INSTRUCTIONS FOR USE

Sherlock™ CRISPR SARS-CoV-2 kit

Rx Only

IVD

For Emergency Use Authorization (EUA) only

CATALOG NUMBER	COMPANY
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Intended Use

The **Sherlock™ CRISPR SARS-CoV-2 kit** is intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (anterior nasal swabs, nasopharyngeal swabs, oropharyngeal swabs, nasopharyngeal wash/aspirate or nasal aspirate) and bronchoalveolar lavage specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The RNA of 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 infective status. Positive results do not rule out bacterial 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 results to the appropriate public health authorities.

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

The **Sherlock™ CRISPR SARS-CoV-2 kit** is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of non-automated molecular in vitro diagnostic procedures. The **Sherlock™ CRISPR SARS-CoV-2 kit** is only for use under the Food and Drug Administration's Emergency Use Authorization.

Summary and Explanation

An outbreak of pneumonia caused by a novel coronavirus (SARS-CoV-2) in Wuhan City, Hubei Province, China was initially reported to WHO on December 31, 2019. On January 31, 2020, Health and Human Services Secretary Alex M. Azar II declared a public health emergency (PHE) for the United States to aid the nation's healthcare community in responding to SARS-CoV-2. The emergence and rapid spread of SARS-CoV-2 to numerous areas throughout the world, has necessitated preparedness and response in public health laboratories, as well as health care and other areas of society in general. The availability of specific and sensitive assays for the detection of the virus are essential for accurate diagnosis of cases, assessment of the extent of the outbreak, monitoring of intervention strategies and surveillance studies.

Principles of the Procedure

The **Sherlock™ CRISPR SARS-CoV-2 kit** has been designed to detect fragments of the Open Reading Frame (ORF1ab, "O") gene and the Nucleocapsid ("N") gene of SARS-CoV-2. An included third target is the human RNase P POP7 gene ("RP") which serves as a control for the extraction of the clinical sample in the absence of a positive SARS-CoV-2 result. A dedicated instrument platform (e.g., thermal cycler) is not required. Amplification can be performed using a heat block, and CRISPR complex activation and reporter cleavage can be run in a standard microplate reader capable of fluorescence detection. The entire reaction from RT-LAMP amplification to CRISPR-based detection of the target analytes can be performed in approximately one hour.

The **Sherlock™ CRISPR SARS-CoV-2 kit** is designed to detect RNA from upper respiratory specimens (anterior nasal swabs, nasopharyngeal swabs, oropharyngeal swabs, nasopharyngeal wash/aspirate or nasal aspirate) and bronchoalveolar lavage specimens from patients suspected of COVID-19 by their healthcare provider. RNA is extracted from clinical samples using the PureLink™ Viral RNA/DNA Mini Kit.

The **Sherlock™ CRISPR SARS-CoV-2 kit** comprises two steps. Step one is a reverse transcriptase loop-mediated amplification (RT-LAMP) where targeted SARS-CoV-2 genomic RNA is reverse transcribed to DNA, and this DNA is amplified by a strand-displacing DNA polymerase. Step two is the transcription of the amplified DNA to activate the collateral cleavage activity of a CRISPR complex programmed to the target RNA sequence. This collateral activity results in cleavage of nucleic acid reporters, resulting in a fluorescent readout detected by a plate reader.

Components and Storage

Materials Required (Provided): Each Sherlock™ CRISPR SARS-CoV-2 kit consists of the following components:

Table 1: Components Included in Kit

Cap Color	Component	Description	Pack Size – 33 Tests per kit	Volume per Vial
Blue	**RP – 10x Primer**	RNase P POP7 gene LAMP Primer Mix	1 vial	100 µL
	N – 10x Primer	N gene LAMP Primer Mix	1 vial	100 µL
	O – 10x Primer	ORF1ab gene LAMP Primer Mix	1 vial	100 µL
	2x RT-LAMP Mix	2x Warm Start RT-LAMP Mix	1 vial	1.1 mL
Yellow	**RP – crRNA**	RNase P POP7 gene crRNA, 1 µM	1 vial	40 µL
	N – crRNA	N gene crRNA, 1 µM	1 vial	40 µL
	O – crRNA	ORF1ab gene crRNA, 1 µM	1 vial	40 µL
	Reporter	RNase Alert, 2 µM	1 vial	200 µL
Red	**CRISPR-Cas enzyme**	LwaCas13a Enzyme, 500 ng/µL	1 vial	50 µL
	T7 RNA Polymerase	T7 Polymerase, 50 U/µL	1 vial	70 µL
	rNTP Mix	rNTP Mix, 25 mM of each rNTP	1 vial	120 µL
	Rnase Inhibitor	Murine RNase Inhibitor, 40 U/µL	1 vial	75 µL
	$MgCl_2$	1 M $MgCl_2$	1 vial	50 µL

Storage and Handling of Kit Components

- The **Sherlock™ CRISPR SARS-CoV-2 kit** is shipped on dry ice. The components of the kit should arrive frozen. If one or more of the components are not frozen upon receipt or are compromised during shipment, contact Sherlock Biosciences for assistance.
- Store all components at or below -20°C to prevent degradation of reagents.
- Based on individual component shelf life, the approximate shelf life of the kit is estimated to be 12 months.
- Always check the expiration date prior to use. Do not use expired reagents.
- Always work with **Sherlock™ CRISPR SARS-CoV-2 kit** components on ice.

Materials Required (But Not Provided)

Control Materials

- **Positive Control:** Quantified extracted SARS-CoV-2 genomic RNA (recommended supplier below)
- **Negative Control:** Molecular grade, nuclease-free water (recommended supplier below)

Table 2: Control Materials

Control	Supplier	Part Number	Description
SARS-CoV-2 Positive Control (*spc*)	BEI Resources ATCC®	NR-52285 VR1986D™	Genomic RNA from SARS-Related Coronavirus 2, Isolate USA-WA1/2020 or equivalent (BEI NR-52285, or ATCC® VR1986D™), diluted to a concentration of 4800 copies/µL
Negative Template Control (*ntc*)	Qiagen	1039480	Nuclease-free water for use in Any Molecular Biology application

RNA Extraction System

Table 3: RNA Extraction System

Extraction System	Manufacturer	Catalog No.
PureLink™ Viral RNA/DNA Mini Kit	Thermo Fisher Scientific	12280050 (50 extractions)

Equipment

Note: Prior to use, ensure that instruments and equipment have been maintained and calibrated according to the manufacturer's recommendations.

Table 4: Equipment

Equipment	Manufacturer	Model
Plate Reader running Gen5 3.08 software	BioTek	NEO2
PC running Microsoft Excel	NA	NA

Table 5: Additional Equipment and Consumables

Equipment and Consumables
0.2 mL strip tubes
1.5 mL snap cap tubes, low bind and nuclease-free
Molecular grade water (nuclease-free)

96–100% ethanol
Dedicated adjustable P-10, P-20, P-100, P-200, and P-1000 pipettes for sample preparation
Dedicated adjustable P-10 or P-20 for dispensing template RNA
Dedicated adjustable P-10, P-20, P-100, P-200, and P-1000 pipettes for preparing and dispensing master mix
Dedicated adjustable M-10 and M-100 multichannel pipettes for transferring RT-LAMP amplified product and CRISPR Cas detection reaction
Dedicated electronic pipettes for dispensing master mixes (OPTIONAL)
Aerosol barrier tips
384 Corning Black Clear Bottom Low Volume Plate
Plate Optical Seal
Biosafety Cabinet Class II, for the extraction
PCR Workstations, for each portion of the assay set up
Heat block with a heated lid capable of maintaining 61°C or PCR instrument with a heated lid
Vortex
Microcentrifuge
Cold blocks or ice
Tube racks
8 strip tube opener**
Dry Bath/Heat Block*
Tabletop Centrifuge*
Serological Pipette*

*required for RNA Extraction only

**recommended to reduce contamination risk

Table 6: Additional Supplies

Dedicated laboratory coat for each area	Powder-free latex, vinyl or nitrile gloves
Disposable booties	20% (v/v) bleach solution (2.0% w/v sodium hypochlorite in water)
Biohazard bag for tip and tube disposal	70% ethanol

Warnings and Precautions

- For *in vitro* diagnostic use (IVD) only.
- For Emergency Use Authorization (EUA) only.
- For prescription use only.
- This product has not been FDA cleared or approved, but has been authorized by FDA under an Emergency Use Authorization (EUA) for use by authorized laboratories; use by laboratories certified under CLIA of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The emergency use of this product is authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or the authorization is revoked sooner.
- Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.
- Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.
- Proper personal protective equipment including lab coats, gowns, gloves, eye protection, and a biological safety cabinet are recommended for manipulation of clinical specimens. Refer to Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition - CDC.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2 https://www.cdc.gov/coronavirus/2019-nCoV/lab- biosafety-guidelines.html.
- Specimen processing should be performed in accordance with national biological safety recommendations.
- If infection with SARS-CoV-2 is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.
- Perform all manipulations of human clinical specimens within a Class II (or higher) biological safety cabinet (BSC). Immediately clean up any spill containing potentially infectious material with 0.5-1% (w/v) sodium hypochlorite (20% v/v bleach). Dispose of cleaning materials in a biohazard waste stockpot.
- Report incident to supervisor and consult a physician immediately in the event that infectious materials are ingested or come into contact with mucus membranes, open lacerations, lesions or other breaks in the skin.
- Use of non-recommended reagent volumes may result in a loss of performance and may also decrease the reliability of the test results.
- Use of non-recommended volumes and concentrations of the RNA/ DNA sample may result in a loss of performance and may also decrease the reliability of the test results.
- Use of non-recommended consumables with instruments may adversely affect test results.
- Do not mix reagents from different lots.
- RNA should be maintained on a cold block or ice during preparation and use to ensure stability.
- Primers, CRISPR guide RNA (crRNA) stocks (including aliquots), enzymes, and RT-LAMP amplification master mix must be thawed and maintained on a cold block at all times during preparation and use.
- Return all components to the appropriate storage condition after preparing the working reagents.

- **Workflow in the laboratory should proceed in a unidirectional manner.**
- Clinical specimens may contain high titers of viral particles. **Care must be taken to minimize the risk of cross contamination of negative clinical specimens from positive clinical specimens during the extraction procedure.**
  - **Do not open more than one clinical specimen at a time.**
  - **Allow adequate spacing between tubes that contain intact or lysed clinical specimens so that there is no contact between the tubes or lids.**
  - **Change gloves frequently.**
  - **Change pipette tips between each specimen when adding lysis or precipitation reagents.**
- Amplification technologies are sensitive to accidental introduction of product from previous amplifications reactions. Incorrect results could occur if either the clinical specimen or reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon).
  - Maintain separate areas for assay setup and handling of nucleic acids.
  - Always check the expiration date prior to use. Do not use expired reagent. Do not substitute or mix reagent from different kit lots or from other manufacturers.
  - Change aerosol barrier pipette tips between all manual liquid transfers.
  - During preparation of samples, compliance with good laboratory techniques is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with nucleic acids.
  - **Maintain separate, dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g., microcentrifuge tubes, pipette tips) for assay setup, handling of extracted nucleic acids, and handling post-amplification products.**
  - **Perform work in a unidirectional workflow in separate locations, from areas without specimen/nucleic acid or amplicon to areas with amplified nucleic acid**
  - Wear a clean lab coat and powder-free disposable gloves (not previously worn) when setting up assays.
  - Change gloves between samples and whenever contamination is suspected.
  - **Change gloves after tubes containing amplified product are handled before touching other tubes, equipment, etc.**
  - Keep reagent and reaction tubes capped or covered as much as possible.
  - Do not interchange vial or bottle caps, as cross-contamination may occur.
  - Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products such as 20% bleach and "RNase AWAY®" to minimize risk of nucleic acid or RNase contamination. Residual cleaning solutions should be removed using 70% ethanol.
- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.

The product contains no substances which at their given concentration, are considered to be hazardous to health or environment.

HMIS

Health	0
Flammability	0
Reactivity	0

Specimen Collection, Handling, and Storage

Adequate, appropriate specimen collection, storage, and transport are important in order to obtain accurate test results. Training in correct specimen collection procedures is highly recommended to assure good quality specimens and results. CLSI MM13-A may be referenced as an appropriate resource.

Collecting the Specimen

- Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (2019-nCoV) https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html
- A sample collection device is not a part of the assay kit. Follow specimen collection device manufacturer instructions for proper collection methods.
- Swab specimens should be collected using only swabs with a synthetic tip, such as nylon or Dacron®, and an aluminum or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing 2-3 ml of viral transport media (i.e. VTM, UTM, M4RT).

Transporting Specimens

- It is the shipper's responsibility to ensure that appropriate shipping materials are used. Please refer to IATA and local regulations.
- Refrigerate specimens at 2-8°C and ship overnight on dry ice.

Storing Specimens

It is recommended that specimens be kept at -20°C for up to 7 days. For storage longer than 7 days, specimens should be frozen at -70°C. Repeated freezing and thawing of a specimen should be avoided. If a specimen is kept for retesting, it should be aliquoted in different tubes to avoid freezing and thawing cycles. The temperature in the storage areas should be monitored and recorded regularly to identify potential fluctuations. Domestic refrigerators/ freezers with wide temperature fluctuations are not suitable for the storage of frozen specimens (CDC, 2020)

Reagent Controls and Preparation

SARS-CoV-2 Positive Control (*spc*) Preparation:

- Precautions: This reagent should be handled with caution to prevent possible contamination. Freeze-thaw cycles should be avoided. Maintain on cold block when thawed.
- Dilute the *spc* with nuclease free water to achieve the working concentration of the *spc* (4,800 gene copies/µL). Make single use aliquots (approximately 20 µL each) and store at ≤ -70^{O}C.
- Thaw a single aliquot of the positive control for each experiment and keep on cold block until adding to the RT-LAMP reaction. Discard any unused portion of the aliquot.

No Template Control (*ntc*)

Nuclease-free water

Quality Control

- Quality control requirements must be performed in conformance with local, state, and federal regulations or accreditation requirements and the user's laboratory's standard quality control procedures. For further guidance on appropriate quality control practices, refer to 42 CFR 493.1256.
- Quality control procedures are intended to monitor reagent and assay performance.
- Always include a negative control (*ntc*) for all three Sherlock^{TM} SARS-CoV-2 kit targets (O, N, and RP), and a positive control (*spc*) for each of the SARS-CoV-2 specific targets (O and N) in each amplification and detection run.
- All clinical samples must be tested for the presence of the human RNase P (RP) target to control for specimen quality and extraction, which acts as the internal control.

Nucleic Acid Extraction and Assay Set up

All procedures should be performed in a BSL2 laboratory, and specimens should be handled within a Biological Safety Cabinet. All necessary safety precautions should be taken according to the Laboratory guidelines. Precautions must also be taken to prevent cross-contamination of samples.

Separate work areas should be used for:

- Nucleic acid extraction
- Reagent preparation (e.g. preparation of master mixes; **NO** amplified reactions, target solutions, or clinical specimens should be brought into this area. After working in this area, laboratory coat and gloves should be changed before moving into the nucleic acid addition

area)
- Nucleic acid addition
- RT-LAMP Amplification (e.g. thermocyclers/heat blocks)
- Post-amplification detection (After working in this area, laboratory coat and gloves should be changed and disposed of)

General Handling

- Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination.
- Always wear powder-free latex, vinyl, or nitrile gloves while handling reagents, tubes and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment.
- Change gloves frequently and keep tubes closed when not in use.
- During the procedure, avoid delays and keep everything on cold blocks or ice when possible to avoid degradation of RNA by endogenous or residual RNases.
- Clean working surfaces, pipettes and equipment with 20% bleach or other solution that can destroy nucleic acids and RNases. To eliminate accelerated deterioration of any plastics and metals, wipe down with 70% ethanol after using 20% bleach.
- Make sure all bleach is removed to eliminate possible chemical reactions between bleach and guanidine thiocyanate which is present in the extraction reagents.

ASSAY PROCEDURE

1. Nucleic Acid Extraction

NOTE: Care must be taken to track patient sample ID#s throughout the extraction process. When processing multiple clinical specimens, ensure that intact and/or lysed samples are adequately spaced in the racks so that there is no contact between tubes containing distinct patient samples.

a. Sample extraction for the **Sherlock™ SARS-CoV-2 kit** is performed using the PureLink™ Viral RNA/DNA Mini Kit (Cat. 12280050).
b. Prepare wash buffer per the instructions on page 10 of the Purelink™ Viral RNA/DNA Mini Kit User Guide.
c. Prepare carrier RNA per the instructions on page 11 of the Purelink™ Viral RNA/DNA Mini Kit User Guide.
d. For a 200 µL sample, prepare a 225 µL aliquot of Lysis Buffer/proteinase K mixture in a sterile microcentrifuge tube per the instructions on page 14 of the Purelink™ Viral RNA/DNA Mini Kit User Guide.

NOTE: For steps 1e and 1f below, add the indicated reagent to each clinical sample consecutively. Never allow more than one clinical sample to be open at a given time in the lysis procedure.

e. Prepare lysate by adding 200 µL of sample that has been equilibrated to room temperature to the aliquot of Lysis Buffer/proteinase K mixture (total input sample lysate volume is 425 µL). Close the tube lid and mix by vortexing for 15 seconds. Incubate at 56°C for 15 minutes.

f. Add 250 µL of 96–100% ethanol to the tube, close the lid, and mix by vortexing for 15 seconds. Incubate the lysate for 5 minutes at room temperature.

NOTE: Change pipette tips between each addition of ethanol

g. Purify the RNA using the following steps:

1. Add the lysate to the Viral Spin Column in a collection tube.
2. Centrifuge the column at 6800 x g for 1 minute. Discard the collection tube. Place the spin column in a new Wash Tube.
3. Wash the column with 500 µL Wash Buffer (WII) with ethanol.
4. Centrifuge at 6800 x g for 1 minute. Discard the flow through.
5. Transfer the column to a **fresh 2 mL waste container** prior to the second wash step.
6. Repeat wash Step 3 with 500 µL Wash Buffer (WII) once.
7. Discard the collection tube and place the spin column in another, clean Wash Tube.
8. Centrifuge the spin column at maximum speed for **3 minutes** to remove any residual Wash Buffer (WII).
9. Place the spin column in a clean 1.7-mL Recovery Tube.
10. **Additional Required Nucleic Acid Extraction Step:** Immediately prior to RNA elution, open the assembly lid and air-dry the column membrane for 15 minutes at room temperature before eluting with 30 µL of nuclease free water.
11. Elute with 30 µL sterile RNase-free water (E3) supplied with the kit (add water to the center of the cartridge).
12. Incubate at room temperature for 1 minute. Centrifuge the spin column at maximum speed for 1 minute to elute nucleic acids. The Recovery Tube contains purified viral nucleic acids. Discard the spin column.
13. Store purified viral RNA/DNA at –80°C or use RNA/DNA for the desired downstream application.

Up to 8 µL of the extracted RNA can be used per reaction. As extracted RNA is extremely sensitive to degradation caused by RNases, follow general laboratory precautions for handling RNA. Store extracted RNA <-70°C if not using immediately.

2. RT-LAMP Master Mix Preparation

a. Label a new 1.5 mL microcentrifuge tube for each target (O, N, and RP) and prepare a RT-LAMP Master Mix consisting of the 2x RT-LAMP Mix and the appropriate 10x Primer Mix using the recipe in Table 7 below. Make enough of each master mix for all samples to be tested and the necessary controls for each run.

b. Pulse vortex RT-LAMP Master Mix for 3 seconds and spin down for 3 seconds in a microcentrifuge after all components are added.

Table 7: Target Specific RT-LAMP Master Mix Recipe

Reagent	Cap Color	Volume per reaction	Volume for N Reactions	Volume for N=10 Reactions	Volume for N=30 Reactions
2x RT-LAMP Mix	Blue	10 µL	10 µL x (*N* + 1)	110 µL	310 µL
10x Primer (N, O, or RP)	Blue	2 µL	2 µL x (*N* + 1)	22 µL	62 µL
Total Volume	NA	12 µL	12 µL x (*N* + 1)	132 µL	372 µL

N = number of extracted samples plus number of controls. Prepare enough for 1 extra (*N* + 1) sample to allow for overage during reaction set-up.

3. **RT-LAMP Amplification**
   a. Label a strip tube (0.2 mL) with the target name (i.e. O) and strip number according to recommended layouts in Figure 1. Add 12 µL of the RT-LAMP Master Mix into one well for each sample and control to be amplified.
   b. Repeat step 3.a. for the remaining 2 targets using a new strip tube for each target (i.e. N, or RP).
   c. Add 8 µL of extracted RNA in each respective strip tube containing the RT-LAMP Master mix. Vortex the strip tube for 3 seconds and spin down for 3 seconds in microcentrifuge with a 0.2 mL tube adaptor.

Table 8: RT-LAMP Assay Components and reaction volume

Reagent	Volume per reaction
RT-LAMP Master Mix	12 µL
RNA Sample or Controls	8 µL
Total Volume	20 µL

   d. Incubate the strip tubes on the thermocycler/heating block set to 61°C for 40 minutes.

Note: Heat the lid to 99°C for the incubation.

   e. Pulse spin the tubes after the 40 minutes incubation to remove any condensation from the lids of the tubes.

4. **CRISPR Cas Reaction Preparation**
   a. Preheat a fluorescence microplate reader to 37°C.
   b. For each target tested label a 1.5 mL tube with the target name (i.e. O) and "Cas Mix". Prepare a CRISPR Cas Master Mix using the following recipe in Table 8 below, scaling as required for the number of assays to be run (one Cas assay for every RT-LAMP reaction). A minimum total volume to complete 10 reactions is recommended (*N* ≥ 10 in Table 9).

Note: The mix, without $MgCl_2$, can be prepared while the RT-LAMP amplification is running and stored in a cool block. Add the $MgCl_2$ to the mix last, immediately before moving on to the next step.

   c. Repeat step 4.b. for the remaining 2 targets using a new 1.5 mL tube for each target (i.e. N, or RP).
   d. Pulse vortex for 3 seconds and spin down for 3 seconds in a microcentrifuge after all components are added.

CAUTION: Do not allow the completed mix to sit for longer than 10 minutes prior to moving on to the next step.

Table 9: Target CRISPR Cas Master Mix Recipe

Reagent	Cap Color	Volume per Reaction	Volume for N Reactions	Volume for N=10 Reactions	Volume for N=30 Reactions
Reporter	Yellow	1.56 µL	1.56 µL x (*N + 1)*	17.16 µL	48.36 µL
crRNA (N or O or RP)	Yellow	0.56 µL	0.56 µL x (*N + 1)*	6.16 µL	17.36 µL
rNTP Mix	Red	1 µL	1 µL x (*N + 1)*	11 µL	31 µL
T7 RNA Polymerase	Red	0.5 µL	0.5 µL x (*N + 1)*	5.5 µL	15.5 µL
RNase Inhibitor	Red	0.63 µL	0.63 µL x (*N + 1)*	6.93 µL	19.53 µL
CRISPR-Cas enzyme	Red	0.32 µL	0.32 µL x (*N + 1)*	3.52 µL	9.92 µL
Water	n/a	15.2 µL	15.2 µL x (*N + 1)*	167.2 µL	471.2 µL
$MgCl_2$	Red	0.23 µL	0.23 µL x (*N + 1)*	2.53 µL	7.13 µL
Total Volume	NA	20 µL	20 µL x (*N + 1)*	220 µL	620 µL

N = number of extracted samples plus number of controls. Prepare enough for 1 extra (*N* + 1) sample to allow for overage during reaction set-up.

5. CRISPR Cas Detection

Caution: Perform work in a unidirectional workflow in separate locations, from areas without specimen/nucleic acid or amplicon to areas with amplified nucleic acid. Gloves should be changed after every manipulation of amplicon-containing tubes prior to touching additional reactions. Ensure that a clean strip tube opener is used for every experiment, and that the strip tube opener is cleaned after use.

a. Label a strip tube (0.2 mL) with the target name (i.e. O) and strip number according to recommend plate layout in Figure 2. Add 20 µL of the CRISPR Cas Master Mix made in step 4 into one well for each sample and control to be amplified. Close strip tube.
b. **Repeat** step 5.a. for the remaining 2 targets using a new strip tube for each target (i.e. N, or RP).
c. In an isolated, clean dead box, carefully open one of the aliquoted CRISPR Cas Master Mix strip tubes (i.e. O) using a strip tube opener.
d. Open the corresponding target (i.e. O) RT-LAMP amplicon strip tube, placed in a different rack after amplification in step 3.c. is complete. **Change gloves.**
e. Using multi-channel pipette, carefully add 5 µL of the RT-LAMP amplicon to the corresponding CRISPR Cas Master Mix strip tube. **Eject tips.**

Table 10: CRISPR Cas Detection Components and reaction volume

Reagent	Volume per reaction
RT-LAMP Amplicon (N, O, RP)	5 µL
CRISPR Cas Master Mix	20 µL
Total Volume	25 µL

f. Carefully close the caps of the CRISPR Cas Detection strip tube (RT-LAMP amplicon + CRISPR Cas Master Mix). Carefully close the amplified RT-LAMP amplicon strip tubes and dispose after use. **Change gloves.**

g. Flick the CRISPR Cas Detection strip tube to mix and spin down for 3 seconds in a mini centrifuge with a 0.2 mL tube adaptor. **Change gloves.**

h. **Repeat** step 5.b. to 5.f. for the remaining CRISPR Cas Master Mix strip tubes.

i. Carefully open each CRISPR Cas Detection strip tube using a strip tube opener. **Change gloves.**

j. Using a multichannel pipette, carefully add 20 µL from the CRISPR Cas Detection strip tube to a Corning® 384 Well Plate (Black/Clear Bottom) according to recommended plate layout, Figure 2.

CAUTION: Do not go to the second stop of the pipette to avoid the introduction of bubbles to the reaction wells.

k. **Repeat** step 5.g. to 5.h. for the remaining CRISPR Cas Detection strip tubes.

l. Seal the plate with an Optical Seal.

m. Open the plate reader software to create a read procedure.

1. Set setpoint temperature to 37°C.
2. Select "Kinetic" run reading with a total read time of 10 minutes, and data collection intervals at 2.5 minutes.

Note: if an error is produced about kinetic read interval, increase the data collection intervals to 5 minutes.

3. Select filter settings in read details to 485nm/528nm filter set with the gains setting set to "extended".
4. Highlight the rows and columns of the plate to be read in the plate settings.
5. If a warning about "Max V" calculations appears, press "OK" and continue.
6. Press green arrow to start, (i.e. "Create experiment and read now").
7. Save experiment in a designated place with an appropriate unique name
8. When plate loader, extends, load plate

CAUTION: Ensure plate is loaded in correct orientation

9. Press "OK" to load plate.

6. Recommended Layouts

Figure 1. RT-LAMP Amplification Layout

	RT-LAMP Mix	ORF1ab	ORF1ab	ORF1ab	ORF1ab	N	N	N	N	RNaseP	RNaseP	RNaseP	RNaseP
	LAMP Strip ID	Strip-1	Strip-2	Strip-3	Strip-4	Strip-5	Strip-6	Strip-7	Strip-8	Strip-9	Strip-10	Strip-11	Strip-12
Well position in Strip	1	*ntc*	Sample 8	Sample 16	Sample 24	*ntc*	Sample 8	Sample 16	Sample 24	*ntc*	Sample 8	Sample 16	Sample 24
	2	Sample 1	Sample 9	Sample 17	Sample 25	Sample 1	Sample 9	Sample 17	Sample 25	Sample 1	Sample 9	Sample 17	Sample 25
	3	Sample 2	Sample 10	Sample 18	Sample 26	Sample 2	Sample 10	Sample 18	Sample 26	Sample 2	Sample 10	Sample 18	Sample 26
	4	Sample 3	Sample 11	Sample 19	Sample 27	Sample 3	Sample 11	Sample 19	Sample 27	Sample 3	Sample 11	Sample 19	Sample 27
	5	Sample 4	Sample 12	Sample 20	Sample 28	Sample 4	Sample 12	Sample 20	Sample 28	Sample 4	Sample 12	Sample 20	Sample 28
	6	Sample 5	Sample 13	Sample 21	Sample 29	Sample 5	Sample 13	Sample 21	Sample 29	Sample 5	Sample 13	Sample 21	Sample 29
	7	Sample 6	Sample 14	Sample 22	Sample 30	Sample 6	Sample 14	Sample 22	Sample 30	Sample 6	Sample 14	Sample 22	Sample 30
	8	Sample 7	Sample 15	Sample 23	*spc*	Sample 7	Sample 15	Sample 23	*spc*	Sample 7	Sample 15	Sample 23	

Figure 2: CRISPR Cas Detection Plate Layout (384 Well plate)

CRISPR Cas Mix:	ORF1ab	ORF1ab	ORF1ab	ORF1ab	N	N	N	N	RNaseP	RNaseP	RNaseP	RNaseP
Cas detection Strip	Strip-1	Strip-2	Strip-3	Strip-4	Strip-5	Strip-6	Strip-7	Strip-8	Strip-9	Strip-10	Strip-11	Strip-12
ROW / Column	1	3	5	7	9	11	13	15	17	19	21	23
A	*ntc*	Sample 8	Sample 16	Sample 24	*ntc*	Sample 8	Sample 16	Sample 24	*ntc*	Sample 8	Sample 16	Sample 24
C	Sample 1	Sample 9	Sample 17	Sample 25	Sample 1	Sample 9	Sample 17	Sample 25	Sample 1	Sample 9	Sample 17	Sample 25
E	Sample 2	Sample 10	Sample 18	Sample 26	Sample 2	Sample 10	Sample 18	Sample 26	Sample 2	Sample 10	Sample 18	Sample 26
G	Sample 3	Sample 11	Sample 19	Sample 27	Sample 3	Sample 11	Sample 19	Sample 27	Sample 3	Sample 11	Sample 19	Sample 27
I	Sample 4	Sample 12	Sample 20	Sample 28	Sample 4	Sample 12	Sample 20	Sample 28	Sample 4	Sample 12	Sample 20	Sample 28
K	Sample 5	Sample 13	Sample 21	Sample 29	Sample 5	Sample 13	Sample 21	Sample 29	Sample 5	Sample 13	Sample 21	Sample 29
M	Sample 6	Sample 14	Sample 22	Sample 30	Sample 6	Sample 14	Sample 22	Sample 30	Sample 6	Sample 14	Sample 22	Sample 30
O	Sample 7	Sample 15	Sample 23	*spc*	Sample 7	Sample 15	Sample 23	*spc*	Sample 7	Sample 15	Sample 23	

7. Data Extraction and Analysis

a. After the completion of the plate reader run, open plate reader software and open experiment file.

b. Select and export data for all samples and controls according to manufacturer's instructions. Export the data from these wells to an excel spreadsheet.

c. For the negative template control ("*ntc*"), SARS-CoV-2 Positive Controls ("*spc*") and patient specimens calculate the ratios in Table 10 below:

Table 11: Ratio calculations for data analysis

Sample Type	Reaction Type	Reaction Name	Ratio Calculation
Negative Template Control (*ntc*)	SARS-CoV-2 N gene target	N^{ntc}	$\frac{N_{t=10}^{ntc}}{N_{t=0}^{ntc}}$
	SARS-CoV-2 ORF1ab gene target	O^{ntc}	$\frac{O_{t=10}^{ntc}}{O_{t=0}^{ntc}}$
	Human RNaseP gene target	RP^{ntc}	$\frac{RP_{t=10}^{ntc}}{RP_{t=0}^{ntc}}$
SARS-CoV-2 Positive Control (*spc*)	SARS-CoV-2 N gene target	N^{spc}	$\frac{N_{t=10}^{spc}}{N_{t=10}^{ntc}}$
	SARS-CoV-2 ORF1ab gene target	O^{spc}	$\frac{O_{t=10}^{spc}}{O_{t=10}^{ntc}}$
Patient Specimen	SARS-CoV-2 N gene target	N	$\frac{N_{t=10}}{N_{t=10}^{ntc}}$
	SARS-CoV-2 ORF1ab gene target	O	$\frac{O_{t=10}}{O_{t=10}^{ntc}}$
	Human RNaseP gene target	RP	$\frac{RP_{t=10}}{RP_{t=10}^{ntc}}$

where,
- N = N target reaction fluorescence
- O = ORF1ab target reaction fluorescence
- RP = RNaseP target reaction fluorescence
- N^{ntc} = N target negative template control reaction fluorescence
- O^{ntc} = ORF1ab target negative template control reaction fluorescence
- RP^{ntc} = RNaseP target negative template control reaction fluorescence
- t = reaction time on fluorescence plate reader (minutes)
- N^{spc} = N target positive template control reaction fluorescence
- O^{spc} = ORF1ab target positive template control reaction fluorescence

Assay Controls and Interpretation of Results

Assay Controls

Negative Template Control Reactions (*ntc*): Negative template control (*ntc*) reactions are used to monitor reagent and/or environmental contamination. There are three negative control reactions, one for each primer/crRNA set – (i) SARS-CoV-2 N gene, (ii) SARS-CoV-2 ORF1ab gene, and (iii) human RNase P gene. Negative template control reactions are created by substituting the volume of sample material in the RT-LAMP reaction with an equal volume of nuclease-free water.

Positive Control Reactions (*spc*): Positive control (*spc*) reactions are used to monitor gross reagent failure, such as reagent degradation, or incorrect assay set-up. There are two positive control reactions, one for each SARS-CoV-2 target – (i) N gene, and (ii) ORF1ab gene. Positive control reactions are created by substituting the volume of sample material in the RT-LAMP reaction with an equal volume of extracted SARS-CoV-2 viral RNA at a stock concentration of 4800 copies/µl.

CAUTION: If any control reaction is **INVALID** as defined in the table below, see troubleshooting.

Table 12: Control Reaction Results Interpretation

Control Type	Reaction Type	Reaction Name	Ratio Calculation	Ratio Calculation Result Interpretation	
				VALID	INVALID
Negative	SARS-CoV-2 N gene target negative control	N^{ntc}	$\frac{N^{ntc}_{t=10}}{N^{ntc}_{t=0}}$	< 3	≥ 3
	SARS-CoV-2 ORF1ab gene target negative control	O^{ntc}	$\frac{O^{ntc}_{t=10}}{O^{ntc}_{t=0}}$	< 3	≥ 3
	Human RNase P gene target negative control	RP^{ntc}	$\frac{RP^{ntc}_{t=10}}{RP^{ntc}_{t=0}}$	< 3	≥ 3
Positive	SARS-CoV-2 N gene target positive control	N^{spc}	$\frac{N^{spc}_{t=10}}{N^{ntc}_{t=10}}$	≥ 5	< 5
	SARS-CoV-2 ORF1ab gene target positive control	O^{spc}	$\frac{O^{spc}_{t=10}}{O^{ntc}_{t=10}}$	≥ 5	< 5

where, N^{ntc} = N target negative template control reaction fluorescence
O^{ntc} = ORF1ab target negative template control reaction fluorescence
RP^{ntc} = RNase P target negative template control reaction fluorescence
N^{spc} = N target positive template control reaction fluorescence
O^{spc} = ORF1ab target positive template control reaction fluorescence
t = reaction time on fluorescence plate reader (minutes)

Interpretation of Sample Results

- Unknown clinical sample results should only be assessed once all control reactions have been determined to be valid and acceptable.
- A result is **Positive** if **either** SARS-CoV-2 target (N or ORF1ab) reaction has a greater than or equal to 5-fold increase in fluorescence measurement at 10 minutes over the corresponding valid negative template control reaction fluorescence measurement at 10 minutes.
- A result is **Negative** if **both** SARS-CoV-2 target (N and ORF1ab) reactions have a less than 5-fold increase in reaction fluorescence measurement at 10 minutes over the corresponding valid negative template control reaction fluorescence measurement at 10 minutes **AND** the RNase P target reaction has a greater than or equal to 5-fold increase in fluorescence measurement at 10 minutes over the corresponding valid negative template control reaction fluorescence measurement at 10 minutes.
- A result is **Invalid** if **all** target (N, ORF1ab, and RNase P) reactions have a less than 5-fold increase in reaction fluorescence measurement at 10 minutes over the corresponding valid negative template control reaction fluorescence measurement at 10 minutes.

CAUTION: If sample reaction is INVALID as defined in the table below, see troubleshooting.

Table 13: Sample Reaction Results Interpretation

Reaction target gene and ratio calculation result			Result Interpretation and Action
SARS-CoV-2 N	SARS-CoV-2 ORF1ab	Human RNaseP	
$\frac{N_{t=10}}{N^{ntc}_{t=10}}$	$\frac{O_{t=10}}{O^{ntc}_{t=10}}$	$\frac{RP_{t=10}}{RP^{ntc}_{t=10}}$	
≥ 5 (DETECTED)	≥ 5 (DETECTED)	N/A	POSITIVE for SARS-CoV-2 ACTION: Report Result
< 5 (not detected)	≥ 5 (DETECTED)	N/A	
≥ 5 (DETECTED)	< 5 (not detected)	N/A	

< 5 (not detected)	< 5 (not detected)	≥ 5 (DETECTED)	NEGATIVE for SARS-CoV-2 ACTION: Report Result
< 5 (not detected)	< 5 (not detected)	< 5 (not detected)	INVALID ACTION: See Troubleshooting

where,
N = N target reaction fluorescence
O = ORF1ab target reaction fluorescence
RP = RNaseP target reaction fluorescence
N^{ntc} = N target negative template control reaction fluorescence
O^{ntc} = ORF1ab target negative template control reaction fluorescence
RP^{ntc} = RNaseP target negative template control reaction fluorescence
t = reaction time on fluorescence plate reader (minutes)
N/A = not applicable

Troubleshooting

User Errors

- Good Clinical Laboratory Practices (GCLP) for Molecular Biology Based Tests Used In Diagnostic Laboratories (Viana & Wallis, 2011) are necessary for the use of this product. This product is not intended to be used by untrained personnel. The user needs to have molecular biology experience and be familiar with the proper pipetting technique to prevent errors, such as splashes, crossover contamination, and errors on volume selection.
- Pipette tips must be replaced after every pipetting. Gloves must be replaced often. Equipment must have calibration up to date for the pipettes and thermocyclers, when applicable.
- A 90 minutes online training for Good Laboratory Practices for Molecular Genetics Testing (Centers for Disease Control and Prevention, 2017) is available at the CDC website at the following link:

https://www.cdc.gov/labtraining/training-courses/good-lab-practices-molecular-genetics-testing.html

Invalid Results

SARS-CoV-2 Positive Control (*spc*) not detected.

Possible causes:

- ☐ Pipetting errors (control in wrong well, missing a well, inadequate amount of a reagent)
- ☐ Incorrect dilution of positive control nucleic acid
- ☐ Incorrect placement of tubes into heat block/PCR machine
- ☐ Incorrect placement of plate in plate reader
- ☐ Degraded reagents due to incorrect storage temperature
- ☐ Use of expired reagents

☐ Use of incorrect reagent

- If the SARS-CoV-2 Positive Control is invalid, the run should be considered invalid and the user should re-test the samples by re-extraction and use a fresh aliquot of the diluted SARS-CoV-2 Positive Control.
- If the positive control fails again, then an investigation should be conducted to identify possible causes for error and depending on the investigation results and risks identified in the process, the samples may need to be re-run.
- If failure of the positive control happens a third time after re-extraction and re-amplification, test with a new lot of **Sherlock™ CRISPR SARS-CoV-2 kit reagents**.
- If still failing, please contact Sherlock Biosciences.

No Template Control (*ntc*) detection of a SARS-CoV-2 target.

Possible causes:

☐ Contamination of one or more reagents during set-up
☐ Pipetting errors (control in wrong well, missing a well, inadequate amount of a reagent)
☐ Cross contamination of sample wells during RT-LAMP
☐ Cross contamination of sample wells during Cas Detection
☐ Incorrect placement of tubes into heat block/PCR machine
☐ Incorrect placement of plate in plate reader
☐ Use of incorrect reagent

- If the *ntc* is invalid, the run should be considered invalid and the user should re-test the samples by re-extraction.
- If the *ntc* fails again, then an investigation should be conducted to identify possible causes for error and depending on the investigation results and risks identified in the process, the samples may need to be re-run.
- If failure of the *ntc* happens a third time after re-extraction and re-amplification, test with a new lot of **Sherlock™ CRISPR SARS-CoV-2 kit reagents** and a new lot of nuclease free water.
- If still failing, please contact Sherlock Biosciences.

RNaseP (PR) not being detected in patient samples.

Possible causes:

☐ Not enough nuclear material in the patient sample
☐ The extraction was performed incorrectly
☐ Inhibitors present in patient sample
☐ Pipetting errors (control in wrong well, missing a well, inadequate amount of a reagent)
☐ Incorrect placement of tubes into heat block/PCR machine
☐ Incorrect placement of plate in plate reader
☐ Use of incorrect reagent

- If the *rp* is invalid, the run should be considered invalid and the user should re-test the samples by re-extraction.

- If the *rp* fails again, then an investigation should be conducted to identify possible causes for error and depending on the investigation results and risks identified in the process, the samples may need to be re-run.
- If failure of the *rp* happens a third time after re-extraction and re-amplification, test with a new lot of **Sherlock™ CRISPR SARS-CoV-2 kit reagents**.
- If still failing, please contact Sherlock Biosciences.

Limitations

- All users should be qualified by training or experience to perform molecular diagnostic test procedures.
- The use of this assay as an in vitro diagnostic under the FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.
- The test was validated for use only with upper respiratory specimens.
- 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. Optimum specimen types and timing for peak viral levels during infections caused by SARS-CoV-2 have not been determined. Collection of multiple specimens (types and time points) from the same patient may be necessary to detect the virus.
- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely when prevalence of disease is high. False positive test results are more likely when prevalence is moderate to low.
- If the virus mutates in the RT-PCR target region, SARS-CoV-2 may not be detected or may be detected less predictably. Inhibitors or other types of interference may produce a false negative result. An interference study evaluating the effect of common cold medications was not performed.
- The performance of this test was established based on the evaluation of a limited number
- of clinical specimens. Clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- Test performance can be affected because the epidemiology and pathology of disease caused by SARS-CoV-2 is not fully known. For example, clinicians and laboratories may not know the optimum types of specimens to collect, and when during the course of infection these specimens are most likely to contain levels of virus that can be readily detected.
- Detection of viral RNA may not indicate the presence of infectious virus or that SARS-CoV- 2 is the causative agent for clinical symptoms.
- The performance of this test has not been established for monitoring treatment of SARS- CoV-2 infection.
- The performance of this test has not been established for screening of blood or blood product for the presence of SARS-CoV-2.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

Conditions of Authorization for the Laboratory

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

However, to assist clinical laboratories using the Sherlock™ CRISPR SARS-CoV-2 kit the relevant Conditions of Authorization are listed below.

- Authorized laboratories using this product[1] must include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using this product must use this product as outlined in the authorized labeling. Deviations from the authorized procedures, including authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use this product are not permitted.
- Authorized laboratories that receive this product must notify the relevant public health authorities of their intent to run this product prior to initiating testing.
- Authorized laboratories using this product must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories must collect information on the performance of this product and report to: DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA- Reporting@fda.hhs.gov) and to Sherlock Biosciences (via phone: 617-702-6263 or via email: support@sherlock.bio) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of this product of which they become aware.
- All laboratory personnel using this product must be appropriately trained in molecular in vitro diagnostic test techniques, use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.
- Sherlock™ Biosciences authorized distributors, and authorized laboratories using this product must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1] This product refers to the Sherlock™ CRISPR SARS-CoV-2 kit. The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests" as "authorized laboratories."

Performance Characteristics

Analytical Sensitivity (Limit of Detection):

The Limit of Detection (LoD) for the Sherlock™ CRISPR SARS-CoV-2 kit was determined to be the lowest concentration of genomic viral RNA (copies/µL of VTM) at which ≥ 95% of all replicates test positive. As the assay contains two SARS-CoV-2 targets (N and O), the LoD for each target was independently determined and confirmed. The LoD claimed for the kit is the higher of the two values.

To determine the LoD, limiting dilutions of quantified extracted genomic RNA were spiked into a clinical matrix composed of pooled nasophrangeal swabs after the initial lysis step of the PureLink™ Viral RNA/DNA Mini Kit (Thermo Fisher) to prevent the lysis of RNA prior to extraction. After extraction, the samples were processed according to the Sherlock™ SARS-CoV-2 kit workflow. Initially three (3) replicates of ten (10) dilutions were tested. The tentative LoD was determined to be the estimated lowest concentration where 3/3 replicates were positive for a given target. This LoD was confirmed by testing at 1x and 1.5x this concentration in the same sample matrix as above. Results are shown in Table 14 below.

Table 14: LoD determination

Viral Copies in Sample (cp/µL VTM)	Total Positive (ORF1ab)	Total Positive (N)	Total Positive (RNaseP)
18.0	3/3	3/3	3/3
10.8	3/3	3/3	3/3
7.2	3/3	3/3	3/3
6.3	3/3	3/3	3/3
5.4	2/3	3/3	3/3
*4.5	3/3	3/3	3/3
3.6	1/3	3/3	3/3
2.7	3/3	3/3	3/3
1.8	1/3	3/3	3/3
**0.9	1/3	3/3	3/3
0.0	0/3	0/3	3/3
Positive Control	3/3	3/3	N/A
Negative Control	0/3	0/3	0/3

*Estimated LoD of ORF1ab target (4.5 cp/uL VTM)

**Estimated LoD of N target (0.9 cp/uL VTM)

The LoD was confirmed by testing at 1x (4.5 cp/µL and 0.9 cp/ µL for ORF1ab and N, respectively) and 1.5x this estimated concentration for each target (6.75 cp/ µL and 1.35 cp/ µL for ORF1ab and N, respectively). The LoD of each target was determined to be the concentration at which ≥19/20 replicates for each assay target was positive. The LoD for the ORF1ab target was determined to be 6.75 cp/µL VTM. The LoD for the N target was determined to be 1.35 cp/µL VTM.

Table 15A: LoD confirmation

Target	Viral Copies in Sample (cp/uL VTM)	# of Samples	# of Detected	Detection Rate (%)
ORF1ab	4.5	20	17	85
ORF1ab	6.75	20	19	95
N	0.9	20	17	85
N	1.35	20	20	100

The confirmed LoD for the **Sherlock™ CRISPR SARS-CoV-2 kit** is 6.75 cp/uL VTM

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. Testing was performed using the PureLink™ Viral RNA/DNA Mini Kit (Cat. 12280050) extraction method and BioTek NEO2 Plate Reader instrument running Gen5 3.08 software. The results are summarized in **Table 15B**.

Table 15B: Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Pooled COVID-19 negative nasopharyngeal swab matrix spiked with a CBER heat-inactivated COVID-19 strain or MERS-CoV in cell culture media	$0.6x10^4$ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not detected

Analytical Inclusivity:

In silico analysis was performed to determine reactivity of the SHERLOCK™ CRISPR SARS-CoV-2 kit. All (9166 individual strains) SARS-CoV-2 genomes available from GISAID on 04/15/2020 and defined as complete by GISAID (>29,000 bp) were downloaded. The 9166 genomes were aligned with all primer and crRNA binding regions for both ORF1ab and N targets, strains with mismatches were identified, and the dataset was refined by removing samples from animals (e.g. pangolin, bat) and ambiguous sequencing data (e.g. N's). N=109 (1.19%) genomes were identified with a single mismatch to the ORF1ab target sequences. N=628 (6.85%) genomes were identified with a single mismatch (627 genomes) or double mismatch (1 genome) to the N target sequences. No genome was identified with a mismatch in both the ORF1ab and N targets.

On December 6, 2021 a bioinformatic analysis was carried out to determine the *in silico* inclusivity of all Sherlock CRISPR SARS-CoV-2-kit molecular components against the Alpha, Beta, Gamma, Delta, lambda, Mu and Omicron SARS-CoV-2 genomic sequences available in the GISAID database from June 2021 to December 2021. All SARS-CoV-2 variants exhibited 100% homology to at least one of the SARS-CoV-2 targets (N or ORF1ab) of the Sherlock CRISPR SARS-CoV-2 kit. The *in silico* analysis predicts that assay performance is unlikely to be impacted by the currently circulating escape variants.

Analytical Specificity:

All primers and crRNAs were analyzed with BLASTn using public domain nucleotide databases and excluding results with the taxonomy ID for SARS-CoV-2. The nt database was used as described above for general exclusivity testing except this database was also filtered to only include sequences for taxonomy identifiers (taxid) of the high priority organisms (see Table 15 below). Search parameters were automatically adjusted for short input sequences. Additional settings included: expect threshold 1000, match score 1 and mismatch scores -3, and penalty to create gap in an alignment 5 and extend a gap in an alignment 2.

Significant homology (>80%) was identified between Bat SARS and a single primer in the ORF1ab target. Significant homology (>80%) was identified between Bat SARS, SARS and Influenza B and a single primer or guide in the N target. Since LAMP amplicon generation is not possible with a single primer there is little risk of cross reactivity with the organisms.

Of the organisms identified with high homology, microbial interference is unlikely from Bat SARS or SARS because they are not common human pathogens. Microbial interference from Influenza B is unlikely because there are 2 mismatches at the terminal 3' end of the primer.

In summary, the ORF1ab and N primers and crRNAs designed for the specific detection of SARS-CoV-2 showed no significant homologies that are likely to cause cross-reactivity or microbial interference with the human genome and transcriptome or with other organisms including bacteria and viruses.

Table 16 – List of High Priority Organisms for Specificity / Exclusivity Assessment

Other high priority pathogens from the same genetic family	High priority organisms likely in the circulating area
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 & B
SARS-coronavirus	Enterovirus (e.g. EV68)
MERS-coronavirus	Respiratory syncytial virus
	Rhinovirus
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococcus pneumoniae
	Streptococcus pyogenes

	Bordetella pertussis
	Mycoplasma pneumoniae
	Pneumocystis jirovecii (PJP)
	Human genome
	Candida albicans
	Pseudomonas aeruginosa
	Staphylococcus epidermis
	Staphylococcus salivarius

Wet testing against high risk pathogenic organisms of the respiratory tract, selected based on disease prevalence, disease risk, homology to assay specific targets and homology to the SARS-CoV-2 genome, was performed to confirm the results of the *in silico* analysis. Each organism identified in **Table 17** below was tested in triplicate with the Sherlock™ CRISPR SARS-CoV-2 kit by spiking diluted organism stock into lysis-treated pooled nasopharyngeal swab matrix. All replicates were negative for SARS-CoV-2 detection.

Table 17: Wet testing of potential cross reactive organisms

Organism	ATCC Cat. Number	Concentration	ORF1ab	N	RNaseP
Human coronavirus 229E	ATCC® VR-740D	1 x 10^5 copies/mL	0/3	0/3	3/3
Human coronavirus OC43	ATCC® VR-1558D	1 x 10^5 copies/mL	0/3	0/3	3/3
Human coronavirus HKU1	ATCC® VR-3262SD	1 x 10^5 copies/mL	0/3	0/3	3/3
Human coronavirus NL63	ATCC® 3263SD	1 x 10^5 copies/mL	0/3	0/3	3/3
Influenza A	VR-95DQ	1 x 10^5 copies/mL	0/3	0/3	3/3
Influenza B	VR-1885DQ	1 x 10^5 copies/mL	0/3	0/3	3/3
Respiratory syncytial virus	ATCC® VR-1580DQ	1 x 10^5 copies/mL	0/3	0/3	3/3
Pseudomonas aeruginosa	ATCC® 27853D-5	1 x 10^6 copies/mL	0/3	0/3	3/3
Staphylococcus epidermis	ATCC® 12228D-5	1 x 10^6 copies/mL	0/3	0/3	3/3
Candida albicans	ATCC® 10231D-5	1 x 10^6 copies/mL	0/3	0/3	3/3

Endogenous Interference Substances Studies:

The **Sherlock™ CRISPR SARS-CoV-2 kit** uses a conventional nucleic acid extraction method; we do not anticipate interference from common endogenous substances using this method.

Clinical Evaluation (Contrived Specimens):

The clinical evaluation was performed on 30 contrived positive and 30 contrived negative nasopharyngeal specimens. Individual nasopharyngeal swab samples collected in the 2018 -2019 Flu season and confirmed to be negative for SARS-CoV-2 were used either unaltered (30 specimens), or spiked with extracted, quantitated SARS-CoV-2 viral genomic RNA to a concentration of 2x LoD (20 specimens), 3x LoD (5 specimens), or 5x LoD (5 specimens). For positive specimens, viral RNA was added to the nasopharyngeal matrix after the initial lysis step for the PureLink™ Viral RNA/DNA Mini Kit (Thermo Fisher) to prevent the degradation of unencapsulated RNA. The samples were randomized, then processed using the **Sherlock™ CRISPR SARS-CoV-2 kit** workflow. The results, as

presented in Table 18 below, showed 100% agreement with the expected results for both the positive and negative specimens.

Table 18: Contrived clinical sample evaluation

Sample Concentration	Number of samples	Number Detected	% Agreement (95% confidence interval)
5x LoD	5	5	100% (NA*)
3x LoD	5	5	100% (NA*)
2x LoD	20	20	100% (83.9% - 100%)
Negative specimens (NS)	30	0	100% (88.6% - 100%)

NA*, confidence intervals not calculated for sample sizes of 5 or less

Clinical Evaluation (Clinical Specimens):

Performance of the Sherlock™ CRISPR SARS-CoV-2 kit was further evaluated using 96 distinct remnant clinical nasopharyngeal swab or nasopharyngeal aspirate specimens, N=30 of which were SARS CoV-2 positive and N=66 of which were SARS CoV-2 negative as determined by a highly sensitive RT-PCR FDA authorized EUA comparator assay. RNA was extracted from the clinical specimens using the PureLink Viral RNA/DNA Mini Kit and tested with the Sherlock™ CRISPR SARS-CoV-2 kit as described in the Instructions for Use. Performance was established by evaluating the positive and negative percent agreement between the Sherlock CRISPR SARS-CoV-2 kit and the FDA authorized EUA comparator assay. Testing resulted in a positive percent agreement of 97.2% (35/36) and negative percent agreement of 97.0% (64/66).

Table 19: Calculation of PPA and NPA for the Sherlock CRISPR SARS-CoV-2 kit (Clinical Specimens)

		FDA EUA RT-PCR Comparator Assay Results	
		+	-
Sherlock CRISPR SARS-CoV-2 Kit Result	+	29	2
	-	1	64
Total		30	66
Positive percent agreement (Sensitivity) = 96.7% (95% CI: 88.3-99.4%) **Negative percent agreement (Specificity) = 97.0% (95% CI: 89.6-99.2%)**			

Disposal

Dispose of hazardous or biologically contaminated materials according to the practices of your institution.

The product contains no substances which at their given concentration, are considered to be hazardous to health.

HMIS

Health	0
Flammability	0
Reactivity	0

Symbols Used In Packaging

Symbol	Definition
Rx	Prescription Only
EUA	Emergency Use Authorization
	In vitro Diagnostic Use
	Catalog Number
	Manufactured By
	Temperature Limitation
	Batch Code
	Expiration Date
	Contains sufficient for <n> tests
1011-11-17	Date Format (year-month-day)
1011-11	Date Format (year-month)

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CDC guidelines for Sample collection –
https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html

FDA EUA guidance –
https://www.fda.gov/regulatory-information/search-fda-guidance-documents/policy-diagnostic-test s-coronavirus-disease-2019-during-public-health-emergency

Thermo Fisher viral RNA extraction kit PureLink™ Viral RNA/DNA Mini Kit (Cat# 12280050) Kit for RNA isolation
https://www.thermofisher.com/order/catalog/product/12280050#/12280050 Catalog Number 12280050 Publication NumberMAN0000562

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Product documentation
- User guides, manuals, and protocols
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