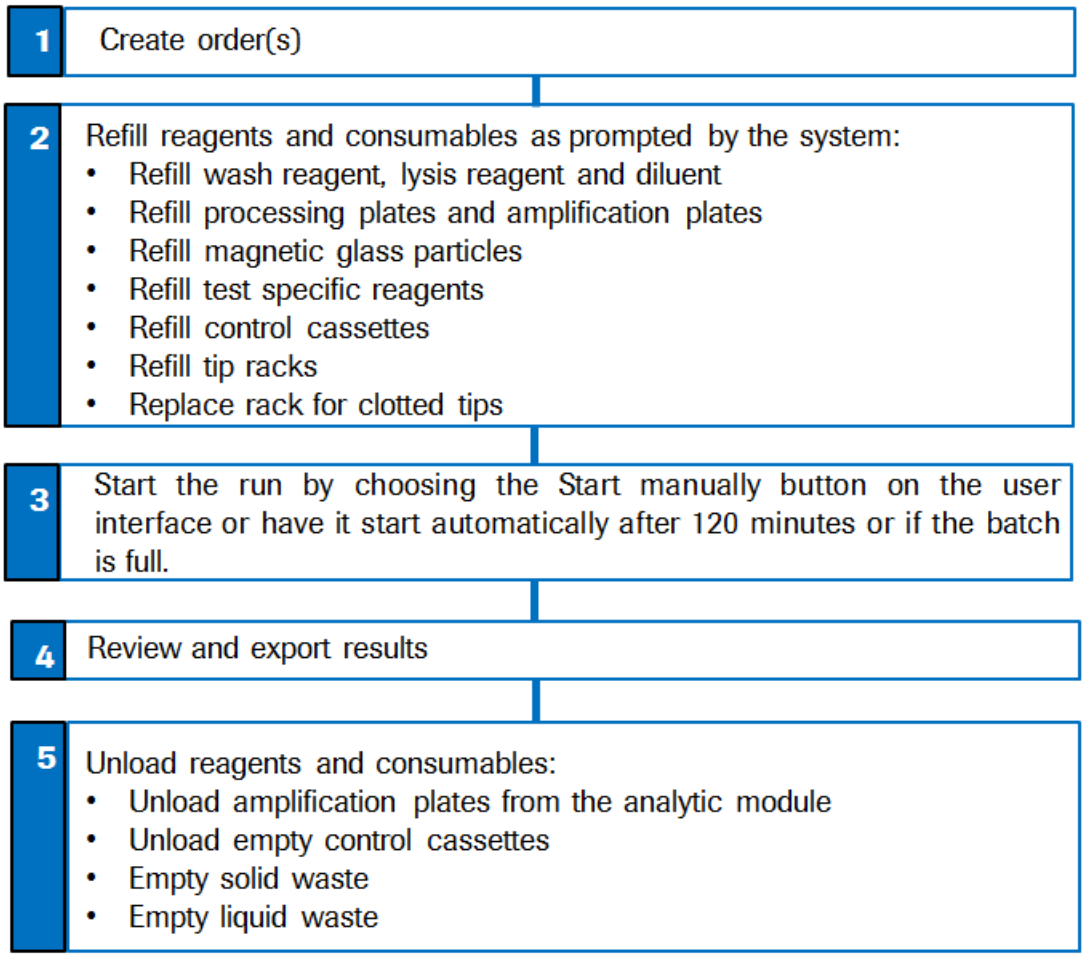
Occasionally, incoming swab specimens contain excessive mucus which may induce a pipetting error (e.g., clot or other obstruction) on the **cobas**® 6800/8800 Systems. Prior to retesting of specimens that exhibited clots during initial processing, remove and discard the swab, then re-cap and vortex these specimens for 30 seconds to disperse the excess mucus.Swab specimens can be processed twice on the **cobas**® 6800/8800 Systems while the swab is in the collection tube. If additional testing is required, or if the first test fails due to specimen pipetting error (e.g., clot or other obstruction), the swab must be removed and the remaining fluid must have a minimum volume of 1.0 mL.

Table 10 Sample type selection in the user interface of the **cobas**® SARS-CoV-2

Collection kit/Matrix type	**Minimum volume (mL)** **Processing tube**	**Process as Sample Type**
Copan Universal Transport Medium BD™ Universal Viral Transport 0.9% physiological saline **cobas**® PCR Media Kit	0.6 mL **cobas omni** Secondary tube	**Swab**
cobas® PCR Media Uni or Dual Swab Sample Kit	1.0 mL Primary tube	**cobas® PCR media swab**

The test procedure is described in detail in the **cobas**® 6800/8800 Systems – User Assistance and/or User Guide. Figure 1 below summarizes the procedure.

Figure 1 **cobas**® SARS-CoV-2 procedure



Results

The **cobas**® 6800/8800 Systems automatically detect the SARS-CoV-2 , for each individually processed sample and control, displaying individual target results for samples as well as test validity and overall results for controls.

Quality control and validity of results

- One **cobas**® Buffer Negative Control [(-) Ctrl] and one [SARS-CoV-2 (+)C] are processed with each batch.
- In the **cobas**® 6800/8800 software and/or report, check for flags and their associated results to ensure the batch validity.
- All flags are described in the **cobas**® 6800/8800 Systems User Guide.
- The batch is valid if no flags appear for any controls. If the batch is invalid, repeat testing of the entire batch.

Validation of results is performed automatically by the **cobas**® 6800/8800 software based on negative and positive control performance.

Interpretation of results

cobas® SARS-CoV-2 for System Software v1.2

Display examples for **cobas**® SARS-CoV-2 for System Software v1.2 or higher are shown in Figure 2.

Figure 2 Example of **cobas**® SARS-CoV-2 results display for System Software v1.2

Test	Sample ID	Valid*	Flags	Sample type	Overall result*	Target 1	Target 2
SARS-CoV-2 400 µL	Swab_01	Yes		Swab	**Negative**	Negative	Negative
SARS-CoV-2 400 µL	Swab _C1	No	Y40T	Swab	**Invalid**	Invalid	Invalid
SARS-CoV-2 400 µL	Swab _B1	Yes		Swab	**Reactive**	Negative	Positive
SARS-CoV-2 400 µL	Swab _B2	Yes		Swab	**Positive**	Positive	Positive
SARS-CoV-2 400 µL	Swab _D1	Yes		Swab	**Negative**	Negative	Negative
SARS-CoV-2 400 µL	Swab _A6	Yes		Swab	**Reactive**	Positive	Negative
SARS-CoV-2 400 µL	Swab _E1	No	C01H2	Swab	**Invalid**	Positive	Invalid
SARS-CoV-2 400 µL	Swab _A2	No	C01H1	Swab	**Invalid**	Invalid	Positive
SARS-CoV-2	C161420284090428828404	Yes		(-) Ctrl	**Valid**	Valid	Valid
SARS-CoV-2	C16142028409300958026 4	Yes		SARS-CoV-2 (+) C	**Valid**	Valid	Valid

* The “Valid” and “Overall Result” columns are not applicable to sample results for the **cobas**® SARS-CoV-2. Values reported in these columns are not applicable and do not impact the validity of results reported within individual Target Result columns. Refer to Table 11, **cobas**® SARS-CoV-2 results interpretation, for specific instructions on test results interpretation.

cobas® SARS-CoV-2 for System Software v1.3 or higher

Display examples for **cobas**® SARS-CoV-2 for System Software v1.3 or higher are shown in Figure 3.

Figure 3 Example of **cobas**® SARS-CoV-2 results display for System Software v1.3 or higher

Test	Sample ID	Valid*	Flags	Sample type	Overall result*	Target 1	Target 2
SARS-CoV-2 400 µL	Swab_01	NA		Swab	**NA**	Negative	Negative
SARS-CoV-2 400 µL	Swab _C1	NA	Y40T	Swab	**NA**	Invalid	Invalid
SARS-CoV-2 400 µL	Swab _B1	NA		Swab	**NA**	Negative	Positive
SARS-CoV-2 400 µL	Swab _B2	NA		Swab	**NA**	Positive	Positive
SARS-CoV-2 400 µL	Swab _D1	NA		Swab	**NA**	Negative	Negative
SARS-CoV-2 400 µL	Swab _A6	NA		Swab	**NA**	Positive	Negative
SARS-CoV-2 400 µL	Swab _E1	NA	C01H2	Swab	**NA**	Positive	Invalid
SARS-CoV-2 400 µL	Swab _A2	NA	C01H1	Swab	**NA**	Invalid	Positive
SARS-CoV-2	C161420284090428828404	Yes		(-) Ctrl	**Valid**	Valid	Valid
SARS-CoV-2	C16142028409300958026 4	Yes		SARS-CoV-2 (+) C	**Valid**	Valid	Valid

* The “Valid” and “Overall Result” columns are not applicable to sample results for the **cobas**® SARS-CoV-2. Values reported in these columns are not applicable and do not impact the validity of results reported within individual Target Result columns. Refer to Table 11, **cobas**® SARS-CoV-2 results interpretation, for specific instructions on test results interpretation.

Interpretation of results

The following result interpretation applies to both **cobas**® 6800/8800 software version 1.2 and **cobas**® 6800/8800 software version 1.3 and higher.

For a valid batch, check each individual sample for flags in the **cobas**® 6800/8800 software and/or report. The result interpretation should be as follows:

- A valid batch may include both valid and invalid sample results.
- **The "Valid" and "Overall Result" columns are not applicable to sample results for the cobas® SARS-CoV-2. Values reported in these columns are not applicable and do not impact the validity of results reported within individual Target Result columns.**
- Invalid results for one or more target combinations are possible and are reported out specifically for each channel.
- Results of this test should only be interpreted in conjunction with information available from clinical evaluation of the patient and patient history.

Results and their corresponding interpretation for detecting SARS-CoV-2 are shown below (Table 11).

Table 11 cobas® SARS-CoV-2 results interpretation

Target 1	Target 2	Interpretation
Positive	**Positive**	All Target Results were valid. Result for SARS-CoV-2 RNA is Detected.
Positive	**Negative**	All Target Results were valid. Result for SARS-CoV-2 RNA is Detected. A positive Target 1 result and a negative Target 2 result is suggestive of 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in the Target 2, target region, or 3) other factors.
Negative	**Positive**	All Target Results were valid. Result for SARS-CoV-2 RNA is Presumptive Positive. A negative Target 1 result and a positive Target 2 result is suggestive of 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in the Target 1 target region in the oligo binding sites, or 3) infection with some other Sarbecovirus (e.g., SARS-CoV or some other Sarbecovirus previously unknown to infect humans), or 4) other factors. For samples with a Presumptive Positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management.
Negative	**Negative**	All Target Results were valid. Result for SARS-CoV-2 RNA is Not Detected.
Positive	**Invalid**	Not all Target Results were valid. Result for SARS-CoV-2 RNA is Detected.
Invalid	**Positive**	Not all Target Results were valid. Result for SARS-CoV-2 RNA is Presumptive Positive. For samples with a Presumptive Positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management.
Negative	**Invalid**	Not all Target Results were valid. Sample should be retested. If the result is still invalid, a new specimen should be obtained.
Invalid	**Negative**	Not all Target Results were valid. Sample should be retested. If the result is still invalid, a new specimen should be obtained.
Invalid	**Invalid**	All Target Results were invalid. Sample should be retested. If the result is still invalid, a new specimen should be obtained.

Procedural limitations

- **cobas**® SARS-CoV-2 has been evaluated only for use in combination with the **cobas**® SARS-CoV-2 Control Kit, **cobas**® Buffer Negative Control Kit, **cobas omni** MGP Reagent, **cobas omni** Lysis Reagent, **cobas omni** Specimen Diluent, and **cobas omni** Wash Reagent for use on the **cobas**® 6800/8800 Systems.
- Reliable results depend on proper sample collection, storage and handling procedures.
- This test is intended to be used for the detection of SARS-CoV-2 RNA in nasal, nasopharyngeal, and oropharyngeal swab samples collected in a Copan UTM-RT System (UTM-RT) or BD™ Universal Viral Transport System (UVT) and nasal swab samples collected in **cobas**® PCR Media and 0.9% physiological saline. Testing of other sample types with **cobas**® SARS-CoV-2 may result in inaccurate results.
- Detection of SARS-CoV-2 RNA may be affected by sample collection methods, patient factors (e.g., presence of symptoms), and/or stage of infection.
- As with any molecular test, mutations within the target regions of **cobas**® SARS-CoV-2 could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. One hundred percent agreement between the results should not be expected due to aforementioned differences between technologies. Users should follow their own specific policies/procedures.
- False negative or invalid results may occur due to interference. The Internal Control is included in **cobas**® SARS-CoV-2 to help identify the specimens containing substances that may interfere with nucleic acid isolation and PCR amplification.
- The addition of AmpErase enzyme into the **cobas**® SARS-CoV-2 Master Mix reagent enables selective amplification of target RNA; however, good laboratory practices and careful adherence to the procedures specified in this Instructions For Use document are necessary to avoid contamination of reagents.

Conditions of Authorization for the Laboratory

The **cobas**® SARS-CoV-2 test 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/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd

To assist clinical laboratories running the **cobas**® SARS-CoV-2 test, the relevant Conditions of Authorization are listed verbatim below, and are required to be met by laboratories performing the EUA test.

A. Authorized laboratories[1] using the **cobas**® SARS-CoV-2 test will include with result reports of the **cobas**® SARS CoV-2 test, 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 **cobas**® SARS-CoV-2 test will perform the **cobas**® SARS-CoV-2 test as outlined in the **cobas**® SARS-CoV-2 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 **cobas**® SARS-CoV-2 test are not permitted.
C. Authorized laboratories that receive the **cobas**® SARS-CoV-2 test must notify the relevant public health authorities of their intent to run the test prior to initiating testing.

D. Authorized laboratories using the **cobas**® SARS-CoV-2 test will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
E. 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 Roche Diagnostics US Customer Technical Support 1-800-526-1247 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 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.
G. RMS, its authorized distributor(s) and authorized laboratories using the **cobas**® SARS-CoV-2 test 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.

[1] For ease of reference, this letter will refer to, "United States (U. S.) laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform moderate complexity tests, and in U.S. laboratories certified under CLIA to perform high complexity tests" as "authorized laboratories."

Non-clinical performance evaluation

Key performance characteristics

Analytical sensitivity

Limit of detection (LoD) studies determine the lowest detectable concentration of SARS-CoV-2 at which greater or equal to 95% of all (true positive) replicates test positive.

To determine the LoD, a cultured virus of an isolate from a US patient (USA-WA1/2020, catalog number NR-52281, lot number 70033175, 2.8E+05 $TCID_{50}$/mL[1]) was serially diluted in simulated clinical matrix. A total of 7 concentration levels, with 3-fold serial dilutions between the levels, were tested with a total of 21 replicates per concentration, with an additional 10 replicates of a blank sample (i.e, simulated clinical matrix).

As shown in Table 12, the concentration level with observed hit rates greater than or equal to 95% were 0.009 and 0.003 $TCID_{50}$/mL for SARS-CoV-2 (Target 1) and pan-Sarbecovirus (Target 2), respectively. As shown in Table 13, the Probit predicted 95% hit rates were 0.007 and 0.004 $TCID_{50}$/mL for SARS-CoV-2 (Target 1) and pan-Sarbecovirus (Target 2), respectively.

Table 12 LoD determination using USA-WA1/2020 strain

Strain	Concentration [$TCID_{50}$/mL]	Total valid results	Hit rate [%]^		Mean Ct*	
			Target 1	Target 2	Target 1	Target 2
USA-WA1/2020 (stock concentration 2.8E+05 $TCID_{50}$/mL)	0.084	21	100	100	31.0	33.0
	0.028	21	100	100	31.8	34.1
	0.009	21	100	100	32.7	35.2
	0.003	21	38.1	100	33.5	36.4
	0.001	21	0	52.4	n/a	37.9
	0.0003	21	0	14.3	n/a	37.2
	0.0001	21	0	9.5	n/a	38.5
	0 (blank)	10	0	0	n/a	n/a

^ All replicates where Target 1 was positive were also positive for Target 2.

* Calculations only include positive results.

Table 13 Probit predicted 95% hit rates using USA-WA1/2020 strain

Strain	Probit Predicted 95% Hit Rate [$TCID_{50}$/mL]	
	Target 1	Target 2
USA-WA1/2020 (stock concentration 2.8E+05 $TCID_{50}$/mL)	0.007 (95% CI: 0.005 – 0.036)	0.004 (95% CI: 0.002 – 0.009)

[1] The following reagent was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH: SARS-Related Coronavirus 2, Isolate USA-WA1/2020, NR-52281.

The analytical sensitivity of the assay was tested with AccuPlex SARS-CoV-2 (Lot #105324), a quantitated reference material – recombinant Sindbis virus particle containing target sequences from the SARS-CoV-2 genome. The concentration level in a dilution series with observed hit rates greater than or equal to 95% was 46 copies/mL for both Target 1 and Target 2. Probit model 95% LoD estimates based on these data were 25 copies/mL (95% CI: 17 – 58 copies/mL) for Target 1 and 32 copies/ml (95% CI: 21 – 73 copies/mL) for Target 2.

Reactivity/inclusivity

In silico analysis concluded that **cobas**® SARS-CoV-2 will detect all analyzed SARS-CoV-2 sequences in NCBI and in GISAID databases.

cobas® SARS-CoV-2 had 100% match to all but one sequence for Target 1 (NCBI (n = 79); GISAID (n = 366)). For the one sequence, a single nucleotide mismatch was found that maps to the 5'-end of the reverse primer, with no predicted impact on the assay performance.

cobas® SARS-CoV-2 had 100% match to all but three sequences for Target 2 (NCBI (n = 81); GISAID (n = 364)). For one sequence, a single nucleotide mismatch was found close to the 3'-end of the probe binding region. For a second sequence, a single mismatch was found at the 3'-end of the forward primer binding region. For a third sequence, a single mismatch was found at the 3'-end of the reverse primer binding region. None of these single base mismatches are predicted to impact the performance.

Cross-reactivity

In silico analysis

The *in silico* analysis for possible cross-reactions with all the organisms listed in Table 14 was conducted by mapping primers in **cobas**® SARS-CoV-2 individually to the sequences downloaded from NCBI and GISAID databases. If any two of the primers were mapped to a sequence on opposite strands with short distance apart, potential amplifications were flagged. No potential unintended cross reactivity is expected based on this *in silico* analysis.

Table 14 In silico analysis for SARS-CoV-2

Strain	*In Silico* Analysis for % Identity to Target 1 (nCoV)	*In Silico* Analysis for % Identity to Target 2 (Pan-Sarbecovirus 1)
CoV 229E	74.47	No alignment was found*
CoV OC43	72.26	No alignment was found*
CoV HKU1	76.52	No alignment was found*
CoV NL63	71.32	No alignment was found*
SARS-CoV	95.04	100
MERS	No alignment was found*	No alignment was found*
AdV	No alignment was found*	No alignment was found*
HMPV	No alignment was found*	No alignment was found*
HPIV1	No alignment was found*	No alignment was found*
HPIV2	No alignment was found*	No alignment was found*
HPIV3	No alignment was found*	No alignment was found*
HPIV4	No alignment was found*	No alignment was found*
Flu A	No alignment was found*	No alignment was found*

Strain	***In Silico* Analysis for % Identity to Target 1 (nCoV)**	***In Silico* Analysis for % Identity to Target 2 (Pan-Sarbecovirus 1)**
Flu B	No alignment was found*	No alignment was found*
EV	No alignment was found*	No alignment was found*
RSV	No alignment was found*	No alignment was found*
RV	No alignment was found*	No alignment was found*
Chlamydia pneumoniae	No alignment was found*	No alignment was found*
Haemophilus influenzae	No alignment was found*	No alignment was found*
Legionella pneumophila	No alignment was found*	No alignment was found*
MTB Mycobacterium bovis subsp. Bovis	No alignment was found*	No alignment was found*
Streptococcus pneumoniae	No alignment was found*	No alignment was found*
Streptococcus pyrogenes	No alignment was found*	No alignment was found*
Bordetella pertussis	No alignment was found*	No alignment was found*
Mycoplasma pneumoniae	No alignment was found*	No alignment was found*
Pneumocystis jirovecii	No alignment was found*	No alignment was found*
Influenza C	No alignment was found*	No alignment was found*
Parechovirus	No alignment was found*	No alignment was found*
Candida albicans	No alignment was found*	No alignment was found*
Corynebacterium diphtheriae	No alignment was found*	No alignment was found*
Legionella non-pneumophila	No alignment was found*	No alignment was found*
Bacillus anthracis (Anthrax)	No alignment was found*	No alignment was found*
Moraxella catarrhalis	No alignment was found*	No alignment was found*
Neisseria elongate and meningitides	No alignment was found*	No alignment was found*
Pseudomonas aeruginosa	No alignment was found*	No alignment was found*
Staphylococcus epidermidis	No alignment was found*	No alignment was found*
Staphylococcus salivarius	No alignment was found*	No alignment was found*
Leptospira	No alignment was found*	No alignment was found*
Chlamydia psittaci	No alignment was found*	No alignment was found*
Coxiella burnetii (Q-Fever)	No alignment was found*	No alignment was found*
Staphylococcus aureus	No alignment was found*	No alignment was found*

Note: * The amplicon sequences were blasted against all the exclusive sequences with very low stringency cutoff (50% and 100bp). No alignment were found passing the cutoff and no concerns for cross-reactivity were observed.

Cross reactivity testing

Cross-reactivity of **cobas**® SARS-CoV-2 was evaluated by testing whole organisms. As listed in Table 15, a panel of multiple unique sub-species of microorganisms were tested. High titer stocks of the potentially cross-reacting microorganisms were spiked into negative simulated clinical matrix to a concentration level of 1.0E+05 units/mL for viruses and 1.0E+06 units/mL for other microorganisms, unless otherwise noted.

None of the organisms tested interfered with **cobas**® SARS-CoV-2 performance by generating false positive results.

Table 15 Cross-reactivity test results

Microorganism	Concentration	Target 1 Result	Target 2 Result
Human coronavirus 229E	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Human coronavirus OC43	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Human coronavirus HKU1	1.0E+05 cp/mL	Negative	Negative
Human coronavirus NL63	1.0E+05 $TCID_{50}$/mL	Negative	Negative
MERS coronavirus	1.0E+05 genomic equivalent/mL	Negative	Negative
SARS coronavirus	1.0E+05 PFU/mL	Negative	Positive
Adenovirus B (Type 34)	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Human Metapneumovirus (hMPV)	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Parainfluenza virus Type 1	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Parainfluenza virus Type 2	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Parainfluenza virus Type 3	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Parainfluenza virus Type 4	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Influenza A (H1N1)	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Influenza B	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Enterovirus E (Type 1)	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Respiratory syncytial virus	1.0E+05 PFU/mL	Negative	Negative
Rhinovirus	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Chlamydia pneumonia	1.0E+06 $TCID_{50}$/mL	Negative	Negative
Haemophilus influenzae	1.0E+06 CFU/mL	Negative	Negative
Legionella pneumophila	1.0E+06 CFU/mL	Negative	Negative
Mycobacterium tuberculosis	1.0E+06 cells/mL	Negative	Negative
Streptococcus pneumonia	1.0E+06 CFU/mL	Negative	Negative
Streptococcus pyrogenes	1.0E+06 CFU/mL	Negative	Negative
Bordetella pertussis	1.0E+06 CFU/mL	Negative	Negative
Mycoplasma pneumoniae	1.0E+06 CFU/mL	Negative	Negative
Pooled human nasal wash	5 - 50%	Negative	Negative

Sample type equivalency

Equivalence between nasopharyngeal swab (NPS) and oropharyngeal swab (OPS) sample types was evaluated using cultured virus (USA-WA1/2020 strain) spiked into paired negative samples (individual samples, not pooled) to prepare contrived low positive (approximately 1.5x Target 1 LoD) and moderate positive (approximately 4x Target 1 LoD) samples for each sample type. A total of 21 low positive paired samples, 11 moderate positive paired samples, and 11 negative paired samples were tested.

As shown in Table 16, all low positive and moderate positive paired samples were positive in both sample matrices. All negative paired samples were negative in both sample types. The observed Ct values for contrived positive samples were comparable in both sample types.

Table 16 Result comparison of nasopharyngeal to oropharyngeal sample types

Sample Type	Sample Concentration	N	Target 1		Target 2	
			% Positive	Mean Ct (95% CI)	% Positive	Mean Ct (95% CI)
NPS	~1.5x LoD (Target 1)	21	100	31.9 (31.7 – 32.0)	100	33.6 (33.5 – 33.7)
OPS			100	32.2 (31.8 – 32.6)	100	33.7 (33.4 – 34.1)
NPS	~4x LoD (Target 1)	11	100	30.9 (30.3 – 31.5)	100	32.2 (31.6 – 32.9)
OPS			100	31.5 (31.2 – 31.9)	100	32.7 (32.4 – 33.0)
NPS	Negative	11	0	n/a	0	n/a
OPS			0	n/a	0	n/a

Matrix equivalency – UTM-RT and cobas® PCR Media

Equivalence between samples collected in UTM-RT and **cobas**® PCR Media (CPM) was evaluated using cultured virus (USA-WA1/2020 strain) spiked into paired negative nasopharyngeal samples from patients with signs and symptoms of an upper respiratory infection (individual samples, not pooled) to prepare contrived low positive (approximately 1.5x LoD) and moderate positive (approximately 4x LoD) samples for each collection media. A total of 21 low positive paired samples, 11 moderate positive paired samples, and 11 negative paired samples were tested.

As shown in Table 17, all low positive and moderate positive paired samples were positive in both sample matrices. All negative paired samples were negative in both sample matrices. The observed Ct values for contrived positive samples were comparable in both sample matrices.

Table 17 Result comparison of UTM-RT to **cobas**® PCR Media

Collection Media	Sample Concentration	N	Target 1		Target 2	
			% Positive	Mean Ct (95% CI)	% Positive	Mean Ct (95% CI)
UTM	~1.5x LoD	21	100	31.8 (31.6 - 32.0)	100	34.0 (33.8 - 34.2)
CPM			100	32.2 (31.9 - 32.4)	100	34.7 (34.4 – 35.0)
UTM	~4x LoD	11	100	30.7 (30.1 - 31.2)	100	32.4 (31.7 - 33.1)
CPM			100	31.6 (31.0 - 32.1)	100	33.7 (32.9 - 34.5)
UTM	Negative	11	0	n/a	0	n/a
CPM			0	n/a	0	n/a

Matrix equivalency –UTM-RT and 0.9% physiological saline

Equivalence between samples collected in UTM-RT and 0.9% physiological saline was evaluated using cultured virus (USA-WA1/2020 strain) spiked into paired negative samples (individual samples, not pooled) to prepare contrived low positive (approximately 1.5x LoD) and moderate positive (approximately 4x LoD) samples for each collection media. Three samples were collected from each of 45 healthy donors using swabs from **cobas**® PCR Media Dual Swab Sample Kit; two nasal samples (NS) collected using dual flocked/woven polyester swabs stored in UTM and one nasal sample (other nostril) collected using a woven polyester swab stored in 0.9% physiological saline. A total of 17 low positive paired samples, 11 moderate positive paired samples, and 45 negative paired samples were tested.

As shown in Table 18, all low positive and moderate positive paired samples were positive in both sample matrices. All negative paired samples were negative in both sample matrices. The observed Ct values for contrived positive samples were comparable in both sample matrices.

Table 18 Result comparison of UTM-RT to 0.9% physiological saline

Collection Device	Sample Concentration	N	Target 1		Target 2	
			% Positive	Mean Ct (95% CI)	% Positive	Mean Ct (95% CI)
Flocked Swab in UTM-RT	~1.5x LoD	17	100	32.2 (32.0 - 32.4)	100	33.6 (33.6 - 33.7)
Woven Swab in UTM-RT		16	100	31.6 (31.1 - 32.1)	100	33.2 (32.7 - 33.8)
Woven Swab in Saline		17	100	31.7 (31.4 - 32.0)	100	33.5 (33.2 - 33.8)
Flocked Swab in UTM-RT	~4x LoD	11	100	31.2 (31.1 - 31.4)	100	32.6 (32.4 - 32.7)
Woven Swab in UTM-RT			100	30.9 (30.4 - 31.4)	100	32.4 (31.9 - 33.0)
Woven Swab in Saline			100	31.0 (30.8 - 31.3)	100	32.6 (32.5 - 32.7)
Flocked Swab in UTM-RT	Negative	45	0	n/a	0	n/a
Woven Swab in UTM-RT			0	n/a	0	n/a
Woven Swab in Saline			0	n/a	0	n/a

Clinical evaluation

The performance of **cobas**® SARS-CoV-2 with prospectively collected nasopharyngeal swab clinical samples was evaluated using 100 individual negative clinical samples and 50 contrived positive clinical samples collected from patients with signs and symptoms of an upper respiratory infection.

Clinical samples were collected by qualified personnel according to the package insert of the collection device. Samples were handled as described in the package insert of the collection device and stored frozen until use. Samples were tested to be negative by a commercially available nucleic acid test for the qualitative detection of microorganisms associated with common upper respiratory tract infections.

Low positive and moderate positive contrived positive clinical samples were prepared by spiking cultured virus (USA-WA1/2020 strain) into individual negative clinical samples to approximately ~1.5x LoD (Target 1) (25 samples) and ~4x LoD (Target 1) (25 samples), respectively.

As shown in Table 19, all low positive and moderate positive samples were positive and all negative samples were negative in the background of individual clinical sample matrix.

Table 19 Clinical evaluation with nasopharyngeal swab samples

Sample Concentration	N	Target 1		Target 2	
		% positive (two-sided 95% CI)	Mean Ct	% positive (two-sided 95% CI)	Mean Ct
~1.5x LoD	25	100 (86.7 – 100)	31.6	100 (86.7 – 100)	33.2
~4x LoD	25	100 (86.7 – 100)	31.1	100 (86.7 – 100)	32.4
Negative	100	0 (n/a)	n/a	0 (n/a)	n/a

Performance against the expected results are:
Positive Percent Agreement 50/50 = 100% (95% CI: 92.9% - 100%)
Negative Percent Agreement 100/100 = 100% (95% CI: 96.3% - 100%)

Additional information

Key test features

Sample type	Nasopharyngeal and oropharyngeal swab samples collected in the Copan UTM-RT System or the BD™ UVT System Nasal swab samples collected in the Copan UTM-RT System, the BD™ UVT System, the **cobas**® PCR Media, and 0.9% physiological saline
Minimum amount of sample required	0.6 or 1.0 mL*
Sample processing volume	0.4 mL
Test duration	Results are available within less than 3.5 hours after loading the sample on the system.

*Dead volume of 0.2 mL is identified for the **cobas omni** Secondary tubes. Dead volume of 0.6 mL is identified for the **cobas**® PCR Media primary tubes. Other tubes compatible with **cobas**® 6800/8800 Systems (consult User Assistance Guide) may have different dead volume and require more or less minimum volume.

Symbols

The following symbols are used in labeling for Roche PCR diagnostic products.

Table 20 Symbols used in labeling for Roche PCR diagnostics products

	Ancillary Software	LLR	Lower Limit of Assigned Range	CONTROL –	Negative Control
EC REP	Authorized representative in the European community	ULR	Upper Limit of Assigned Range	CONTROL +	Positive Control
BARCODE	Barcode Data Sheet		Store in the dark	CONTROL	Control
LOT	Batch code		Contains sufficient for <*n*> tests	Assigned Range [copies/mL]	Assigned Range (copies/mL)
	Biological risks		Temperature limit	Assigned Range [IU/mL]	Assigned Range (IU/mL)
REF	Catalogue number		Test Definition File	Procedure Standard	Standard Procedure
	Consult instructions for use		Manufacturer	Procedure UltraSensitive	Ultrasensitive Procedure
Cont.	Contents of kit		Use-by date	QS copies/PCR	QS copies per PCR reaction, use the QS copies per PCR reaction in calculation of the results.
D	Distributed by	GTIN	Global Trade Item Number	QS IU/PCR	QS IU per PCR reaction, use the QS International Units (IU) per PCR reaction in calculation of the results.
	For IVD performance evaluation only	SN	Serial number	CE	This product fulfills the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices.
Rx Only	US Only: Federal law restricts this device to sale by or on the order of a physician.		Date of manufacture		
IVD	*In Vitro* diagnostic medical device		Do not reuse		

US Customer Technical Support 1-800-526-1247

Manufacturer and distributors

Table 21 Manufacturer and distributors

Roche Molecular Systems, Inc.
1080 US Highway 202 South
Branchburg, NJ 08876 USA
www.roche.com

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

Roche Diagnostics
9115 Hague Road
Indianapolis, IN 46250-0457 USA
(For Technical Assistance call the
Roche Response Center
toll-free: 1-800-526-1247)

Trademarks and patents

See http://www.roche-diagnostics.us/patents

Copyright

References

1. Center for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories, 5th ed. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health HHS Publication No. (CDC) 21-1112, revised December 2009.
2. Clinical and Laboratory Standards Institute (CLSI). Protection of laboratory workers from occupationally acquired infections. Approved Guideline-Fourth Edition. CLSI Document M29-A4:Wayne, PA;CLSI, 2014.

Document revision

Document Revision Information	
Doc Rev. 1.0 03/2020	First Publishing.
Doc Rev. 2.0 04/2020	Corrected typographical errors, organism names, and table references. Added nasal swabs (self-collected on site or by the physician), collected in UTM-RT, UVT, **cobas**® PCR Media and 0.9% physiological saline. Addition of the analytical performance data related to the added specimen and media types. Replaced "container" with "collection tube" to improve clarity. Please contact your local Roche Representative if you have any questions.
Doc Rev. 3.0 05/2020	Workflow descriptions of the new sample type "**cobas**® PCR Media swab". Workflow to prepare the **cobas**® PCR Media tubes for processing. Data for analytical sensitivity for AccuPlex added. Removal of duplicate ingredients in formulation for positive control. Removal of repeat testing for samples with presumptive positive results. Update of figures in **Sample collection, transport, and storage** section with gloved hands. Removal of the limitation regarding nasal and mid-turbinate nasa collection in **Procedural limitations** section. Moved **Conditions of Authorization for the laboratory** section to the **Procedural limitations** section. Please contact your local Roche Representative if you have any questions.

Berlin, 13.01.2020

Diagnostic detection of Wuhan coronavirus 2019 by real-time RT-PCR

-Protocol and preliminary evaluation as of Jan 13, 2020-

Victor Corman, Tobias Bleicker, Sebastian Brünink, Christian Drosten
Charité Virology, Berlin, Germany

Olfert Landt, Tib-Molbiol, Berlin, Germany

Marion Koopmans
Erasmus MC, Rotterdam, The Netherlands

Maria Zambon
Public Health England, London

Additional advice by Malik Peiris, University of Hong Kong

Contact: christian.drosten@charite.de
https://virologie-ccm.charite.de/en/

Positive control material is available from Charité, Berlin, via EVAg (https://www.european-virus-archive.com/).

We acknowledge the originators of sequences in GISAID (www.gisaid.org): National Institute for Viral Disease Control and Prevention, China, Institute of Pathogen Biology, Chinese Academy of Medical Sciences, Peking Union Medical College, China, and Wuhan Jinyintan Hospital Wuhan Institute of Virology, Chinese Academy of Sciences, China). We acknowledge Professor Yong-Zhen Zhang, Shanghai Public Health Clinical Center & School of Public Health, Fudan University, Shanghai, China for release of another sequence (MN908947).

Abbreviations and taxonomy related to the Wuhan virus are not used in any systematic way, i.e., there are multiple different designations and abbreviations for the "Wuhan virus" in this document. They all relate to the same viral agent. We use the term "SARS-related Coronavirus" to include the SARS virus as well as the clade of betacoronaviruses known to be associated with (mainly) rhinolophid bats across the Palearctic. The latest taxonomy classifies these viruses in a subgenus termed *Sarbecovirus*.

Berlin, 13.01.2020

Background

We used known SARS- and SARS-related coronaviruses (bat viruses from our own studies as well as literature sources) to generate a non-redundant alignment (excerpts shown in Annex). We designed candidate diagnostic RT-PCR assays before release of the first sequence of the Wuhan virus. Upon sequence release, three assays were selected based on their matching to the Wuhan virus as per inspection of the sequence alignment (Figures 1 and 2).

All assays can use SARS-CoV genomic RNA as positive control. Synthetic control RNA for Wuhan virus will be provided shortly.

First line screening assay: E gene assay
Confirmatory assay: RdRp gene assay
Additional confirmatory assay: N gene assay

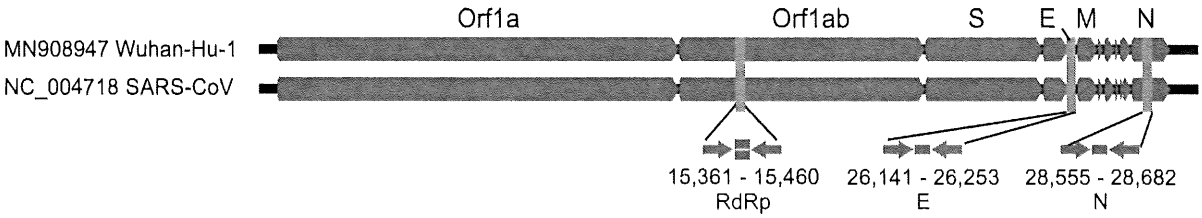


Figure 1 relative positions of amplicon targets on SARS-CoV ad Wuhan-CoV genome. N: nucleocapsid; ORF: open reading frame; RdRp: RNA-dependent RNA polymerase. Numbers below amplicon are genome positions according to SARS-CoV, NC_004718.

Materials and assay formulation

Clinical samples and CoV cell culture supernatants

Respiratory samples were obtained during 2019 from patients hospitalized at Charité medical center and tested by the NxTAG® Respiratory Pathogen Panel (Luminex) or in cases of MERS-CoV by the MERS-CoV upE assay as published before (1).

Cell culture supernatants from typed coronaviruses were available at our research and clinical laboratories. The typed avian influenza virus RNA (H5N1) was obtained from the German Society for Promotion of Quality Assurance in Medical Laboratories (INSTAND) proficiency testing panels. RNA was extracted from clinical samples by using the MagNA Pure 96 system (Roche) and from cell culture supernatants by the viral RNA mini kit (Qiagen).

Assay design

For oligonucleotide design and in-silico evaluation we downloaded all complete and partial (if >400 nucleotides) SARS-related virus sequences available at GenBank by January 1st, 2020. The list (n=729 entries) was manually checked and artificial sequences (lab-derived,

synthetic etc.), as well as sequence duplicates removed, resulting in a final list of 375 sequences. These sequences were aligned and the alignment used for assay design. The alignment was later complemented by sequences released from the Wuhan cluster. All presently release sequences match the amplicons (Figure 2). An overview of oligonucleotide binding sites in all unique sequences of bat-associated SARS-related viruses is shown in the appendix.

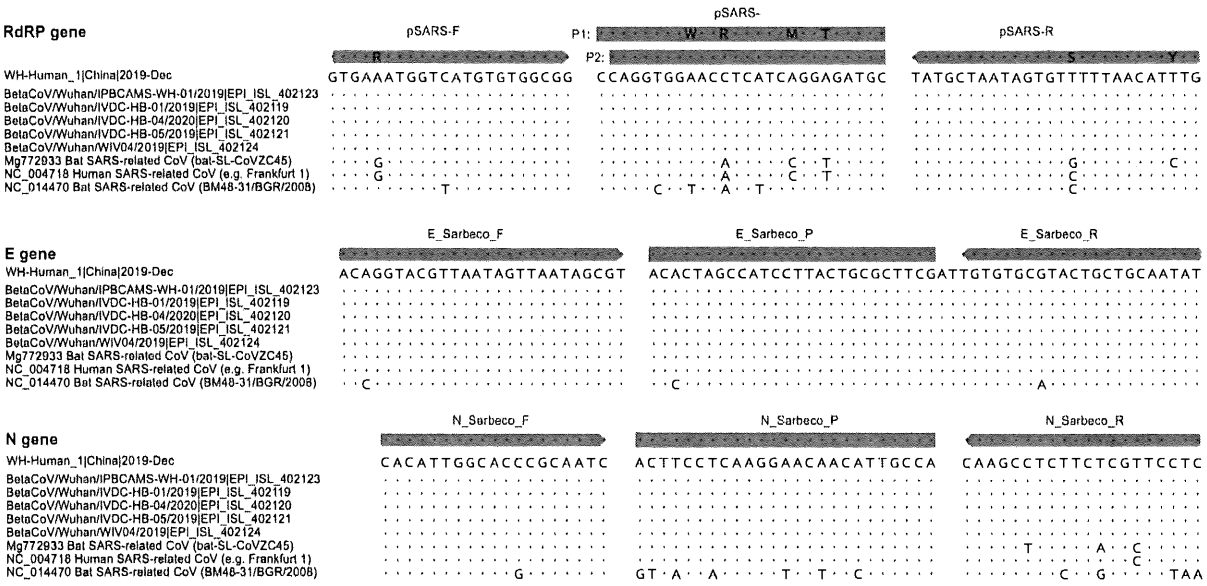


Figure 2 Partial alignments of oligonucleotide binding regions. Panels show six available sequences of the Wuhan-CoV, aligned to the corresponding partial sequences of SARS-CoV strain Frankfurt 1, which can be used as a positive control for all three RT-PCR assays. The alignment also contains the most closely-related bat virus (Bat SARS-related CoV isolate bat-SL-CoVZC45, GenBank Acc.No. MG772933.1) as well as the most distant member within the SARS-related bat CoV clade, detected in Bulgaria (GenBank Acc. No. NC_014470). Dots represent identical nucleotides compared to Wuhan-Hu 1. Substitutions are specified. More comprehensive alignments in the Appendix.

Real-time reverse-transcription polymerase chain reaction

All assays used the same conditions. Primer and probe sequences, as well as optimized concentrations are shown in Table 1. A 25-µl reaction was set up containing 5 µl of RNA, 12.5 µl of 2 X reaction buffer provided with the Superscript III one step RT-PCR system with Platinum Taq Polymerase (Invitrogen; containing 0.4 mM of each deoxyribonucleotide triphosphates (dNTP) and 3.2 mM magnesium sulfate), 1 µl of reverse transcriptase/Taq mixture from the kit, 0.4 µl of a 50 mM magnesium sulfate solution (Invitrogen – not provided with the kit), and 1 µg of nonacetylated bovine serum albumin (Roche). All oligonucleotides were synthesised and provided by Tib-Molbiol, Berlin. Thermal cycling was performed at 55°C for 10 min for reverse transcription, followed by 95°C for 3 min and then 45 cycles of 95°C for 15 s, 58°C for 30 s.

Table 1. Primers and probes

Optimized concentrations are mol per liter of final reaction mix.

(e.g., 1.5 microliters of a 10 micromolar (uM) primer stock solution per 25 microliter (ul) total reaction volume yields a final concentration of 600 nanomol per liter (nM) as indicated in the table)
-note that standard, non-optimized reaction conditions as indicated by suppliers of one-step RT-PCR kits will generally yield sufficient sensitivity-

Assay/ Use	Oligonucleotide ID	Sequence (5'–3')	Comment
RdRP gene	RdRP_SARSr-F2	GTGARATGGTCATGTGTGGCGG	use 600 nM per reaction
	RdRP_SARSr-R1	CARATGTTAAASACACTATTAGCATA	use 800 nM per reaction
	RdRP_SARSr-P2	FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ	Specific for Wuhan-CoV, will not detect SARS-CoV use 100 nM per reaction and mix with P1
	RdRP_SARSr-P1	FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ	Pan Sarbeco-Probe, will detect Wuhan virus, SARS-CoV and bat-SARS-related CoVs use 100 nM per reaction and mix with P2
E gene	E_Sarbeco_F1	ACAGGTACGTTAATAGTTAATAGCGT	use 400 nM per reaction
	E_Sarbeco_R2	ATATTGCAGCAGTACGCACACA	use 400 nM per reaction
	E_Sarbeco_P1	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ	use 200 nM per reaction
N gene	N_Sarbeco_F1	CACATTGGCACCCGCAATC	use 600 nM per reaction
	N_Sarbeco_R1	GAGGAACGAGAAGAGGCTTG	use 800 nM per reaction
	N_Sarbeco_P1	FAM-ACTTCCTCAAGGAACAACATTGCCA-BBQ	use 200 nM per reaction

W is A/T; R is G/A; M is A/C ; FAM, 6-carboxyfluorescein; BBQ, blackberry quencher

Technical sensitivity testing

Preliminary assessment of analytical sensitivity for RdRp assay.

We tested purified cell culture supernatant containing SARS-CoV strain Frankfurt-1 virions grown on Vero cells, and quantified by real-time RT-PCR assay as described in Drosten et al. (2) using a specific *in-vitro* transcribed RNA quantification standard. The results are shown in Figure 3. All assays are highly sensitive.

Berlin, 13.01.2020

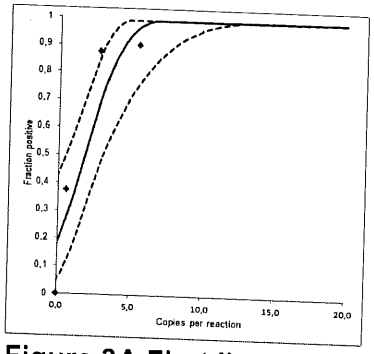
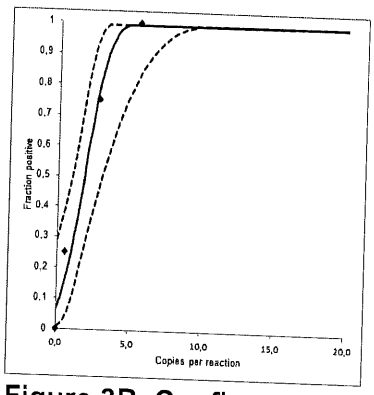


Figure 3A First line assay: E gene
Technical limit of detection (LOD) = 5.2 RNA copies/reaction, at 95% hit rate; 95% CI: 3.7-9.6 RNA copies/reaction.



Legend to these figures: X-axis shows input RNA copies per reaction. Y-axis shows positive results in all parallel reactions performed, squares are experimental data points resulting from replicate testing of given concentrations (x-axis) in parallels assays (8 replicate reactions per datum point). The inner line is a probit curve (dose-response rule). The outer dotted lines are 95% confidence intervals.

Figure 3B. Confirmatory assay: RdRP gene
Technical LOD = 3.8 RNA copies/reaction, at 95% hit rate; 95% CI: 2.7-7.6 RNA copies/reaction.

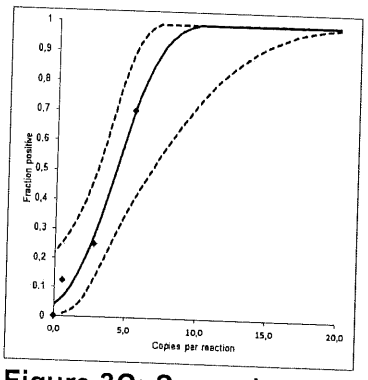


Figure 3C: Second confirmatory assay: N gene
Technical LOD = 8.3 RNA copies/reaction, at 95% hit rate; 95% CI: 6.1-16.3 RNA copies/reaction.

Breadth of detection

To show that the assays will detect other bat-associated SARS-related viruses, we tested bat-derived fecal samples available from Drexler et al., (3) und Muth et al., (4) using the novel assays.

KC633203, Betacoronavirus BtCoV/Rhi_eur/BB98-98/BGR/2008
KC633204, Betacoronavirus BtCoV/Rhi_eur/BB98-92/BGR/2008
KC633201, Betacoronavirus BtCoV/Rhi_bla/BB98-22/BGR/2008
GU190221 Betacoronavirus Bat coronavirus BR98-19/BGR/2008
GU190222 Betacoronavirus Bat coronavirus BM98-01/BGR/2008
GU190223, Betacoronavirus Bat coronavirus BM98-13/BGR/2008

All samples were successfully tested positive by the E gene assay. Detection of these relatively distant members of the SARS-related CoV clade suggests that all Asian viruses are likely to be detected.

Specificity testing

1. Chemical stability
To exclude non-specific reactivity of oligonucleotides among each other, all assays were tested 40 times in parallel with water and no other nucleic acid except the provided oligonucleotides. In none of these reactions was any positive signal detected.

2. Cross-reactivity with other coronaviruses
Cell culture supernatants containing human coronaviruses (HCoV)-229E, -NL63, -OC43, and -HKU1 as well as MERS-CoV were tested in all three assays (Table 2). For the non-cultivable HCoV-HKU1, supernatant from human airway culture was used. Virus RNA concentration in all samples was determined by specific real-time RT-PCRs and in-vitro transcribed RNA standards designed for absolute viral load quantification.

Table 2. Cell-culture supernatants tested by all assays

Cell culture supernatants	Tested concentration	Result
Alphacoronaviruses		
Human coronavirus NL63	4x10^9 RNA copies/ml	No reactivity with any of three assays
Human coronavirus 229E	3x10^9 RNA copies/ml	No reactivity with any of three assays
Betacoronaviruses		
Betacoronavirus 1 (strain HCoV-OC43)	1x10^10 RNA copies/ml	No reactivity with any of three assays
Human coronavirus HKU1 (HCOV-HKU1)	1x10^5 RNA copies /ml	No reactivity with any of three assays
Middle East respiratory syndrome-related coronavirus (strain EMC/2012)	1x10^8 RNA copies/ml	No reactivity with any of three assays

3. Tests of human clinical samples previously tested to contain respiratory viruses

All assays were applied on human clinical samples from our own diagnostic services, previously tested positive for the viruses listed in Table 3. All tests returned negative results.

Table 3. Tests of known respiratory viruses and bacteria in clinical samples

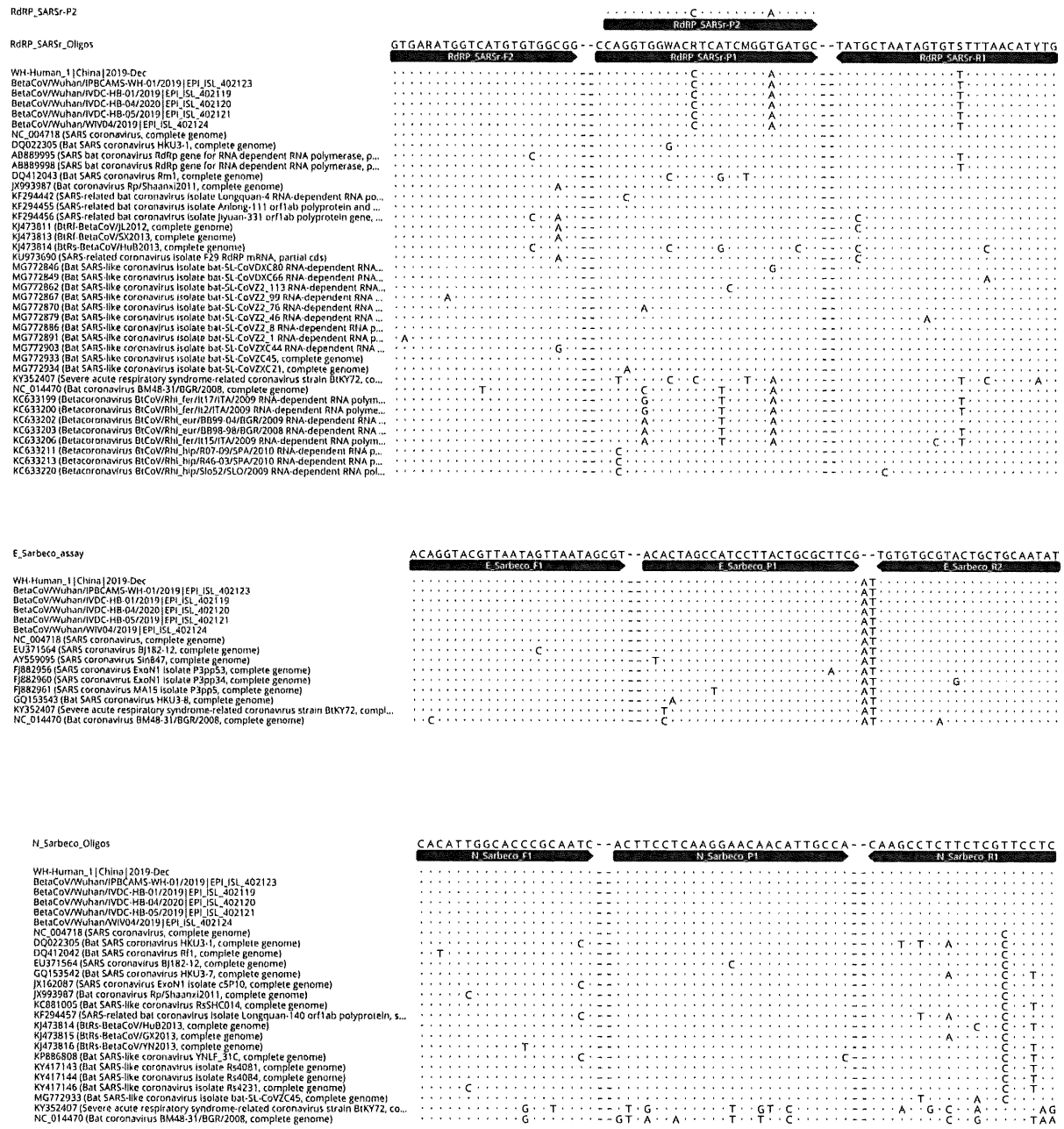
Clinical samples with known viruses	Number of samples tested in all three assays
HCoV-HKU1	2
HCoV-OC43	5
HCoV-NL63	5
HCoV-229E	5
MERS-CoV	5
Influenza A (H1N1/09)	6
Influenza A (H3N2)	5
Influenza A(H5N1)	1
Influenza B	3
Rhinovirus/Enterovirus	3
Respiratory syncytial virus (A/B)	6
Parainfluenza 1 virus	3
Parainfluenza 2 virus	3
Parainfluenza 3 virus	3
Parainfluenza A or -B virus	5
Human metapneumovirus	3
Adenovirus	3
Human Bocavirus	3
Legionella spp.	3
Mycoplasma spp.	3
Total clinical samples	75

References

1. Corman VM, Eckerle I, Bleicker T, Zaki A, Landt O, Eschbach-Bludau M, et al. Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. Euro Surveill. 2012;17(39).
2. Drosten C, Gunther S, Preiser W, van der Werf S, Brodt HR, Becker S, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med. 2003;348(20):1967-76.
3. Drexler JF, Gloza-Rausch F, Glende J, Corman VM, Muth D, Goettsche M, et al. Genomic characterization of severe acute respiratory syndrome-related coronavirus in European bats and classification of coronaviruses based on partial RNA-dependent RNA polymerase gene sequences. J Virol. 2010;84(21):11336-49.
4. Muth D, Corman VM, Roth H, Binger T, Dijkman R, Gottula LT, et al. Attenuation of replication by a 29 nucleotide deletion in SARS-coronavirus acquired during the early stages of human-to-human transmission. Sci Rep. 2018;8(1):15177.

Berlin, 13.01.2020

Annex:



Annex figure. Non-redundant alignments of SARS-related CoVs focused on oligonucleotide binding sites of all assays (top to bottom: RdRp, E, N). Viruses not present in these alignments have been removed because their binding sites are 100% identical to one of the members of the alignment. ("--") means sequence gaps not covered by oligonucleotides. Note that these alignments contain only one sequence of the Wuhan virus while Figure 2 above contains all presently released sequences. We will fuse this into one figure.

Berlin, 13.01.2020

Annex 2: Bench Protocol

Real-time rtPCR for Betacoronavirus (Wuhan Betacoronavirus, Wu-Hu-1)

Example formulation:
Thermo Fischer /Invitrogen SuperScriptIII OneStep RT-PCR System with Platinum Taq DNA Polymerase

E assay:

MasterMix:	**25µl single rxn, µl**
H_2O (RNAse free)	2.6
2x Reaction mix*	12.5
$MgSO_4$(50mM)	0.4
BSA (1 mg/ml)**	1
Fwd primer (10 µM)	1
Rev primer (10 µM)	1
Probe (10 µM)	0.5
SSIII/Taq EnzymeMix*	1
	20
Template RNA	5

Cycler:

55°C	10'	
94°C	3'	
94°C	15"	45x
58°C	30"	
40°C	30"	

' = minutes; " = seconds

RdRp- and N assay:

MasterMix:	**25µl single rxn, µl**
H_2O (RNAse free)	1.1
2x Reaction mix*	12.5
$MgSO_4$(50mM)	0.4
BSA (1 mg/ml)**	1
Fwd primer (10 µM)	1.5
Rev primer (10 µM)	2
Probe (10 µM)	0.5
SSIII/Taq EnzymeMix*	1
	20
Template RNA	5

Cycler:

55°C	10'	
94°C	3'	
94°C	15"	45x
58°C	30"	
40°C	30"	

' = minutes; " = seconds

* Thermo Fischer/Invitrogen: SuperScriptIII OneStep RT-PCR System with Platinum® Taq DNA Polymerase
** MgSO4 (50 mM) [Sigma], This component is not provided with the OneStep RT-PCR kit
*** non-acetylated [Roche]. This component is only necessary when using glass capillaries with LightCycler. Can be replaced with water in plastic vessel machines such as ABI 7500, LC 480, etc.

Primers / probe: See table

Berlin, 13.01.2020

Positive Control: SARS-CoV (e.g. strain Frankfurt 1)
References: Corman/Drosten, unpublished

Assay/ Use	Oligonucleotide ID	Sequence (5'–3')	Comment
RdRP gene	RdRP_SARSr-F2	GTGARATGGTCATGTGTGGCGG	use 600 nM per reaction
	RdRP_SARSr-R1	CARATGTTAAASACACTATTAGCATA	use 800 nM per reaction
	RdRP_SARSr-P2	FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ	Specific for Wuhan-CoV, will not detect SARS-CoV use 100 nM per reaction and mix with P1
	RdRP_SARSr-P1	FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ	Pan Sarbeco-Probe, will detect Wuhan virus, SARS-CoV and bat-SARS-related CoVs use 100 nM per reaction and mix with P2
E gene	E_Sarbeco_F1	ACAGGTACGTTAATAGTTAATAGCGT	use 400 nM per reaction
	E_Sarbeco_R2	ATATTGCAGCAGTACGCACACA	use 400 nM per reaction
	E_Sarbeco_P1	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ	use 200 nM per reaction
N gene	N_Sarbeco_F1	CACATTGGCACCCGCAATC	use 600 nM per reaction
	N_Sarbeco_R1	GAGGAACGAGAAGAGGCTTG	use 800 nM per reaction
	N_Sarbeco_P1	FAM-ACTTCCTCAAGGAACAACATTGCCA-BBQ	use 200 nM per reaction

Australian Government
Department of Health
Therapeutic Goods Administration

How testing works for COVID-19

8 October 2020

COVID-19 is the disease caused when a person is infected by a new coronavirus called SARS-CoV-2.

There are two kinds of tests that can detect whether a person has been infected with SARS-CoV-2 and has the COVID-19 virus.

1. Tests that detect the presence of the actual SARS-CoV-2 virus in your body. This is usually done by testing if the virus is present in your throat, nose, nasal secretions (snot) or sputum (saliva/spit).
2. Tests that detect whether your body has produced antibodies to the SARS-CoV-2 infection. This is usually done by taking a sample of your blood and testing your blood for specific antibodies.

Detecting the presence of SARS-CoV-2 virus

Two types of tests that detect the presence of the SARS-CoV-2 virus include - nucleic acid tests that detect the virus's genetic material and antigen tests that detect specific viral proteins.

Nucleic acid tests

These tests detect the presence of the genetic material, called nucleic acids, of the actual SARS-CoV-2 virus. Such tests are good at detecting the virus early in the infection and can sometimes even detect the virus in a person before they become unwell. There are several types of nucleic acid tests that can be used to detect the SARS-CoV-2 virus, including polymerase chain reaction (PCR) tests and isothermal nucleic acid amplification tests (e.g., loop-mediated isothermal amplification (LAMP) tests).

PCR tests are generally considered better at detecting the presence of the SARS-CoV-2 virus and are currently the gold standard for diagnosis of COVID-19.

Nucleic acid tests are complicated to do and usually need specialist scientists to run the tests in a laboratory to get an accurate result. The laboratory scientists can sometimes run these tests on automated machines that can do many tests at once. This means that you can test lots of people

quickly.

There are now some SARS-CoV-2 nucleic tests available that can be used outside of a laboratory by trained people. Most of these systems give results quickly but cannot do many tests at once.

Rapid Antigen tests

These tests detect the presence of specific proteins of the SARS-CoV-2 virus in symptomatic patients. Rapid antigen tests can be performed by health professionals outside of a laboratory and may produce a result within 15 30 minutes, although their ability to detect the virus may not be as good as a nucleic acid test.

Rapid antigen tests are generally best performed within the first 5-7 days from the time symptoms first appear. Negative results, and some positive results, may require further testing by a nucleic acid test to confirm if a patient is infected with the SARS-CoV-2 virus.

Rapid antigen tests, are not intended for home testing. They are designed to be used by trained health professionals or laboratory scientists.

Detecting antibodies to the SARS-CoV-2 virus

These tests look in our blood to see if our body has started fighting a SARS-CoV-2 infection, rather than detecting the actual SARS-CoV-2 virus. They do this by seeing if our blood contains specific antibodies that attach to parts of the virus.

It takes time for our bodies to make antibodies, so people can already have the SARS-CoV-2 virus and be spreading the infection to other people before we can detect their antibodies.

Scientists usually run viral antibody tests in laboratories, however since the beginning of the COVID-19 pandemic, a number of manufacturers have developed tests that can be used outside of the laboratory at the point of care. These are called rapid or Point of Care tests (PoC or PoCT). You might also hear these tests called IgG or IgM tests. IgG and IgM are different kinds of antibodies that are made by our bodies to fight infection.

These rapid and PoC antibody tests are not intended for self-testing. They are designed to be used by a health professional.

Important things to remember

It is illegal for someone to sell you a test claiming that you can test yourself for a COVID-19 infection. In Australia, the supply of self-tests (i.e. home use tests) for COVID-19 is prohibited under the Therapeutic Goods (Medical Devices—Excluded Purposes) Specification 2020 (https://www.legislation.gov.au/Series/F2020L01150).

As you can see from the above information, it is complicated to accurately detect a SARS-CoV-2 infection. This is why we need to make sure that all COVID-19 testing is done with a health

professional. The health professional can provide you with appropriate advice and treatment if required, and importantly, the health professional can alert the relevant health authorities. This way we can trace infections and take action to stop further spread of the infection.

Please see the Department of Health website (https://www.health.gov.au/news/health-alerts/novel-coronavirus-2019-ncov-health-alert) for all COVID-19 updates.

The TGA will take action in relation to any report of poor or faulty performance of these devices. Reports can be submitted via the TGA website (//www.tga.gov.au/reporting-problems).

Category: Medical devices/IVDs
Tags: COVID-19 tests
URL: https://www.tga.gov.au/node/904152 (https://www.tga.gov.au/node/904152)

Queensland Health

COVID-19

Laboratory testing for SARS-CoV-2: Information and FAQs

29 June 2020

The capacity to identify and isolate cases of COVID-19 is critical to limit the spread of the virus, protect vulnerable persons in the community and ensure healthcare systems maintain the capacity to deliver quality healthcare.

Laboratory testing for SARS-CoV-2 has evolved over the course of the pandemic and continues to advance as more is known about the virus and testing capability is enhanced.

Testing for SARS-CoV-2 should be done in conjunction with assessment and testing for other potential causes of the person's presentation, as deemed appropriate by the treating clinician and in line with local protocols.

What types of tests are available for SARS-CoV-2?

Testing can be broadly grouped into:

- SARS-CoV-2 specific testing (testing directly for the virus), done by nucleic acid testing AND
- Serology (testing for antibody response).

Nucleic acid testing (NAT)

NAT is performed by real time polymerase chain reaction (PCR). This method involves amplification of RNA of the SARS-CoV-2 virus. PCR is the appropriate test for diagnosis of acute COVID-19 infection.

PCR is most commonly performed on upper respiratory tract specimens. For best results from upper respiratory tract sampling, both deep nasal and throat swabs should be collected. Both sites can be sampled using the same swab.

PCR can also be performed on lower respiratory tract specimens including sputum, bronchoalveolar lavage and tracheal aspirate. Lower respiratory tract specimens contain higher viral loads in SARS-CoV-2, and therefore should be tested wherever appropriate.

PCR is occasionally performed on faecal and tissue specimens in special circumstances. Testing of faeces or tissue is only available by special request on the advice of a microbiologist, infectious diseases physician or public health physician.

Serology

Serology tests are performed on serum to look for antibodies that are produced by the person against SARS-CoV-2. They do not detect the virus itself.

A validated SARS-CoV-2 serology assay is now available at Queensland Health Forensic and Scientific Services (QHFSS). The assay detects both IgM and IgG antibodies in serum from patients who have been infected with the virus. Serology assays are currently being assessed by other laboratories in Queensland and will likely become available soon.

Serology is not recommended for diagnosis of acute infection. Acute and convalescent specimens collected 10–14 days apart can assist in diagnosis of COVID-19 in persons with negative PCR tests where there remains a high suspicion of COVID-19, or in persons suspected to have recovered from COVID-19 who did not undergo PCR at the time they were unwell.

Serology testing is currently limited in Queensland and must be prioritised for epidemiological investigations where the results will be used to provide information relating to current community transmission. Examples of this include:

- an active outbreak or cluster investigation, or
- investigation of potential upstream contacts of a confirmed case of COVID-19 where the case has no apparent epidemiological links (i.e. contact with other known cases or travel to an area where there is known community transmission of the virus).

Serology test requests via QHFSS must be discussed with the local public health unit and approved by the QHFSS microbiologist.

Point of care (POC) serology tests

Point of care (POC) serology tests, or finger-prick tests, are yet to be validated. The accuracy and clinical utility of these tests is unknown and therefore they are not endorsed for use by Queensland Health outside of the research setting.

Please refer to the following Chief Health Officer direction in relation to the use of POC serology tests in Queensland, effective from 23 April 2020, available at https://www.health.qld.gov.au/system-governance/legislation/cho-public-health-directions-under-expanded-public-health-act-powers/point-of-care-serology-tests

Is there a possibility of false negative or false positive PCR results?

There are no pathology tests that are completely accurate, however the accuracy of PCR assays used to detect SARS-CoV-2 in diagnostic laboratories in Queensland perform very well.

Factors that can influence the accuracy of PCR testing and correct classification of cases include:

- a variable presence of virus in different body sites at different phases of illness
- the quality of the sampling (how well the swab is taken)
- technical factors specific to the assay
- specimen handling and processing.

If the results of testing do not fit with the clinical and epidemiological context of the person being evaluated, this should be discussed with a microbiologist, infectious diseases physician or public health physician and consideration given to repeat testing.

Despite more than 250 000 tests for SARS-CoV-2 having been performed in Queensland to date, fewer than 10 have been identified as false positive reports. For further information on false positive PCR tests, refer to the "Public Health Laboratory Network Statement on Nucleic Acid Test False Positive Results for SARS-CoV-2", available at: https://www1.health.gov.au/internet/main/publishing.nsf/Content/2FCDB8DA4EB40BA9CA257BF000211F2A/$File/Nucleic-Acid-Test-False-Positive-Results-SARS-CoV-2-PHLN.pdf

What platforms are available for PCR?

There are a variety of platforms in use for PCR testing across different laboratories. These platforms are continually being evaluated and enhanced as new information on the virus and testing methods becomes available.

PCR is principally performed using high throughput instruments, processing specimens in large batches. These tests take approximately six hours to process once they are loaded onto the instrument in the laboratory.

In regional sites and some hospitals across Queensland, there are also GeneXpert instruments available. GeneXpert can process a small number of specimens with a quicker processing time than high throughput instruments.

The availability of testing kits for GeneXpert are currently very limited worldwide, and therefore their use in Queensland must be prioritised for investigation of suspected cases of COVID-19 where the confirmation of a positive or negative result would prompt:

- a rapid public health response, or
- a significant change to immediate clinical management.

The use of GeneXpert tests require approval from a clinical microbiologist, infectious diseases physician, public health physician or executive director of medical services (EDMS), depending on processes in place at your facility.

GeneXpert are sometimes referred to as 'point of care' PCR tests. Specimens being evaluated for SARS-CoV-2 using the GeneXpert must still be processed in a laboratory or clinic environment with adequate safety precautions in place to protect the operator of these tests from contracting COVID-19 from the specimens they are handling.

Can a serology test prove that a person is immune to COVID-19?

The presence of antibodies detected on serology testing demonstrates recent or past COVID-19 infection. They cannot be used to demonstrate immunity to the virus. Some people may never make antibodies to the virus, particularly if they are immunosuppressed.

It is currently not known how long antibodies remain in the body, and there is a possibility that they wane over time.

Serology results are not used to shorten a person's period of quarantine.

Can test results be fast-tracked?

Laboratory turn-around times are very good for SARS-CoV-2 testing and continue to improve. Laboratories in Queensland operate extended hours, seven days per week. Queensland currently tests around 5000 people per day.

If the testing processes were interrupted in order to fast-track one or more specimens, this would slow down the turn-around time for results for all the other specimens being processed on that run. Therefore, prioritisation of testing is not done.

Is there a shortage of SARS-CoV-2 tests?

There are worldwide shortages of some of the reagents used for SARS-CoV-2 testing. Diagnostic laboratories in Queensland have responded to these shortages by diversifying their testing platforms and by using innovative processes within the laboratory to ensure there is enough testing capacity to respond to the COVID-19 pandemic.

Testing should continue to be requested for persons who have symptoms of COVID-19 infection in accordance with state and national guidelines.

Should I test asymptomatic people for SARS-CoV-2?

Testing asymptomatic persons should only occur in specific circumstances, for example, under the guidance of your public health unit during investigation of an outbreak or prior to organ donation. Although asymptomatic and pre-symptomatic shedding of SARS-COV-2 is described, as the current prevalence of COVID-19 in Australia is so low, the predictive value of testing asymptomatic persons without epidemiological risk factors is significantly limited. Increased testing of asymptomatic persons in a low prevalence setting can lead to an increased proportion of false positive tests results.

Where can I find further information about SARS-CoV-2 testing?

- PHLN guidance on laboratory testing for SARS-CoV-2, https://www.health.gov.au/sites/default/files/documents/2020/05/phln-guidance-on-laboratory-testing-for-sars-cov-2-the-virus-that-causes-covid-19_1.pdf
- Queensland Health public health alerts, https://www.health.qld.gov.au/clinical-practice/guidelines-procedures/novel-coronavirus-qld-clinicians/public-health-alerts
- Coronavirus Disease 2019 (COVID-19) - CDNA National guidelines for public health units, https://www1.health.gov.au/internet/main/publishing.nsf/Content/cdna-song-novel-coronavirus.htm
- Australian Health Protection Principal Committee (AHPPC) coronavirus (COVID-19) statements on 14 May 2020, https://www.health.gov.au/news/australian-health-protection-principal-committee-ahppc-coronavirus-covid-19-statements-on-14-may-2020

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

For i*n vitro* diagnostic use.

For U.S. Export only.

CONTENTS

General Information

Intended Use

The Aptima™ SARS-CoV-2 assay is a nucleic acid amplification *in vitro* diagnostic test intended for the qualitative detection of RNA from SARS-CoV-2 isolated and purified from nasopharyngeal (NP), nasal, mid-turbinate and oropharyngeal (OP) swab specimens, nasopharyngeal wash/ aspirate or nasal aspirates obtained from individuals meeting COVID-19 clinical and/or epidemiological criteria.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA, clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

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

Summary and Explanation of the Test

Coronaviruses are a large family of viruses which may cause illness in animals or humans. In humans, several coronaviruses are known to cause respiratory infections ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). The most recently discovered coronavirus, SARS-CoV-2, causes the associated coronavirus disease COVID-19. This new virus and disease were unknown before the outbreak began in Wuhan, China, in December 2019.[1]

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

The virus that causes COVID-19 is infecting people and spreading easily from person to person.[3] On March 11, 2020, the COVID-19 outbreak was characterized as a pandemic by the World Health Organization (WHO).[4,5]

Principles of the Procedure

The Aptima SARS-CoV-2 assay combines the technologies of target capture, Transcription Mediated Amplification (TMA), and Dual Kinetic Assay (DKA).

Specimens are collected and transferred into their respective specimen transport tubes. The transport solutions in these tubes release the RNA target and protect them from degradation during storage. When the Aptima SARS-CoV-2 assay is performed in the laboratory, the target RNA molecules are isolated from specimens by use of capture oligomers via target capture that utilizes magnetic microparticles. The capture oligomers contain sequences complementary to specific regions of the target molecules as well as a string of deoxyadenosine residues. A separate capture oligomer is used for each target. During the hybridization step, the sequence specific regions of the capture oligomers bind to specific regions of the target molecules. The capture oligomer:target complex is then captured out of solution by decreasing the temperature of the reaction to room temperature. This temperature reduction allows hybridization to occur between the deoxyadenosine region on the capture oligomer and the poly-deoxythymidine molecules that are covalently attached to the magnetic particles. The microparticles, including the captured target molecules bound to them, are pulled to the side of the reaction vessel using magnets and the supernatant is aspirated. The particles are washed to remove residual specimen matrix that may contain amplification reaction inhibitors. After the target capture steps are completed, the specimens are ready for amplification.

Target amplification assays are based on the ability of complementary oligonucleotide primers to specifically anneal and allow enzymatic amplification of the target nucleic acid strands. The Aptima SARS-CoV-2 assay replicates specific regions of the RNA from SARS-CoV-2 virus. Detection of the RNA amplification product sequences (amplicon) is achieved using nucleic acid hybridization. Single-stranded chemiluminescent nucleic acid probes, which are unique and complementary to a region of each target amplicon and Internal Control (IC) amplicon, are labeled with different acridinium ester (AE) molecules. The AE labeled probes combine with amplicon to form stable hybrids. The Selection Reagent differentiates hybridized from unhybridized probe, eliminating the generation of signal from unhybridized probe. During the detection step, light emitted from the labeled hybrids is measured as photon signals in a luminometer, and are reported as Relative Light Units (RLU). In DKA, differences in the kinetic profiles of the labeled probes allow for the differentiation of signal; kinetic profiles are derived from measurements of photon output during the detection read time. The chemiluminescent detection reaction for the IC signal has very rapid kinetics and has the “flasher” kinetic type. The chemiluminescent detection reaction for the SARS-CoV-2 signal is relatively slower and has the “glower” kinetic type. Assay results are determined by a cut-off based on the total RLU and the kinetic curve type.

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

Warnings and Precautions

A. For *in vitro* diagnostic use. Carefully read this entire package insert and the *Panther/Panther Fusion System Operator's Manual*.

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

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

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

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

F. Use only supplied or specified disposable laboratory ware.

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

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

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

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

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

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

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

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

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

P. Avoid microbial and ribonuclease contamination of reagents.

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

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

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

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

Reagent Storage and Handling Requirements

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

Aptima SARS-CoV-2 Amplification Reagent

Aptima SARS-CoV-2 Enzyme Reagent

Aptima SARS-CoV-2 Probe Reagent

Aptima SARS-CoV-2 Internal Control

Aptima SARS-CoV-2 Positive Control

Aptima SARS-CoV-2 Negative Control

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

Aptima SARS-CoV-2 Amplification Reconstitution Solution

Aptima SARS-CoV-2 Enzyme Reconstitution Solution

Aptima SARS-CoV-2 Probe Reconstitution Solution

Aptima SARS-CoV-2 Selection Reagent

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

Aptima SARS-CoV-2 Target Capture Reagent

Aptima Wash Solution

Aptima Buffer for Deactivation Fluid

Aptima Oil Reagent

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

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

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

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

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

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

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

K. Do not freeze the reagents.

Specimen Collection and Storage

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

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

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

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

Swab Specimen Collection

Collect NP swab, nasal swab, and OP swab specimens according to standard technique using a polyester-, rayon-, or nylon-tipped swab. Immediately place the swab specimen into 3mL of VTM or UTM. Swab specimens may alternatively be added to saline, Liquid Amies or

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

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

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

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

The following types of VTM/UTM can be used.

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

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

Nasopharyngeal Wash/aspirate and Nasal Aspirate Specimen Collection

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

Specimen Processing using the Panther Fusion Specimen Lysis Tube

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

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

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

Specimen Processing using the Hologic Specimen Lysis Tube with Solid Cap

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

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

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

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

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

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

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

Specimen Processing using a Custom Specimen Lysis Tube

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

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

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

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

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

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

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

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

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

Specimen Processing using the Aptima Specimen Transfer Tube

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

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

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

B. Recap the Aptima Specimen Transfer Tube tightly.

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

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

Specimen Processing for Specimen Collected with the Aptima Multitest Collection Kit

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

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

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

Sample Storage

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

B. Storing samples before or after testing

1. Samples in the Aptima Multitest Tube, Aptima Specimen Tube, or Specimen Lysis Tube should be stored upright in the rack under the following condition:
   - 2°C to 30°C up to 6 days
2. The samples should be covered with a new, clean plastic film or foil barrier.
3. If assayed samples need to be frozen or shipped, remove the penetrable cap and place a new non-penetrable cap on the specimen tubes. If samples need to be shipped for testing at another facility, recommended temperatures must be maintained. Prior to uncapping, specimen transport tubes must be centrifuged for 5 minutes at 420 Relative Centrifugal Force (RCF) to bring all of the liquid down to the bottom of the tube. Avoid splashing and cross-contamination.

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

Specimen Transport

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

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

Panther System

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

Reagents and Materials Provided

Aptima SARS-CoV-2 Assay Kit PRD-06419

250 tests (2 boxes)

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

Symbol	Component	Quantity 250 test kit
A	**Aptima SARS-CoV-2 Amplification Reagent** *Non-infectious nucleic acids dried in buffered solution containing < 5% bulking agent.*	1 vial
E	**Aptima SARS-CoV-2 Enzyme Reagent** *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing < 10% bulking reagent.*	1 vial
P	**Aptima SARS-CoV-2 Probe Reagent** *Non-infectious chemiluminescent DNA probes dried in succinate buffered solution containing < 5% detergent.*	1 vial
IC	**Aptima SARS-CoV-2 Internal Control**	1 vial

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

Symbol	Component	Quantity 250 test kit
AR	**Aptima SARS-CoV-2 Amplification Reconstitution Solution** *Aqueous solution containing preservatives.*	1 x 27.7 mL
ER	**Aptima SARS-CoV-2 Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 x 11.1 mL
PR	**Aptima SARS-CoV-2 Probe Reconstitution Solution** *Succinate buffered solution containing < 5% detergent.*	1 x 35.4 mL
S	**Aptima SARS-CoV-2 Selection Reagent** *600 mM borate buffered solution containing surfactant.*	1 x 108 mL
TCR	**Aptima SARS-CoV-2 Target Capture Reagent** *Buffered salt solution containing solid phase and capture oligomers.*	1 x 54 mL
	Reconstitution Collars	3
	Master Lot Barcode Sheet	1 sheet

Materials Required and Available Separately

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

	Cat. No.
Panther System	303095
Aptima Assay Fluids Kit *(Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent)*	303014 (1000 tests)
Aptima Auto Detect Kit	303013 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther Waste Bag Kit	902731
Panther Waste Bin Cover	504405
Or Panther Run Kit *contains MTUs, waste bags, waste bin covers, assay fluids, and auto detects*	303096 (5000 tests)
Tips, 1000 µL conductive, liquid sensing	10612513 (Tecan)
Aptima SARS-CoV-2 Controls Kit *PC - Aptima SARS-CoV-2 Positive Control. Non-infectious nucleic acid in a buffered solution containing < 5% detergent. Quantity 5 x 1.7 mL* *NC - Aptima SARS-CoV-2 Negative Control. A buffered solution containing <5% detergent. Quantity 5 x 1.7 mL*	PRD-06420
Aptima Multitest Swab Specimen Collection Kit	PRD-03546
Aptima Specimen Transfer Kit	301154C
Aptima Specimen Transfer Kit - printable	PRD-05110
Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens	301041
Panther Fusion Specimen Lysis Tubes, 100 per bag *tube contains 0.71 mL of STM with a penetrable cap*	PRD-04339
Hologic Specimen Lysis Tube, 100 each *tube contains 0.71 mL of STM with a solid cap*	PRD-06554
Hologic Specimen Lysis Tube, 1200 each *tube contains 0.71 mL of STM with a solid cap*	PRD-06660
Specimen Transport Medium, 1 bottle, 80 mL	PRD-04423
Specimen Transport Medium, 1 bottle, 120 mL	PRD-06657
Bleach, 5% to 7% (0.7M to 1.0M) sodium hypochlorite solution	—
Disposable gloves	—
Replacement non-penetrable caps	504415
Fisherbrand VersaClosure Tube Closures*, 1000 per pack **a single-use tube cover for the Hologic Specimen Lysis Tube (PRD-06554 only) after testing*	02-707

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

Optional Materials

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

Panther System Test Procedure

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

A. Work Area Preparation

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

B. Reagent Reconstitution/Preparation of a New Kit

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

1. To reconstitute Amplification, Enzyme, and Probe Reagents, combine the bottles of lyophilized reagent with the reconstitution solution. If refrigerated, allow the reconstitution solutions to reach room temperature before use.
   a. Pair each reconstitution solution with its lyophilized reagent. Ensure that the reconstitution solution and reagent have matching label colors before attaching the reconstitution collar.
   b. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired.
   c. Open the lyophilized reagent vial and firmly insert the notched end of the reconstitution collar into the vial opening (Figure 1, Step 1).
   d. Open the matching reconstitution solution, and set the cap on a clean, covered work surface.
   e. While holding the reconstitution solution bottle on the bench, firmly insert the other end of the reconstitution collar into the bottle opening (Figure 1, Step 2).

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

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

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

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

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

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

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

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

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

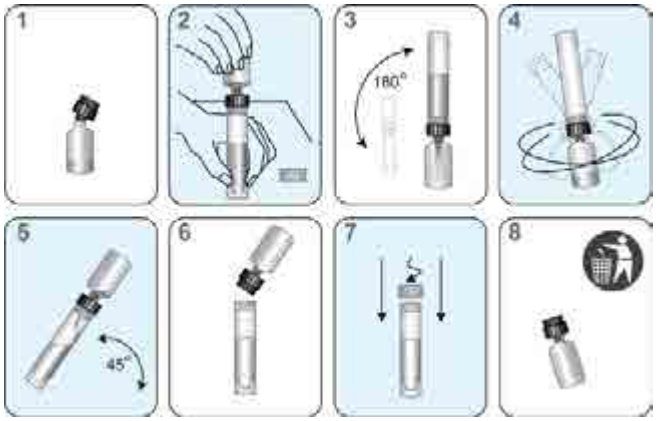


Figure 1. Panther System Reconstitution Process

2. Prepare Working Target Capture Reagent (wTCR)

a. Pair the appropriate bottles of TCR and IC.

b. Check the reagent lot numbers on the Master Lot Barcode Sheet to make sure that the appropriate reagents in the kit are paired.

c. Open the bottle of TCR, and set the cap on a clean, covered work surface.

d. Open the IC bottle and pour the entire contents into the bottle of TCR. Expect a small amount of liquid to remain in the IC bottle.

e. Cap the bottle of TCR and gently swirl the solution to mix the contents. Avoid creating foam during this step.

f. Record operator initials and the current date on the label.

g. Discard the IC bottle and cap.

3. Prepare Selection Reagent
   a. Check the lot number on the reagent bottle to make sure it matches the lot number on the Master Lot Barcode Sheet.
   b. Record operator initials and the current date on the label.

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

C. Reagent Preparation for Previously Reconstituted Reagents

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

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

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

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

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

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

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

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

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

Note: *For samples transferred to the Hologic Specimen Lysis Tube or a custom Specimen Lysis Tube, to avoid a processing error, ensure adequate specimen volume is added to the tube. When adequate collected specimen is added to the tube, there is sufficient volume to perform 2 nucleic acid extractions*

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

F. System Preparation

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

Procedural Notes

A. Controls

1. To work properly with the Aptima Assay software for the Panther System, one pair of controls is required. The Aptima SARS-CoV-2 positive and negative controls can be loaded in any rack position or in any Sample Bay Lane on the Panther System. Patient specimen pipetting will begin when one of the following two conditions has been met:
   a. A pair of controls is currently being processed by the system.
   b. Valid results for the controls are registered on the system.
2. Once the control tubes have been pipetted and are processing for a specific reagent kit, patient specimens can be run with the associated kit up to 24 hours unless:
   a. Controls results are invalid.
   b. The associated assay reagent kit is removed from the system.
   c. The associated assay reagent kit has exceeded stability limits.
3. Each Aptima control tube can be tested once. Attempts to pipette more than once from the tube can lead to processing errors.
4. Patient specimen pipetting begins when one of the following two conditions is met:
   a. Valid results for the controls are registered on the system.
   b. A pair of controls is currently in process on the system.

B. Temperature

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

C. Glove Powder

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

D. Lab Contamination Monitoring Protocol for the Panther System

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

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

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

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

Quality Control

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

Negative and Positive Controls

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

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

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

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

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

Internal Control

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

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

Interpretation of Results

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

Table1 shows the possible results reported in a valid run with result interpretations.

Table 1: Result Interpretation

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

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

Limitations

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

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

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

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

Panther SARS-CoV-2 Assay Performance

Analytical Sensitivity

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

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

Table 2: Analytical Sensitivity Evaluation of Commercial Reference Material

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

Analytical Sensitivity with the Aptima Specimen Transfer Tube Workflow

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

Table 3: LoD Confirmation with the Aptima Specimen Transfer Workflow

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

Inclusivity

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

Analytical Specificity and Microbial Interference

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

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

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

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

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

[2] In place of evaluating pooled human nasal wash, testing of 30 individual negative clinical NP swab specimens was performed to represent diverse microbial flora in the human respiratory tract.

Clinical Performance

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

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

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

Table 5: Aptima SARS-CoV-2 Clinical Agreement

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

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

Clinical Performance with Contrived Panel

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

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

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

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

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

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

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

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

Table 8: Detection Characteristics for Contrived Nasopharyngeal Swab Specimens

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

Clinical Performance with Natural Infected Positive Specimens

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

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

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.

IVD

CE

EC REP
Hologic BVBA
Da Vincilaan 5
1930 Zaventem
Belgium

Hologic, Inc.
10210 Genetic Center Drive
San Diego, CA 92121 USA

Customer Support: +1 800 442 9892
customersupport@hologic.com

Technical Support: +1 888 484 4747
molecularsupport@hologic.com

For more contact information visit www.hologic.com.

AW-21491-001 Rev. 002
2020-06

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

For Emergency Use Authorization (EUA) only

For *in vitro* diagnostic use only

Rx only

CONTENTS

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 nasopharyngeal (NP), nasal, and oropharyngeal (OP) swab specimens and lower respiratory tract (LRT) specimens obtained from individuals who meet COVID-19 clinical and/or epidemiological criteria. The Panther Fusion SARS-CoV-2 Assay is for use only under Emergency Use Authorization (EUA) in the US laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C.§263a, to perform high complexity tests.

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, 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 land on objects and surfaces around the person. Other people may acquire SARS-CoV-2 by touching these objects or surfaces, then touching their eyes, nose, or mouth.

Person to person spread was subsequently reported outside Hubei province and in countries outside China, including in the United States.[2] Some international destinations and the United States now have apparent community spread of SARS-CoV-2 that is not related to travel.[3,4]

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

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

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

E. Handle 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.

F. Specimens may be infectious. Use Universal Precautions when performing this assay. Proper handling and disposal methods should be established by the laboratory director. Only personnel adequately trained in handling infectious materials should be permitted to perform this diagnostic procedure.[5]

G. 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.

H. Use only supplied or specified disposable laboratory ware.

I. 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).

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

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

L. 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.

M. 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.

N. 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.

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

P. Store assay components at the recommended storage condition. See *Reagent Storage and Handling Requirements (*page 7), and *Panther Fusion System Test Procedure* (page 12) for more information.

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

R. Avoid microbial and ribonuclease contamination of reagents.

S. 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.

T. 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.

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

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

W. 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.

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

Note: *For information on any hazard and precautionary statements that may be associated with reagents, refer to the Safety Data Sheet Library at www.hologicsds.com.*

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

B. LRT specimen collection

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

C. Specimen processing

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.*

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

E. 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 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)
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. **Do not vortex samples.**
2. 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.

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 swabs and mid-turbinate nasal swabs are considered acceptable specimen types for use with the Panther Fusion SARS-CoV-2 Assay but performance with the specimen type has not been established. Testing of nasal and mid-turbinate nasal swabs (self-collected under supervision of or collected by a healthcare provider) is limited to patients with symptoms of COVID-19. Please refer to FDA's FAQs on Diagnostic Testing for SARS-CoV-2 for additional information.

Conditions of Authorization for Labs

The Panther Fusion SARS-CoV-2 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/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd.

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

A. Authorized laboratories[1] using the Panther Fusion SARS-CoV-2 will include with result reports of the Panther Fusion SARS-CoV-2, 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 Panther Fusion SARS-CoV-2 will perform the Panther Fusion SARS-CoV-2 as outlined in the Panther Fusion SARS-CoV-2 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 are not permitted.

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

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

E. 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.

F. 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.

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

[1] For ease of reference, this letter will refer to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, 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.

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 2). 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 3. The *in silico* analysis showed no probable cross activity to any of the 183 GenBank sequences evaluated.

Table 2: 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 3: 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 4. Detection characteristics for positive contrived specimens were calculated by target concentration, as shown in Table 5.

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 6. Detection characteristics for positive contrived specimens were calculated by target concentrations, as shown in Table 7.

Table 4: 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 5: 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 6: 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 7: 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%

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.** 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.
3. **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.
4. **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.
5. **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.

Hologic, Inc.
10210 Genetic Center Drive
San Diego, CA 92121 USA

Customer Support: +1 800 442 9892
customersupport@hologic.com

Technical Support: +1 888 484 4747
molecularsupport@hologic.com

For more contact information visit www.hologic.com.

AW-21159-001 Rev. 003
2020-04

GeneXpert
Powered By CEPHEID INNOVATION

Xpert® Xpress SARS-CoV-2

Instructions for Use

REF XPRSARS-COV2-10

For Use with GeneXpert Dx or GeneXpert Infinity Systems

Cepheid

***In Vitro* Diagnostic Medical Device** CE IVD

302-3787, Rev. A May 2020

Trademark, Patents and Copyright Statements

Cepheid®, the Cepheid logo, GeneXpert®, and Xpert® are trademarks of Cepheid.

AccuPlex™ is a trademark of SeraCare Life Sciences.

Windows® is a trademark of Microsoft Corporation.

THE PURCHASE OF THIS PRODUCT CONVEYS TO THE BUYER THE NON-TRANSFERABLE RIGHT TO USE IT IN ACCORDANCE WITH THIS INSTRUCTIONS FOR USE. NO OTHER RIGHTS ARE CONVEYED EXPRESSLY, BY IMPLICATION OR BY ESTOPPEL. FURTHERMORE, NO RIGHTS FOR RESALE ARE CONFERRED WITH THE PURCHASE OF THIS PRODUCT.

Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089
USA
Phone: +1 408 541 4191
Fax: +1 408 541 4192

EC REP
Cepheid Europe SAS
Vira Solelh
81470 Maurens-Scopont
France
Phone: +33 563 825 300
Fax: +33 563 825 301

Xpert® Xpress SARS-CoV-2

1 Proprietary Name

Xpert® Xpress SARS-CoV-2

2 Common or Usual Name

Xpert Xpress SARS-CoV-2

3 Intended Use

The Xpert Xpress SARS-CoV-2 test is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasopharyngeal swab, nasal swab, or nasal wash/aspirate specimen collected from individuals who are suspected of COVID-19 infection.

Results are for the identification of SARS-CoV-2 RNA. 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.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The Xpert Xpress SARS-CoV-2 test is intended to be performed by trained users in both laboratory and near patient testing settings.

4 Summary and Explanation

An outbreak of respiratory illness of unknown etiology in Wuhan City, Hubei Province, China was initially reported to the World Health Organization (WHO) on December 31, 2019.[1] Chinese authorities identified a novel coronavirus (2019-nCoV) which was later renamed SARS-CoV-2 by the International Committee for Taxonomy of Viruses (ICTV).[2] The WHO declared the outbreak a global health emergency on January 30, 2020. SARS-CoV-2 has been responsible for over a million reported cases of Coronavirus infectious disease 2019 (COVID-19) worldwide. The morbidity and mortality of COVID-19 varies by patient age and risk factors, with the elderly and those with co-morbidities such as hypertension, diabetes, and respiratory disease at most risk.

The Xpert Xpress SARS-CoV-2 test is a molecular *in vitro* diagnostic test that aids in the detection and diagnosis of SARS-CoV-2 and is based on widely used nucleic acid amplification technology. The Xpert Xpress SARS-CoV-2 test contains primers and probes and internal controls used in RT-PCR for the *in vitro* qualitative detection of SARS-CoV-2 RNA in nasopharyngeal (NP) swab, nasal swab, or nasal wash/aspirate specimens.

5 Principle of the Procedure

The Xpert Xpress SARS-CoV-2 test is an automated *in vitro* diagnostic test for qualitative detection of nucleic acid from SARS-CoV-2. The Xpert Xpress SARS-CoV-2 test is performed on GeneXpert Instrument Systems.

The GeneXpert Instrument Systems automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in simple or complex samples using real-time PCR assays. The systems consist of an instrument, computer, and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable cartridges that hold the RT-PCR reagents and host the RT-PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the systems, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*.

The Xpert Xpress SARS-CoV-2 test includes reagents for the detection of RNA from SARS-CoV-2 in NP swab, nasal swab, or nasal wash/aspirate specimen. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge utilized by the GeneXpert instrument. The SPC is present to control for adequate processing of the sample and to monitor for the presence of potential inhibitor(s) in the RT-PCR reaction. The SPC also ensures that the RT-PCR reaction conditions (temperature and time) are appropriate for the amplification reaction and that the RT-PCR reagents are functional. The PCC verifies reagent rehydration, PCR tube filling, and confirms that all reaction components are present in the cartridge including monitoring for probe integrity and dye stability.

The NP swab, nasal swab, or nasal wash/aspirate specimen is collected and placed into a transport tube containing 3 mL of viral transport medium or 3 mL of saline. The specimen is briefly mixed by rapidly inverting the collection tube 5 times. Using the supplied transfer pipette, the sample is transferred to the sample chamber of the Xpert Xpress SARS-CoV-2 cartridge. The GeneXpert cartridge is loaded onto the GeneXpert Instrument System platform, which performs hands-off, automated sample processing, and real-time RT-PCR for detection of viral RNA.

6 Reagents and Instruments

6.1 Materials Provided

The Xpert Xpress SARS-CoV-2 kit contains sufficient reagents to process 10 specimens or quality control samples. The kit contains the following:

Xpert Xpress SARS-CoV-2 Cartridges with Integrated Reaction Tubes	**10**
• Bead 1, Bead 2, and Bead 3 (freeze-dried)	1 of each per cartridge
• Lysis Reagent	1.5 mL per cartridge
• Binding Reagent	1.5 mL per cartridge
• Elution Reagent	3.0 mL per cartridge
Disposable Transfer Pipettes	**10-12 per kit**
CD	**1 per kit**
• Assay Definition Files (ADF)	
• Instructions to import ADF into GeneXpert software	
Flyer	**1 per kit**
• Directions to locate the Product Insert on www.cepheid.com	

Note Safety Data Sheets (SDS) are available at www.cepheidinternational.com under the **SUPPORT** tab.

Note The bovine serum albumin (BSA) in the beads within this product was produced and manufactured exclusively from bovine plasma sourced in the United States. No ruminant protein or other animal protein was fed to the animals; the animals passed ante- and postmortem testing. During processing, there was no mixing of the material with other animal materials.

7 Storage and Handling

- Store the Xpert Xpress SARS-CoV-2 cartridges at 2-28°C.
- Do not open a cartridge lid until you are ready to perform testing.
- Do not use a cartridge that is wet or has leaked.

8 Materials Required but Not Provided

- Nylon flocked swab (Copan P/N 502CS01, 503CS01) or equivalent
- Viral transport medium, 3 mL (Copan P/N 330C) or equivalent
- 0.85% (w/v) saline, 3 mL
- Sample Collection Kit for Viruses (Cepheid P/N SWAB/B-100, SWAB/F-100)
- GeneXpert Dx or GeneXpert Infinity systems (catalog number varies by configuration): GeneXpert instrument, computer, barcode scanner, operator manual.

  For GeneXpert Dx System: GeneXpert Dx software version 4.7b or higher

  For GeneXpert Infinity-80 and Infinity-48s systems: Xpertise software version 6.4b or higher

9 Materials Available but Not Provided

SeraCare AccuPlex™ Reference Material Kit, catalog number 0505-0126 (Order Code CEPHEID)

10 Warnings and Precautions

10.1 General

- For *in vitro* diagnostic use.
- Positive results are indicative of presence of SARS-CoV-2 RNA.
- Report all positive results to the appropriate health authorities as required.
-  Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be handled using standard precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention[3] and the Clinical and Laboratory Standards Institute.[4]
- Follow safety procedures set by your institution for working with chemicals and handling biological specimens.
- Consult your institution's environmental waste personnel on proper disposal of used cartridges, which may contain amplified material. This material may exhibit characteristics of federal EPA Resource Conservation and Recovery Act (RCRA) hazardous waste requiring specific disposal requirements. Check state and local regulations as they may differ from federal disposal regulations. Institutions should check the hazardous waste disposal requirements within their respective countries.

10.2 Specimens

- Maintain proper storage conditions during specimen transport to ensure the integrity of the specimen (see Section 12, Specimen Collection, Transport, and Storage). Specimen stability under shipping conditions other than those recommended has not been evaluated.

10.3 Assay/Reagent

- Do not open the Xpert Xpress SARS-CoV-2 cartridge lid except when adding specimen.
- Do not use a cartridge that has been dropped after removing it from the packaging.
- Do not shake the cartridge. Shaking or dropping the cartridge after opening the cartridge lid may yield non-determinate results.
- Do not place the sample ID label on the cartridge lid or on the barcode label on the cartridge.
- Do not use a cartridge with a damaged barcode label.
- Do not use a cartridge that has a damaged reaction tube.
-  Each single-use Xpert Xpress SARS-CoV-2 cartridge is used to process one test. Do not reuse processed cartridges.
-  Each single-use disposable pipette is used to transfer one specimen. Do not reuse disposable pipettes
- Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
- Wear clean lab coats and gloves. Change gloves between the handling of each specimen.

- In the event of a spill of specimens or controls, wear gloves and absorb the spill with paper towels. Then, thoroughly clean the contaminated area with a 10% freshly prepared household chlorine bleach. Allow a minimum of two minutes of contact time. Ensure the work area is dry before using 70% denatured ethanol to remove bleach residue. Allow surface to dry completely before proceeding. Or, follow your institution's standard procedures for a contamination or spill event. For equipment, follow the manufacturer's recommendations for decontamination of equipment.
- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents requiring standard precautions. Follow your institution's environmental waste procedures for proper disposal of used cartridges and unused reagents. These materials may exhibit characteristics of chemical hazardous waste requiring specific disposal. If country or regional regulations do not provide clear direction on proper disposal, biological specimens and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.

11 Chemical Hazards[5,6]

- Signal Word: WARNING
- **UN GHS Hazard Statements**
  - Harmful if swallowed.
  - May be harmful in contact with skin.
  - Causes eye irritation.
- **UN GHS Precautionary Statements**
  - **Prevention**
    - Wash hands thoroughly after handling.
  - **Response**
    - Call a POISON CENTER or doctor/physician if you feel unwell.
    - If skin irritation occurs: Get medical advice/attention.
    - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
    - If eye irritation persists: Get medical advice/attention.

12 Specimen Collection, Transport, and Storage

Proper specimen collection, storage, and transport are critical to the performance of this test. Inadequate specimen collection, improper specimen handling and/or transport may yield a false result. See Section 12.1 for nasopharyngeal swab collection procedure and Section 12.2 for nasal swab collection procedure, and Section 12.3 for nasal wash/aspirate procedure.

Nasopharyngeal swab, nasal swab and nasal wash/aspirate specimens can be stored in viral transport medium or saline, at room temperature (15-30 °C) for up to 8 hours and refrigerated (2-8 °C) up to 7 days until testing is performed on the GeneXpert Instrument Systems.

Refer to the WHO Laboratory Biosafety Guidance Related to the Coronavirus Disease 2019 (COVID-19).

https://www.who.int/publications-detail/laboratory-biosafety-guidance-related-to-coronavirus-disease-2019-(covid-19)

12.1 Nasopharyngeal Swab Collection Procedure

Insert the swab into either nostril, passing it into the posterior nasopharynx (see Figure 1). Rotate swab by firmly brushing against the nasopharynx several times. Remove and place the swab into the tube containing 3mL of viral transport medium or 3 mL of saline. Break swab at the indicated break line and cap the specimen collection tube tightly.

Figure 1. Nasopharyngeal Swab Collection

12.2 Nasal Swab Collection Procedure

1. Insert a nasal swab 1 to 1.5 cm into a nostril. Rotate the swab against the inside of the nostril for 3 seconds while applying pressure with a finger to the outside of the nostril (see Figure 2).

Figure 2. Nasal Swab Collection for First Nostril

2. Repeat on the other nostril with the same swab, using external pressure on the outside of the other nostril (see Figure 3). To avoid specimen contamination, do not touch the swab tip to anything other than the inside of the nostril.

Figure 3. Nasal Swab Collection for Second Nostril

3. Remove and place the swab into the tube containing 3 mL of viral transport medium or 3 mL of saline. Break swab at the indicated break line and cap the specimen collection tube tightly.

12.3 Nasal Wash/Aspirate Procedure

1. Nasal wash/aspirate specimens can be collected following the user institution standard procedure. Also, refer to the WHO guidelines for the collection of human nasal wash/aspirate specimens.

   https://www.who.int/influenza/human_animal_interface/virology_laboratories_and_vaccines/guidelines_collection_h5n1_humans/en/

2. Using a transfer pipette, transfer 600 µL of the undiluted nasal wash/aspirate specimen into the tube containing 3 mL of viral transport medium or 3 mL of saline and then cap the tube.

13 Procedure

13.1 Preparing the Cartridge

Important **Start the test within 30 minutes of adding the sample to the cartridge.**

1. Remove a cartridge from the package.
2. Check the specimen transport tube is closed.
3. Mix specimen by rapidly inverting the specimen transport tube 5 times. Open cap on the specimen transport tube.
4. Open the cartridge lid.
5. Remove the transfer pipette from the wrapper.
6. Squeeze the top bulb of the transfer pipette completely and then place the pipette tip in the specimen transport tube (see Figure 4).

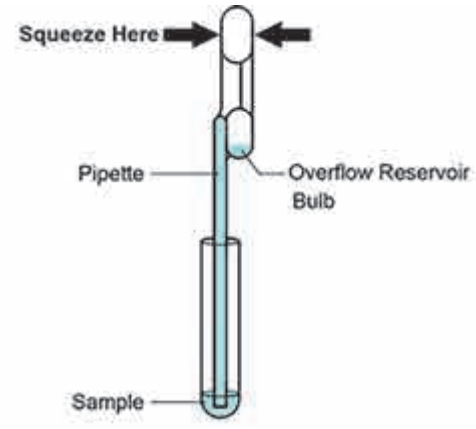


Figure 4. Transfer Pipette

7. Release the top bulb of the pipette to fill the pipette before removing from the tube. After filling pipette, excess sample will be seen in the overflow reservoir bulb of the pipette (see Figure 4). Check that the pipette does not contain bubbles.
8. To transfer the sample to the cartridge, squeeze the top bulb of the transfer pipette completely again to empty the contents of the pipette (300 µL) into the large opening (Sample Chamber) in the cartridge shown in Figure 5. Dispose of the used pipette.

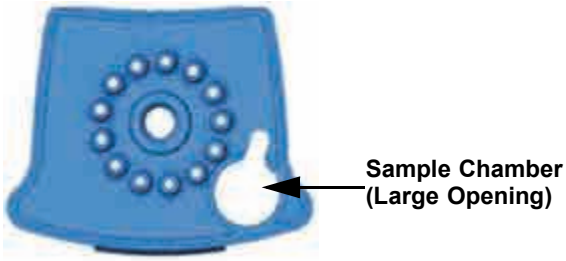


Figure 5. Xpert Xpress SARS-CoV-2 Cartridge (Top View)

Note Take care to dispense the entire volume of liquid into the Sample Chamber. False negative results may occur if insufficient sample is added to the cartridge.

9. Close the cartridge lid.

13.2 External Controls

External controls described in Section 9 are available but not provided and may be used in accordance with local, state, and federal accrediting organizations, as applicable.

To run a control using the Xpert Xpress SARS-CoV-2 test, perform the following steps:

1. Mix control by rapidly inverting the external control tube 5 times. Open cap on external control tube.
2. Open the cartridge lid.
3. Using a clean transfer pipette, transfer one draw of the external control sample (300 µL) into the large opening (Sample Chamber) in the cartridge shown in Figure 5.
4. Close cartridge lid.

13.3 Starting the Test

Note Before you start the test, make sure that the system contains modules with GeneXpert Dx software version 4.7b or higher or Infinity Xpertise software 6.4b or higher, and that the Xpert Xpress SARS-CoV-2 Assay Definition File is imported into the software.

This section lists the default steps to operate the GeneXpert Instrument System. For detailed instructions, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*, depending on the model that is being used.

Note The steps you follow may be different if the system administrator has changed the default workflow of the system.

1. Turn on the GeneXpert Instrument System:
   - **GeneXpert Dx:**
     If using the GeneXpert Dx instrument, first turn on the instrument and then turn on the computer. Log into the Windows operating system. The GeneXpert software may launch automatically or may require double- clicking on the GeneXpert Dx shortcut icon on the Windows® desktop.

     or
   - **GeneXpert Infinity System:**
     If using the GeneXpert Infinity instrument, power up the instrument by turning the power switch clockwise to the **ON** position. On the Windows desktop, double-click the Xpertise Software shortcut icon to launch the software.
2. Log on to the System software. The login screen appears. Type your user name and password.
3. In the GeneXpert System window, click **Create Test** (GeneXpert Dx) or **Orders** followed by **Order Test** (Infinity).
4. Scan or type in the Patient ID (optional). If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is shown on the left side of the View Results window and is associated with the test result.
5. Scan or type in the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is shown on the left side of the View Results window and is associated with the test result.
6. Scan the barcode on the Xpert Xpress SARS-CoV-2 cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Reagent Lot ID, Cartridge SN, Expiration Date and Selected Assay.

Note If the barcode on the Xpert Xpress SARS-CoV-2 cartridge does not scan, then repeat the test with a new cartridge.

7. Click **Start Test** (GeneXpert Dx) or **Submit** (Infinity) if Auto-Submit is not enabled. In the dialog box that appears, type your password, if required.

   For the GeneXpert Dx Instrument

   A. Locate the module with the blinking green light, open the instrument module door and load the cartridge.

   B. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off and the door will unlock. Remove the cartridge.

   C. Dispose of used cartridges in the appropriate sample waste containers according to your institution's standard practices.

   or

   For the GeneXpert Infinity System

   A. After clicking **Submit**, you will be asked to place the cartridge on the conveyor belt. After placing the cartridge, click **OK** to continue. The cartridge will be automatically loaded, the test will run and the used cartridge will be placed onto the waste shelf for disposal.

   B. When all samples are loaded, click on the **End Order Test** icon.

Note Do not turn off or unplug the instruments while a test is in progress. Turning off or unplugging the GeneXpert instrument or computer will stop the test.

14 Viewing and Printing Results

For detailed instructions on how to view and print the results, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*.

15 Quality Control

15.1 Internal Controls

CONTROL

Each cartridge includes a Sample Processing Control (SPC) and Probe Check Control (PCC).

Sample Processing Control (SPC) - Ensures that the sample was processed correctly. The SPC verifies that sample processing is adequate. Additionally, this control detects sample-associated inhibition of the real-time PCR assay, ensures that the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction, and that the PCR reagents are functional. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria.

Probe Check Control (PCC) - Before the start of the PCR reaction, the GeneXpert System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the validated acceptance criteria.

15.2 External Controls

External controls should be used in accordance with local, state, and federal accrediting organizations as applicable.

16 Interpretation of Results

The results are interpreted automatically by the GeneXpert System and are clearly shown in the **View Results** window. The Xpert Xpress SARS-CoV-2 test provides test results based on the detection of two gene targets according to the algorithms shown in Table 1.

Table 1. Xpert Xpress SARS-CoV-2 Possible Results

Result Text	N2	E	SPC
SARS-CoV-2 POSITIVE	+	+/-	+/-
SARS-CoV-2 PRESUMPTIVE POS	-	+	+/-
SARS-CoV-2 NEGATIVE	-	-	+
INVALID	-	-	-

See Table 2 to interpret test result statements for the Xpert Xpress SARS-CoV-2 test.

Table 2. Xpert Xpress SARS-CoV-2 Results and Interpretation

Result	Interpretation
SARS-CoV-2 POSITIVE	The 2019 novel coronavirus (SARS-CoV-2) target nucleic acids are detected. • The SARS-CoV-2 signal for the N2 nucleic acid target or signals for both nucleic acid targets (N2 and E) have a Ct within the valid range and endpoint above the minimum setting • SPC: NA; SPC is ignored because coronavirus target amplification occurred • Probe Check: PASS; all probe check results pass
SARS-CoV-2 PRESUMPTIVE POS	The 2019 novel coronavirus (SARS-CoV-2) nucleic acids may be present. Sample should be retested according to the Retest Procedure in Section 17.2. For samples with a repeated presumptive positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management. • The SARS-CoV-2 signal for only the E nucleic acid target has a Ct within the valid range and endpoint above the minimum setting • SPC: NA; SPC is ignored because a target amplification has occurred. • Probe Check: PASS; all probe check results pass
SARS-CoV-2 NEGATIVE	The 2019 novel coronavirus (SARS-CoV-2) target nucleic acids are not detected. • The SARS-CoV-2 signals for two nucleic acid targets (N2 and E) do not have a Ct within the valid range and endpoint above the minimum setting • SPC: PASS; SPC has a Ct within the valid range and endpoint above the minimum setting • Probe Check: PASS; all probe check results pass

Table 2. Xpert Xpress SARS-CoV-2 Results and Interpretation (Continued)

Result	Interpretation
INVALID	SPC does not meet acceptance criteria. Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. • SPC: FAIL; SPC and SARS-CoV-2 signals do not have a Ct within valid range and endpoint below minimum setting • Probe Check - PASS; all probe check results pass
ERROR	Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. • SARS-CoV-2: NO RESULT • SPC: NO RESULT • Probe Check: FAIL[1]; all or one of the probe check results fail [1] If the probe check passes, the error is caused by the maximum pressure limit exceeding the acceptable range, no sample added, or by a system component failure.
NO RESULT	Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress. • SARS-CoV-2: NO RESULT • SPC: NO RESULT • Probe Check: NA (not applicable)

The Xpert Xpress SARS-CoV-2 test includes an Early Assay Termination (EAT) function which will provide earlier time to results in high titer specimens. When SARS-CoV-2 titers are high enough to initiate the EAT function, the SPC amplification curve may not be seen and its results may not be reported.

17 Retests

17.1 Reasons to Repeat the Assay

If any of the test results mentioned below occur, repeat the test once according to instructions in Section 17.2, Retest Procedure.

- A **PRESUMPTIVE POS** result indicates the 2019 novel coronavirus (SARS-CoV-2) nucleic acids may be present. Only one of the SARS-CoV-2 nucleic acid target was detected (E gene) while the other SARS-CoV-2 nucleic acid target (N2 gene) was not detected.
- An **INVALID** result indicates that the control SPC failed. The sample was not properly processed, PCR is inhibited, or the sample was not properly collected.
- An **ERROR** result could be due to, but not limited to, Probe Check Control failure, system component failure, no sample added, or the maximum pressure limits were exceeded.
- A **NO RESULT** indicates that insufficient data were collected. For example, cartridge failed integrity test, the operator stopped a test that was in progress, or a power failure occurred.

If an External Control fails to perform as expected, repeat external control test and/or contact Cepheid for assistance.

17.2 Retest Procedure

To retest a non-determinate result (**INVALID**, **NO RESULT**, or **ERROR)** or a **PRESUMPTIVE POS** result, use a new cartridge.

Use the leftover sample from the original specimen transport medium tube or new external control tube.

1. Put on a clean pair of gloves. Obtain a new Xpert Xpress SARS-CoV-2 cartridge and a new transfer pipette.
2. Check the specimen transport tube or external control tube is closed.

3. Mix the sample by rapidly invert the specimen transport medium tube or external control tube 5 times. Open the cap on the specimen transport tube or external control tube.
4. Open the cartridge lid.
5. Using a clean transfer pipette (supplied), transfer sample (one draw) to the sample chamber with the large opening in the cartridge.
6. Close the cartridge lid.

18 Limitations

- Performance characteristics of this test have been established with the specimen types listed in the Intended Use Section only. The performance of this assay with other specimen types or samples has not been evaluated.
- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if inadequate numbers of organisms are present in the specimen.
- As with any molecular test, mutations within the target regions of Xpert Xpress SARS-CoV-2 could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

19 Performance Characteristics

19.1 Clinical Evaluation

The performance of the Xpert Xpress SARS-CoV-2 test was evaluated using contrived clinical NP swab specimens in viral transport medium obtained from US patients with signs and symptoms of respiratory infection. The samples were prepared by spiking each individual negative clinical NP swab sample with live SARS-CoV-2 virus (USA_WA1/2020) at 2x LoD, 3x LoD and 5x LoD levels. The NP swab samples were determined to be negative for SARS-CoV-2 prior to spiking. Individual negative NP swab samples were also tested in the study. All positive and negative samples in the study were tested in a randomized and blinded fashion.

Table 3 shows the number of concordant results out of the total number of samples tested for each target concentration of live SARS-CoV-2 virus, the mean Ct values for each of the E and N2 nucleic acid targets as well as the percent agreement with the 95% confidence interval (95% CI), where appropriate. At each target concentration, the results show 100% agreement with the expected results in the live SARS-CoV-2 virus spiked samples and 100% agreement with the expected results in the negative samples. The overall performance of Xpert Xpress SARS-CoV-2 for all 30 samples combined shows a positive percent agreement (PPA) of 100% (95% CI: 88.7% - 100%) and a negative percent agreement (NPA) of 100% (95% CI: 88.7% - 100%).

Table 3. Xpert SARS-CoV-2 Test Agreement with the Expected Results by Sample Concentration

Target Concentration	Number Concordant/ Number Tested	E Mean Ct	N2 Mean Ct	% Agreement [95% CI]
2x LoD	20/20	35.4	38.4	100% [83.9% - 100%]
3x LoD	5/5	34.2	37.2	100% [NA*]
5x LoD	5/5	33.9	37.0	100% [NA*]
Negative	30/30	NA	NA	100% [88.7% - 100%]

*95% CI not computed for sample concentrations with sample size of 5 or less.

20 Analytical Performance

20.1 Analytical Sensitivity (Limit of Detection)

Studies were performed to determine the analytical limit of detection (LoD) of the Xpert Xpress SARS-CoV-2. The LoD of Xpert Xpress SARS-CoV-2 was established using one lot of reagent and limiting dilutions of live SARS-CoV-2 virus (USA_WA1/2020) prepared in viral transport medium and NP swab clinical matrix. Verification of the estimated LoD claim was performed on one reagent lot in replicates of 22 prepared in NP swab clinical matrix. The LoD is the lowest concentration (reported as PFU/mL) of live SARS-CoV-2 virus samples that can be reproducibly distinguished from negative samples ≥ 95% of the time with 95% confidence. The claimed LoD for the assay is 0.0100 PFU/mL (Table 4).

Table 4. Limit of Detection of the Xpert Xpress SARS-CoV-2

Strain	Claimed LoD (PFU/mL)	Positives/ Replicates
SARS-CoV-2 virus (USA_WA1/2020)	0.0100	22/22

20.2 Analytical Reactivity (Inclusivity)

The inclusivity of Xpert Xpress SARS-CoV-2 was evaluated using *in silico* analysis of the assay primers and probes in relation to 324 SARS-CoV-2 sequences available in the GISAID gene database for two targets, E and N2.

For the E target, Xpert Xpress SARS-CoV-2 had 100% match to all sequences with the exception of 4 sequences that had a single mismatch. For the N2 target, Xpert Xpress SARS-CoV-2 had 100% match to all sequences with the exception of 2 sequences that had a single mismatch. None of these mismatches found for both targets are predicted to have a negative impact on the performance of the assay, given the location of the mutations in the primer and probe regions respectively for the two variants. These mutations are not predicted to adversely affect the probe and primer binding to the sequences or reduce assay efficiency.

20.3 Analytical Specificity (Exclusivity)

An *in silico* analysis for possible cross-reactions with all the organisms listed in Table 5 was conducted by mapping primers and probes in the Xpert Xpress SARS-CoV-2 test individually to the sequences downloaded from the GISAID database. E primers and probes are not specific for SARS-CoV-2 and will detect Human and Bat SARS-coronavirus. No potential unintended cross reactivity with other organisms listed in Table 5 is expected based on the *in silico* analysis.

Table 5. Xpert Xpress SARS-CoV-2 Analytical Specificity Microorganisms

Microorganisms from the Same Genetic Family	High Priority Organisms
Human coronavirus 229E	Adenovirus (e.g. C1 Ad. 71)
Human coronavirus OC43	Human Metapneumovirus (hMPV)
Human coronavirus HKU1	Parainfluenza virus 1-4
Human coronavirus NL63	Influenza A
SARS-coronavirus	Influenza B
MERS-coronavirus	Influenza C
Bat coronavirus	Enterovirus (e.g. EV68)
	Respiratory syncytial virus
	Rhinovirus
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococcus pneumoniae
	Streptococcus pyogenes

Table 5. Xpert Xpress SARS-CoV-2 Analytical Specificity Microorganisms

Microorganisms from the Same Genetic Family	High Priority Organisms
	Bordetella pertussis
	Mycoplasma pneumoniae
	Pneumocystis jirovecii (PJP)
	Parechovirus
	Candida albicans
	Corynebacterium diphtheriae
	Legionella non-pneumophila
	Bacillus anthracis (Anthrax)
	Moraxella catarrhalis
	Neisseria elongate and meningitidis
	Pseudomonas aeruginosa
	Staphylococcus epidermidis
	Staphylococcus salivarius
	Leptospira
	Chlamydia psittaci
	Coxiella burnetii (Q-Fever)
	Staphylococcus aureus

20.4 Interfering Substances

Potentially interfering substances studies have been conducted for previous Xpert Flu/RSV tests developed for the GeneXpert system, including Xpert Xpress Flu/RSV and Xpert Flu/RSV XC tests and assay interference was not observed in these studies. Further testing evaluating potentially interfering substances was not conducted with the Xpert Xpress SARS-CoV-2 test. The Xpert Xpress SARS-CoV-2 test uses conventional well-established nucleic acid extraction methods that are utilized with the Xpert Xpress Flu/RSV and Xpert Flu/RSV XC tests. In addition, the Xpert Flu/RSV tests are validated for use with the same specimen types, nasopharyngeal swabs and/or nasal wash/aspirates specimens, as the Xpert Xpress SARS-CoV-2 test. Therefore, assay interference from these substances is not expected for the Xpert Xpress SARS-CoV-2 test.

20.5 Carry-over Contamination Study

Carry-over studies have been conducted for previous Xpert tests developed for the GeneXpert system, including Xpert Xpress Flu/RSV, and no contamination due to carry-over was observed. Further testing for carry-over contamination was not conducted for Xpert Xpress SARS-CoV-2. To minimize test-to-test contamination, specimen and fluids including amplicons are contained within the single-use, disposable cartridge. The self-contained cartridge design prevents the GeneXpert instrument coming into contact with any fluids within the cartridge. Precise fluidic handling within the enclosed cartridge is driven by the syringe and valve, commanded by the assay definition file (ADF) and automated by the GeneXpert instrument. No manual pipetting step is required other than the addition of the specimen to the cartridge by the user prior to the cartridge being placed on the instrument. Once the specimen is added to the cartridge the lid is closed. Thus the instrument and cartridge design are a closed system which minimizes the potential for carry-over.

21 Reproducibility

The reproducibility of the Xpert Xpress SARS-CoV-2 test was established at three sites using a 5-member panel including one negative sample, two low positive (~1.5x LoD) and two moderate positive (~3x LoD) samples. The negative sample consisted of simulated matrix without target microorganism or target RNA. The positive samples were contrived samples in a simulated matrix using either AccuPlex™ SARS-CoV-2 reference material (targeting the N2 and E genes) or inactivated SARS-CoV Urbani strain (targeting the E gene).

Testing was conducted over six (6) days, using three (3) lots of Xpert Xpress SARS-CoV-2 cartridges at three (3) participating sites each with two (2) operators to yield a total of 144 observations per panel member (3 Sites x 2 Operators x 3 Lots x 2 Days/Lot x 2 Runs x 2 Reps = 144 observations/panel member). The results from the study are summarized in Table 6.

Table 6. Summary of Reproducibility Results - % Agreement by Study Site/Operator

Sample	Site 1			Site 2			Site 3			% Total Agreement[a] by Sample
	Op1	Op2	Site	Op1	Op2	Site	Op1	Op2	Site	
Negative	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)
SARS-CoV-2 Low Pos	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	95.8% (23/24)	97.9% (47/48)	95.8% (23/24)	100% (24/24)	97.9% (47/48)	98.6% (142/144)
SARS-CoV-2 Mod Pos	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)
SARS-CoV-2 Low Pos	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)
SARS-CoV-2 Mod Pos	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)

a. Agreement was calculated as the percentage of observed results that were in agreement with the expected results.

22 References

1. Centers for Disease Control and Prevention. https://www.cdc.gov/coronavirus/2019-ncov/index.html. Accessed February 9, 2020.
2. bioRxiv. (https://www.biorxiv.org/content/10.1101/2020.02.07.937862v1). Accessed March 3, 2020.
3. Centers for Disease Control and Prevention. *Biosafety in Microbiological and Biomedical laboratories* (refer to latest edition). http://www.cdc.gov/biosafety/publications/
4. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline.* Document M29 (refer to latest edition).
5. REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on the classification labeling and packaging of substances and mixtures amending and repealing, List of Precautionary Statements, Directives 67/548/EEC and 1999/45/EC (amending Regulation (EC) No 1907/2007).
6. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z).

23 Cepheid Headquarters Locations

Corporate Headquarters	European Headquarters
Cepheid 904 Caribbean Drive Sunnyvale, CA 94089 USA	Cepheid Europe SAS Vira Solelh 81470 Maurens-Scopont France
Telephone: +1 408 541 4191	Telephone: +33 563 825 300
Fax: +1 408 541 4192	Fax: +33 563 825 301
www.cepheid.com	www.cepheidinternational.com

24 Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

- Product name
- Lot number
- Serial number of the instrument
- Error messages (if any)
- Software version and, if applicable, Computer Service Tag number

Region	Telephone	Email
US	+1 888.838.3222	techsupport@cepheid.com
France	+33 563 825 319	support@cepheideurope.com

Contact information for all Cepheid Technical Support offices is available on our website: www.cepheid.com/en/CustomerSupport.

25 Table of Symbols

Symbol	Meaning
REF	Catalog number
IVD	*In vitro* diagnostic medical device
	Do not re-use
LOT	Batch code
CE	CE marking - European Conformity
EC REP	Authorized representative in the European Community
	Consult instructions for use
	Caution
	Manufacturer
	Country of manufacture
	Contains sufficient for <n> tests
CONTROL	Control
	Expiration date
	Temperature limitation
	Biological risks

Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089
USA
Phone: +1 408 541 4191
Fax: +1 408 541 4192

EC REP
Cepheid Europe SAS
Vira Solelh
81470 Maurens-Scopont
France
Phone: +33 563 825 300
Fax: +33 563 825 301

CE IVD