INSTRUCTIONS FOR USE

SARS-CoV-2 DETECTR™ Reagent Kit

Rx Only

IVD

For Emergency Use Authorization (EUA) only

CATALOG NUMBER: MBF00007

COMPANY: Mammoth Biosciences, Inc.

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Intended Use

The SARS-CoV-2 DETECTR™ Reagent Kit is a CRISPR-based, reverse transcription and loop-mediated amplification (RT-LAMP) test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in upper respiratory specimens (nasopharyngeal swabs, oropharyngeal (throat) swabs, mid-turbinate nasal swabs, anterior nasal swabs, nasopharyngeal wash/aspirate or nasal aspirate) 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 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. 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 SARS-CoV-2 DETECTR™ Reagent Kit is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of RT-LAMP, CRISPR detection and *in vitro* diagnostic procedures. The SARS-CoV-2 DETECTR™ Reagent Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

Summary and Explanation

On December 31, 2019 an outbreak of unexplained pneumonia cases was reported to the World Health Organization in Wuhan City, Hubei Province, China. The cause of the outbreak was identified as a novel coronavirus, which was first named 2019-nCoV (1), and then renamed to SARS coronavirus 2 (SARS-CoV-2) (2). The outbreak spread first locally within China, then into multiple countries globally. The disease caused by SARS-CoV-2 was named COVID-19 by the World Health Organization, and the virus is now the cause of a global pandemic infecting over 17.6 million people and causing the deaths of 679 thousand worldwide as of August 1st, 2020. Although most infections are asymptomatic or associated only with mild illness (80%) (3,4), some COVID-19 patients can develop severe pneumonia leading to fatality, with the highest risk in individuals who are elderly and/or have

comorbidities such as cardiac, lung, or liver disease. Asymptomatic infection and transmission have also been described (5,6). In the United States, there have been 4.7 million reported cases and 156 thousand deaths as of August 1st, and "shelter-in-place" policies imposed by nearly all states have devastated the economy, with job losses totaling 22 million.

Laboratory testing for COVID-19 infection is an important part of both the individual patient care and public health responses to this emerging outbreak. Results are used to guide containment efforts, including isolation and contact tracing, and make clinical diagnoses for supportive management and experimental therapies.

Principles of the Procedure

The one milliliter amplification master mixes (SARS-CoV-2 RT-LAMP Master Mix 1 – N Gene and SARS-CoV-2 Control RT-LAMP Master Mix) are activated by the addition of 6 µL of Amp-Activator (Figure 1A), after which 20 µL is placed into parallel wells of a 96-well plate, maintaining separation between the N and RP targets (Figure 1A). 5 µL of each sample is placed in a well with the N-amp and a well with the RP-amp master mix (Figure 1A). The amplification reaction is incubated at 62°C for 30 minutes (Figure 1A). Similarly, 18 µL of the DETECTR™ Master Mixes (SARS-CoV-2 DETECTR Master Mix 1 – N Gene and SARS-CoV-2 Control DETECTR Master Mix) are plated into 96-well plates, maintaining separation between the N and RP targets, with 2 µL of amplification reaction added to the DETECTR™ plate (Figure 1B). Fluorescence data is collected using the JUN channel every 30 seconds for 15 minutes of incubation at 37°C using the QuantStudio 7500 Fast Dx Real-Time PCR system. CRISPR is a revolutionary gene-editing tool that is now being touted as a next-generation diagnostic tool. The CRISPR–Cas12-based assay for detection of SARS-CoV-2 from extracted patient sample RNA, called SARS-CoV-2 RNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR™, trademarked by Mammoth Biosciences, Inc.) performs simultaneous reverse transcription and isothermal amplification using loop-mediated amplification (RT–LAMP) for RNA extracted from upper respiratory specimens in universal transport medium (UTM), followed by Cas12 detection of predefined coronavirus sequences, after which cleavage of a reporter molecule confirms detection of the virus.

The SARS-CoV-2 DETECTR™ Reagent Kit contains master mixes for the two enzymatic reactions used for detection of the coronavirus, SARS-CoV-2. After extraction of the sample with the EZ1 Virus Mini Kit v2.0 (48), the first step in the process is to simultaneously reverse transcribe and amplify a specific portion of the N-gene from the viral genome and RNase P (RP)-gene from patient RNA collected with the sample in parallel. The second enzymatic reaction involves CRISPR-Cas ribonucleoproteins specifically targeting the viral N-gene or the RP-gene. Identification of the N-gene indicates the presence of the SARS-CoV-2 coronavirus.

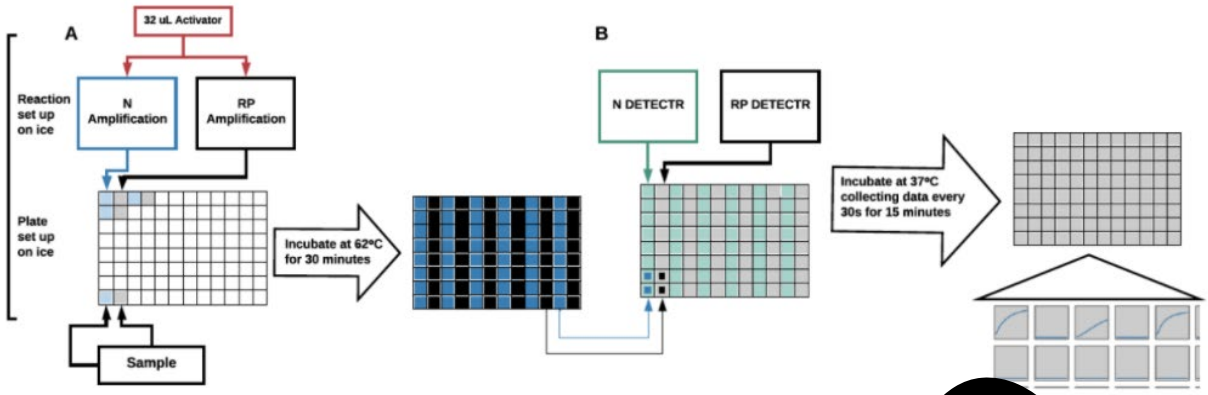

Figure 1. Workflow using the SARS-CoV-2 DETECTR™ Reagent Kit. A. The first half of the workflow focuses on the amplification reaction. B. The second reaction uses material from DETECTR™ workflow.

Components and Storage

Materials Required (Provided)

Each SARS-CoV-2 DETECTR™ Reagent Kit consists of the following components listed in Table 1.

Table 1. SARS-CoV-2 DETECTR™ Reagent Kit

Amplification Reagents	Vial Color	# of vials	Volume (µL/vial)	Storage conditions
SARS-CoV-2 RT-LAMP Master Mix 1 – N Gene	Red	1	1000	-20°C
SARS-CoV-2 Control RT-LAMP Master Mix	Yellow	1	1000	-20°C
SARS-CoV-2 Amp Activator	Clear	1	150	-20°C
Assay Control	Vial Color	# of vials	Volume (µL/vial)	
Assay Control – RNase P (DNA)	Lavender	2	100	-20°C
DETECTR™ Reagents	Vial Color	# of vials	Volume (µL/vial)	
SARS-CoV-2 DETECTR Master Mix 1 – N Gene	Red	1	1000	-20°C
SARS-CoV-2 Control DETECTR Master Mix	Yellow	1	1000	-20°C

Storage and Handling of Kit Components

The formal name is SARS-CoV-2 DETECTR™ Reagent Kit. Each reagent kit is formulated for 48 tests. The reagent kit is designed for single use and any excess material must be discarded after use. Each vial is designated with a lot number based on the formulation date. Each reagent kit is designated with a lot number based on packaging date.

Control Materials

- The SARS-CoV-2 DETECTR™ Reagent Kit provides an Assay Control – RNase P (AC-RP) which is human Hela cell line, extracted genomic DNA at 5ng/µL.
- Positive Template Control (PTC) for SARS-CoV-2 (not provided) can be commercially purchased, such as SeraCare AccuPlex™ SARS-CoV-2 Reference Material (Cat# 0505-0168).
- Negative control: molecular grade, DNase/RNase free water (not provided).

Equipment

Required Equipment and Consumables (but not provided)

Table 2. Components required but not included with the test

Equipment	Manufacturer	Catalog Number
Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument	Thermo Fisher Scientific	4406985
Applied Biosystems™ MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL	Thermo Fisher Scientific	4346907 or 4366906 (with barcode)
MicroAmp™ Optical Adhesive Film	Thermo Fisher Scientific	4311971
Coolrack® Modules, Cooling®, CoolRack XT PCR96, Holds 12 8-well strips	VWR	75779-732

Warnings and Precautions

- For *in vitro* diagnostic use (IVD).
- For use under an Emergency Use Authorization (EUA) only.
- For prescription use only.
- This test has not been FDA cleared or approved.
- This test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by

laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

- This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and
- This test is only 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 authorization is terminated or revoked sooner.
- Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.
- 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).
- Use personal protective equipment such as (but not limited to) gloves, eye protection, and lab coats when handling kit reagents while performing this assay and handling materials including samples, reagents, pipettes, and other equipment and reagents.
- Refer to Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition - CDC.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- 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 the 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.

Risk Mitigation: Amplicon Contamination

Three approaches of decreasing the risk of amplicon contamination include physical (using hoods), chemical (using DNA zap) and procedural (workflow, PPE, GLP) strategies. We recommend three levels of control: 1. Separate locations for pre- and post-amplification activities, 2. Separate environmental control between the rooms and 3. Separate equipment and personnel. For example, this standard operating procedure (SOP) will provide detailed guidelines that will minimize the risk of amplicon contamination.

Directional workflow (Personnel, reagents, equipment)

- The ideal workflow is from the main lab (personnel, reagents) to the pre-amp room to the post-amp area

A. Personnel:

- One-way directional workflow is critical to minimize carrying amplicons to less contaminated and amplicon-free (pre-amp room) zones.
- Operators will don and doff separate disposable lab coats when entering and exiting the pre-amp or post-amp rooms.
- If possible, operators could exclusively work in the pre-amp or post-amp room on any given shift.
- If only one operator is doing the amplification and DETECTR™, it is recommended that the operator do all amplifications first, and then proceed to the DETECTR™ portion.
- Proper PPE should be worn to minimize amplicon movement on clothing, including gloves to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment.
- Immediately don a disposable lab coat when entering the pre-amp room or prior to working in the post-amp area and dispose of the lab coat prior to leaving the pre-amp room.
- Immediately don a disposable lab coat when entering the post-amp room or prior to working in the post-amp area and dispose of the lab coat prior to leaving the post-amp room.
- 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.

B. Reagents:

- Ensure your reagents also have a one-way directional flow from receiving/main lab to the pre-amp room.
- Amplification reagents may be stored in the pre-amp room in the 2-8° C refrigerator or the -20°C freezer.
- 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., pipets, microcentrifuges) and supplies (e.g., microcentrifuge tubes, pipet tips) for assay setup and handling of extracted nucleic acids.
- 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.
- Keep reagent and reaction tubes capped or covered as much as possible.
- RNA should be maintained on a cold block or ice during preparation and use to ensure stability.
- Amplification and DETECTR™ master mix must be thawed and maintained on a cold block at all times during preparation and use.
- Use only reagents from SARS-Cov-2 DETECTR™ Reagent Kit from the same lot
- 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.

C. Equipment:

- Experimental plates should be sealed before moving them into the main laboratory for amplification.
- All equipment found in the pre-amp room stays in the pre-amp room.

D. Workspace:

- Work surfaces, pipets and centrifuges should be cleaned and decontaminated with cleaning products such as 10% bleach, "DNA Zap™" or "RNase AWAY®" to minimize risk of nucleic acid contamination.
- Make sure all bleach is removed to eliminate possible chemical reactions between bleach and guanidine thiocyanate which is present in the extraction reagents.
- To eliminate accelerated deterioration of any plastics and metals, wipe down with 70% ethanol after using 20% bleach.
- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.

Specimen Collection, Handling, and Storage

Inadequate or inappropriate specimen collection, storage, and transport are likely to yield false test results. Training in specimen collection is highly recommended due to the importance of specimen quality. CLSI MM13-A may be referenced as an appropriate resource.

Collecting the Specimen

- Nasopharyngeal swabs, oropharyngeal (throat) swabs, mid-turbinate nasal swabs, anterior nasal swabs, nasopharyngeal wash/aspirate and nasal aspirate are considered acceptable specimen types.
- 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

- 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 1-3 ml of universal transport media.

Transporting Specimens

- Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential 2019-nCoV specimens. Store specimens at 2-8°C and ship overnight on ice pack. If a specimen is frozen at -70°C or lower, ship overnight on dry ice.

Storing Specimens

- Specimens can be stored at 2-8°C for up to 72 hours after collection.
- If a delay in extraction is expected, store specimens at -70°C or lower.
- Extracted nucleic acid should be stored at -70°C or lower.

Reagent Controls and Preparation

Quality Control

Controls for the assay include Assay Control – RNase P (AC-RP, HeLa extracted genomic DNA, NEB, cat# N4006S), SARS-CoV-2 Positive Template Control (PTC, AccuPlex™ SARS-CoV-2 Reference Material, SeraCare, Cat# 0505-0168), universal transport medium (UTM, Copan, Cat# 3C038NHL) and negative template/extraction water control (NTC).

Nucleic Acid Extraction and Assay Set up

Performance of the SARS-CoV-2 DETECTR™ Reagent Kit is dependent upon the amount and quality of template RNA purified from human specimens. Extractions are performed using the Qiagen EZ1 DSP Virus Kit (Catalog # 955134) on the Qiagen EZ1 Advanced benchtop automated extraction

instrument according to the manufacturer's instructions with 200 µL input volume and 90 µL elution volume.

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.

General Handling

- Clean and decontaminate all work surfaces, pipets, centrifuges, and other equipment prior to use. Decontamination agents should be used including 10% bleach, 70% ethanol, and DNAzap™ or RNase AWAY® to minimize the risk of nucleic acid contamination.
- Always wear powder-free latex, vinyl, or nitrile gloves while handling reagents, tubes and RNA samples.
- 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, pipets and equipment with 10% bleach or other solution that can destroy nucleic acids and RNases.

Procedure

DETECTR™ Process:

Preparation of Pre-amp workspace prior to use

- Treat the hood with the U.V. light for 15 minutes.
- Wipe down all pipets and surface of the pre-amp hoods with RNA zap or 10% bleach followed by 70% ethanol

Preparation of Amplification reagents

- Remove amplification reagents from the -20°C storage and place in a rack at room temperature to thaw for >5 minutes. Place on ice as soon as they are thawed.
- Place the cooling rack on ice to chill.
- Prepare the amplification master mix: **Note:** All steps must be performed on ice.
    - Add 60µL Amp-A to each tube of target specific amplification master mix, Amp-N and Amp-RP
    - Vortex to mix for 5-8 seconds, quick spin for 15 seconds

Amplification of patient samples

- Place a 96-well plate into the cooling rack.
- Dispense 20µL of target specific amplification master mix into a single well in a 96-well plate

(Cat# 83009-676) (Figure 2).

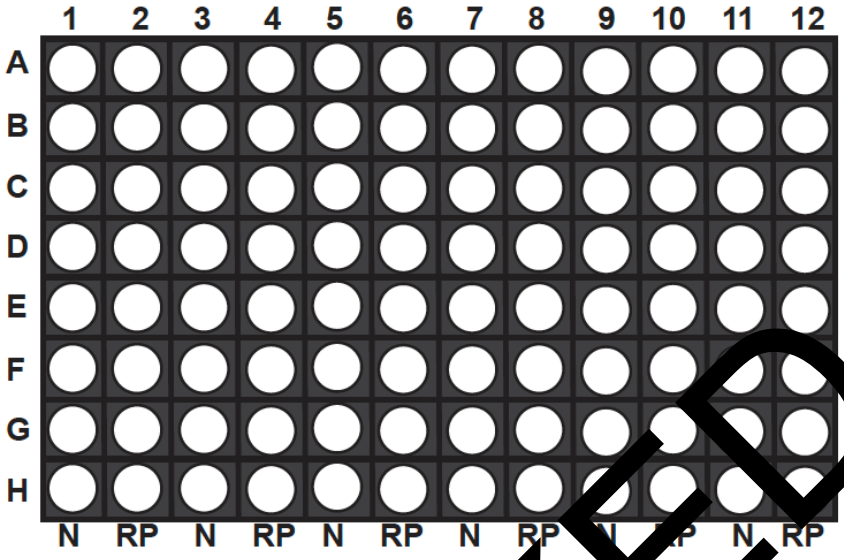

Figure 2. Example of a 96-well amplification plate with gridding to assist accurate loading of samples for SARS-CoV-2 N-gene and sample input control RNase P analysis (N=N-gene, RP=RNase P).

- Add 5µL of target to each well (Sample, Non-Template Control (NTC), Assay Control – RNase P (AC-RP) or Positive template control (PTC))
    - Seal plate with Pierceable Polyethylene Film or Plain Polyethylene Film, Sterile (Cat# 60941-116). Pending plates should be sealed before transport into the main laboratory for amplification.

Centrifuge the plate and place in the ABI 7500 96-well PCR system.
Set the amplification program as follows:

- Volume of reaction: 25 µL
- Time of run: 30 minutes
    - Temperature of run: 62°C
    - Hold temperature: 4°C
- Remove the amplification reaction plate from the instrument and place on ice until ready to begin testing by DETECTR™.
    - Plates can be stored at -20°C for subsequent DETECTR™ analysis.
    - Wipe down all pipettes and surfaces of the Pre-amp hoods with DNA zap or 10% bleach followed by 70% ethanol.
    - Treat the hood with the U.V. light for 15 minutes

Cleaning the Pre-amp workspace between users

- Treat the hood with the U.V. light for 15 minutes.

- Wipe down all pipets and surface of the Pre-amp hoods with DNA zap or 10% bleach followed by 70% ethanol

Preparation of DETECTR™ reagents

- Remove DETECTR™ reagents from the -20°C storage and place in a rack at room temperature to thaw for >30 minutes. Place on ice as soon as they are thawed.
- Place the cooling rack on ice to chill.

DETECTR™ analysis of patient samples

- Make a "loading grid" on a 96-well (Figure 3) plate using a marking pe

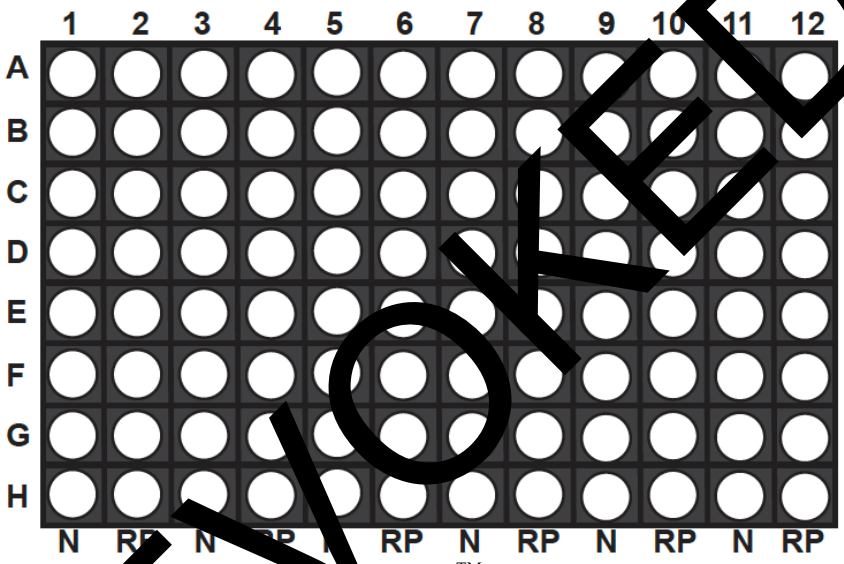

Figure 3. Example of an ABI 96-well DETECTR™ plate with gridding to ensure accurate loading of samples for SARS-CoV-2 N-gene and sample input control RNase P analysis (N=N-gene, RP=RNase P).

- Place a 96-well DETECTR™ plate in the cooling rack
  Note: All steps must be performed on ice.
- Add 18µL of DETECTR™ master mix to each well
  - In the 'post-amp' hood, pierce the amplification plate with multi-channel pipet and transfer 2.0µL of product from the amplification plate to the corresponding DETECTR™ master mix.
  - Seal the plate with Heat-Resistant Films for Real-Time qPCR, Ultra-Clear
  - Leave the plate on ice, while setting up the ABI 7500 Fast Dx.

Preparing the ABI 7500 Fast Dx for DETECTR™ analysis

- Log onto the ABI 7500 Fast Dx.
- Open the 7500 Fast system software icon by double clicking the icon.

- Use the appropriate username and password to log into the program.
- Select 'Open Existing Document'.
- Select 'SARS-CoV-2 DETECTR.sds' to open the program template.
- Using the 'File' drop down menu, select 'save as' and name the program appropriately ensuring that saves as a .sds file.
- Close down the software and reopen as described in above.
- Find designated .sds file, select and open.
- Alternatively, the designated .sds file may appear on the 'Quick Startup menu' under 'Recent document(s)'. If so, select your file name.
- Opening your file should be concurrent with a note showing "7500 Fast System initializing, please wait..."
- Load the DETECTR™ plate into the instrument.
- When the software opens, select the 'Instrument' tab and click the 'Start' button.
- The data is collected every 30 seconds using a 2-stage cycling scheme with 5 seconds at 37°C followed by 25 seconds at 37°C for 30 cycles (total run time ~45 min).

Creating a sample ID import sheet

- Using the 'File' drop down menu, export the Sample Setup (.txt file)
- From your plate map, select and copy the cells in each row containing the sample IDs.
- Under 'Sample Select the appropriate 'Cell' on the Sample Setup sheet (row A = 1 – 12, row B = 13 – 24, etc.)
- Using the 'Paste' drop down menu, select 'Transform'. The sample IDs should populate the column in a row-wise manner.
- Save the Sample Setup file ensuring it remains a Text delimited (.txt) file.
- Using the 'File' drop down menu, open the appropriate .sds file.
- Using the 'File' drop down menu, open 'Import' then, Sample Setup.
- Select the file containing the appropriate sample IDs to load them onto the plate.
- Save the program.

Cleaning the Post-amp workspace between users/runs

- Wipe all pipets and surface of the Pre-amp hoods with DNAzap or 10% bleach (0.5% sodium hypochlorite) followed by 70% ethanol.
- Treat the hood with the U.V. light for 15 minutes

Control Materials to be Used with the SARS-CoV-2 DETECTR™ Reagent Kit

Controls for the assay include Positive Template Control (PTC), Assay Control – RNase P (AC-RP), universal transport medium (UTM) and negative template/extraction water control (NTC). All are extracted together with patient samples with each extraction run. All test controls are examined prior to

interpretation of patient results. If the controls are not valid, the patient results are not interpreted. The results of the expected control reactions are shown in Table 3.

- The PTC consists of AccuPlex™ SARS-CoV-2 Verification Panel reference material (SeraCare, Catalog # 0505-0168) diluted in the negative AccuPlex™ sample (SeraCare, Catalog# 0505-0168) corresponding to 30 copies/µL or 1.5x the claimed LoD of 20 copies/µL. The AccuPlex™ SARS-CoV-2 Verification Panel reference material consists of a target synthetic viral RNA, including the N gene, encapsulated in a viral protein coat. As the reference material spiked into a negative matrix is fully extractable, this reference material serves as a full- process control for the DETECTR™ Reagent Kit.
- The PTC serves as a Positive Template Control for amplification of the N gene target.
- The Assay Control – RNase P (AC-RP, HeLa extracted genomic DNA, NEB, cat# N4006S) serves as a positive control for the presence of human nucleic acid in the sample, which is determined by detection of the RP gene.
- The UTM serves as a control for contamination in the extraction or amplification.
- The NTC serves as a control for contamination during both the extraction and PCR reagent preparation.
- The RP target also serves as an internal control for each patient sample, ensuring that human nucleic acid is present in the sample.

Table 3. Expected Control Results

Fluorescence units (FU) Control	**Target**	**N gene**	**RP gene**
AccuPlex™ SARS-CoV-2 Reference Material (PTC)	N gene	Positive (≥500,000 FU)	Negative (<500,000 FU)
AC-RP	RP gene	Negative (<500,000 FU)	Positive (≥500,000 FU)
UTM	None	Negative (<500,000 FU)	Negative (<500,000 FU)
NTC	None	Negative (<500,000 FU)	Negative (<500,000 FU)

Interpretation of Results

The criteria for determining assay results, actions and interpretation of SARS-CoV-2 DETECTR™ Reagent Kit data collected from the ABI 7500 Fast Dx are provided in Table 4. These data fall into a range of possible assay results and interpretations outlined in Table 5. The two possibilities for a positive SARS-CoV-2 result both require a positive value for the N-gene, which remains positive with or without a positive signal for the RP-gene. A SARS-CoV-2 negative result requires a negative value

for N-gene and a positive value for the RP-gene. An invalid interpretation, known as a quality control (QC) failure occurs if there is a negative result for both the N-gene and RP-gene, in which the same specimen undergoes a repeat extraction and assay.

Table 4: Criteria for determining assay results

Result Interpretation N gene	Result Interpretation RP gene	Interpretation	Action
Positive (≥500,000 FU*)	Positive (≥500,000 FU) or Negative (<500,000 FU)	Detected	Report as SARS CoV-2 Detected
Negative (<500,000 FU)	Positive (≥500,000 FU)	Not Detected	Report as SARS CoV-2 Not Detected
Negative (<500,000 FU)	Negative (<500,000 FU)	Invalid	Repeat extraction; if same invalid result. Report as Invalid and obtain a new specimen

*FU = fluorescence units

Table 5: Guide for interpreting assay results and action

N gene	RP gene	Interpretation	Action
Positive	Positive or Negative	Detected	Report as Detected
Negative	Positive	Not Detected	Report as Not Detected
Negative	Negative	Invalid	Repeat with new extraction; if same invalid result report as Invalid and obtain a new specimen

Control Evaluation (Assay controls, Negative control template reaction (NTC), Positive control reactions (PTC, AC-RP)):

- NTC should be negative and exhibit no fluorescence.
    - If a false positive occurs, sample contamination may have occurred.
- UTM should be negative and exhibit no fluorescence.
    - If a false positive occurs, sample contamination may have occurred.

- PTC reaction should produce a positive N gene result and exhibit fluorescence.
    - If the PTC does not exhibit positivity, the run is invalid.
- RP should be positive for all negative clinical samples and AC-RP. Acceptable results for the RP in patient samples indicate the presence of sufficient nucleic acid from the human RP-gene, and thus, acceptable specimen quality.
    - Failure of RP detection in AC-RP may indicate improper assay set up or instrument malfunction.
    - Failure of RP in patient samples but detection in AC-RP may indicate extraction failure, assay inhibition, or absence of sufficient human cellular material.
    - Samples that are positive for viral detection do not require amplification of RP target to be valid.
- AC-RP should be negative for N-gene.
    - Fluorescence may indicate contamination of reagents or cross-contamination of samples.
    - If the AC-RP exhibits a positive signal, the run is invalid.

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.
- Pipet tips must be replaced after every pipetting. Gloves must be replaced often. Equipment must have calibration up to date for the pipets 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

Limitations

- This assay is for *in vitro* diagnostic use under FDA Emergency Use Authorization only. 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.
- All users, analysts, and any person reporting diagnostic results should be trained to perform this procedure by a competent instructor and should demonstrate their ability to perform the test and

interpret the results prior to performing the assay independently.

- Performance of the SARS-CoV-2 DETECTR™ Reagent Kit was established using nasopharyngeal and oropharyngeal swabs. Mid-turbinate nasal swabs, anterior nasal swabs, nasopharyngeal wash/aspirate or nasal aspirate are also considered acceptable specimen types but performance has not been established.
- 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-AMP 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.
- Test performance can be affected because the epidemiology and clinical spectrum of infection 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, during the course of infection, when these specimens are most likely to contain levels of viral RNA 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 products 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 SARS-CoV-2 DETECTR™ Reagent 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/vitro-diagnostics-euas

However, to assist clinical laboratories using the **SARS-CoV-2 DETECTR™ Reagent Kit**, the relevant Conditions of Authorization are listed below:

- Authorized laboratories will include with result reports of the test, 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 the **SARS-CoV-2 DETECTR™ Reagent Kit** will perform the test as outlined in the Instructions for Use. Deviations from the authorized procedures, including authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use the **SARS-CoV-2 DETECTR™ Reagent Kit** are not permitted.
- Authorized laboratories that receive the **SARS-CoV-2 DETECTR™ Reagent Kit** will notify the relevant public health authorities of their intent to run your product prior to initiating testing.
- Authorized laboratories will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- 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 to Mammoth Biosciences, Inc. (via phone: +1-650-204-4983 and via email: support@mammoth.bio) any suspected occurrence of false positive and false negative results and significant deviations from the established performance characteristics of the test of which they become aware.
- All laboratory personnel using the test must be appropriately trained in RT-LAMP techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.
- Mammoth Biosciences, Inc., authorized distributors, and authorized laboratories 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.

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

Limit of Detection (LoD)

Quantified AccuPlex™ SARS-CoV-2 Verification Panel reference material (SeraCare, Cat# 0505-0168), an encapsulated synthetic RNA containing the N-gene, at a stock concentration of 100,000 copies/mL was diluted in pooled negative patient NPS/OPS in UTM and run in 20 contrived replicates of pooled extracted RNA per dilution. The limit of detection was defined as the lowest concentration where at least 19 of 20 replicates were positive. This showed an LoD of 20 copies/µL in contrived samples (Table 6).

Table 6. LoD based on AccuPlex™ reference material (spiked into NPS matrix and extracted)

Sample ID	Concentration equivalent in UTM (copies/mL)	Copies / Reaction	Copies / µL	Total # of Reactions	# of Positive Reactions	# of Negative Reactions
Dil2	18,000	150	30	20	20	0
Dil3	12,000	100	20	20	20	0
Dil4	9,000	75	15	20	16	4
Dil5	6,000	50	10	20	16	4

Inclusivity (analytical sensitivity)

To demonstrate the predicted inclusivity, *in silico* analysis of the primer and gRNA sequences with 53,503 SARS-CoV-2 genomes available on GISAID as of August 18th, 2020 (defined as >29 kbp of sequence), using NC_045512 from GenBank as a reference for SARS-CoV-2. A total of 1,794 variants were found containing single nucleotide variants (SNVs) in the primer and gRNA regions in the N gene target amplicon used by the DETECTR™ Reagent Kit, representing 3.33% (1,794 of 53,503) of all sequences in GISAID. Among the variants, 1,628 have a single SNV within one of the 6 primer regions (F3, B3, LF, LB, FIP (F2-F1c), or BIP (B2-B1c)), 11 have two SNV within one of the 6 primer regions, and 155 have a single SNV within the gRNA region. Results are summarized in Table 7.

A single SNV in a primer region is unlikely to significantly affect the sensitivity of the assay unless it is at the 3' end of the primer. Among the variants with a single SNV within one of the 6 primer regions, only 41 (0.07%) have an SNV at the 3' end of the primer. A sequence containing a SNV in the gRNA region may also affect sensitivity given the single nucleotide specificity of the gRNA for a CRISPR-Cas12 reaction. Thus, the sensitivity of detection of the DETECTR™ Reagent Kit is likely to be affected in only 155 sequences out of 53,503, constituting only 0.29% of all available sequences in GISAID as of August 18th, 2020. Note that the SNV in the gRNA region may also affect sensitivity of the detection of the CDC N2 assay as well, as it also overlaps with the N2 probe region.

Table 7. Frequency of mismatches in primer and gRNA sequences for all SARS-CoV-2 sequences in GISAID as of August 18th, 2020

Primer Name	F3	B3	FIP	BIP	LF	LB	gRNA
Primer Length	18	22	41	39	19	18	20
Viral strains evaluated	53500	53501	53499	53495	53503	53491	53501
% with no mismatch	99.63%	99.73%	98.91%	99.60%	99.34%	99.70%	99.71%
0 mismatch	53302	53356	52916	53279	53151	53332	53346
1 mismatch	200	145	584	206	354	160	155
2 mismatches	0	0	1	10	0	0	[illegible]
3 mismatches	0	0	0	0	0	0	0
>3 mismatches	0	0	0	0	0	[illegible]	0

Cross-reactivity (Analytical Specificity)

In Silico BLASTn analysis queries of the SARS-CoV-2 DETECTR™ Reagent Kit N gene primers and gRNAs were performed against public domain nucleotide sequences in NCBI (National Center for Biotechnology Information) nucleotide collection (nt) using default parameters. Sequences from the following organisms were analyzed (Table 8).

Table 8: Organisms analyzed in-silico for Cross-Reactivity with other high priority pathogens from the same genetic family

Other high priority pathogens from the same genetic family	High priority organisms likely co-circulating areas
SARS-CoV-2	Adenovirus
bat-SL-CoVCZ45	Influenza A virus
SARS-CoV	Influenza B virus
MERS-CoV	Influenza C virus
HCoV-OC43	RSV (A + B)
HCoV-NL63	Human parainfluenza virus (1-4)
HCoV-229E	Enterovirus
HCoV-HKU1	Rhinovirus
	Bacillus anthracis
	Bordetella pertussis
	Candida albicans
	Chlamydia pneumoniae
	Chlamydia psittaci

	Chlamydia trachomatis
	Corynebacterium diphtheriae
	Coxiella burnetii
	Haemophilus influenzae
	Human metapneumovirus (hMPV)
	Legionella longbeachae
	Legionella pneumophila
	Leptospira interrogans
	Moraxella catarrhalis
	Mycobacterium tuberculosis
	Mycoplasma pneumoniae
	Neisseria elongata
	Neisseria gonorrhoeae
	Parechovirus
	Pneumocystis jirovecii
	Pseudomonas aeruginosa
	Staphylococcus aureus
	Staphylococcus epidermidis
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Streptococcus salivarius

Summary of the results are shown below.

N gene primers and gRNAs:

- F3 → 83.3% homology to a sequence in the *Haemophilus influenzae* genome and the *Homo sapiens* genome. No significant homology to other organisms of interest.
- B3 → 81.8% identity to a sequence in the *Homo sapiens* genome.
- FIP (F2-F1c) → 100% homology to bat coronaviruses and SARS-CoV. No significant homology to other organisms of interest.
- BIP (B2-B1c) → 100% homology for the B1c portion of the BIP primer to bat coronaviruses and SARS-CoV. No significant homology to other organisms of interest.
- LF → homology to SARS-CoV (94%), *Chlamydia pneumoniae* (84%), *Streptococcus pyogenes* (84%), and *Homo sapiens* (89% genomes).
- LB → 88.8% homology to bat coronaviruses. No significant homology to other organisms of interest.
- N-gene gRNA → 80% homology to a sequence in the *Homo sapiens* genome. This sequence lacks the PAM required for Cas12 activity.

Although some primers have partial homology to the organisms of interest, it is unlikely for cross-reactivity to occur with these organisms as RT-LAMP requires complementarity to at least 4 of the 6 primers. In addition, the specificity of the RT-LAMP amplicon is benefited by the sequence specificity of the Cas enzyme.

Although most of the N gene primers (B3, BIP, FIP, LF, and LB) have 100% identity to SARS coronavirus and other bat coronavirus, cross-reactivity with these other coronaviruses would not be expected given the lack of sequence homology in F3 and the N-gene gRNA. The gRNA sequence, in particular, has single-nucleotide specificity.

Cross-reactivity with human, other coronaviruses, and bacterial sequences would also not be expected given the lack of sequence homology in several primers (B3, BIP, FIP) and in the N- gene gRNA.

Cross-reactivity was also assessed by wet-testing in triplicate 18 nasopharyngeal swab patient specimens, positive for the organisms listed in Table 9. An additional three cultured microorganisms listed in Table 10 quantified by microbial culture, spiked into NPS patient specimen and extracted using QIAGEN EZ1were included in the study. Viruses were detected by the FDA cleared Luminex NxTAG Respiratory Pathogen Panel and CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, Influenza A/B Typing Kit. Each sample was then assayed using the SARS-CoV-2 DETECTR™ Reagent Kit. All 18 clinical specimens were negative for SARS-CoV-2.

Table 9: Patient specimens with potentially cross-reactive viral species and summary of wet-testing for the analytical specificity of the SARS-CoV-2 DETECTR™ Reagent Kit

Sample ID	Viruses Detected	SARS-CoV-2 DETECTR™ Result
RespV-001	Human coronavirus OC43	Negative
RespV-002	Human rhinovirus/enterovirus	Negative
RespV-003	Influenza B virus	Negative
RespV-004	Human coronavirus NL63	Negative
RespV-005	Respiratory syncytial virus A	Negative
	Respiratory syncytial virus B	
RespV-006	Respiratory syncytial virus B	Negative
RespV-007	Human rhinovirus/enterovirus	Negative
RespV-008	Human metapneumovirus	Negative
RespV-009	Human coronavirus HKU1	Negative
RespV-010	Human coronavirus HKU1	Negative
	Human adenovirus	
	Human bocavirus	
RespV-011	Human metapneumovirus	Negative
RespV-012	Respiratory syncytial virus A	Negative
RespV-013	Human metapneumovirus	Negative
RespV-014	Human bocavirus	Negative
RespV-015	Human rhinovirus/enterovirus	Negative
RespV-016	Human metapneumovirus	Negative
RespV-017	Human adenovirus	Negative
RespV-018	Human rhinovirus/enterovirus	Negative

Table 10: Negative patient matrix spiked with potentially cross- reactive microorganisms

Cross-Reactive Species Sample ID	Organism Tested	Final Concentration (CFU/mL)	SARS-CoV-2 DETECTR™ Result
RespV-019	*Staphylococcus epidermidis*	1.5 x 10^7	Negative
RespV-020	*Pseudomonas aeruginosa*	1.5 x 10^7	Negative
RespV-021	*Candida albicans*	1.5 x 10^7	Negative

Clinical Evaluation

A total of 102 individual nasopharyngeal swabs (NPS) specimens, sourced from the UCSF CLIA certified high complexity clinical laboratory were tested with the SARS-CoV-2 DETECTR™ Reagent

Kit. These patient samples were collected prospectively after January 2020 in accordance with human subject protection regulations and under IRB approval during routine testing of individuals presenting with COVID-19 symptoms. A single replicate of each blinded sample was tested in the UCSF clinical laboratory and positive and negative agreement were based on the comparator result. RNA was extracted using the Qiagen EZ1 DSP virus kit on the Qiagen EZ1 Advanced benchtop automated extraction instrument. Of the 40 positive NPS patient samples, 38 (95.0%) were detected by the DETECTR™ Reagent Kit and 62/62 (100%) negative NPS samples were confirmed negative. No invalid results were obtained during this study. Results are summarized in Table 11.

Table 11. Evaluation with Clinical NPS Specimens

		Comparator Assay	
		Positive	**Negative**
DETECTR™ Reagent Kit	**Positive**	38	0
	Negative	2	62
	Total	40	62
Positive Agreement		38/40 = 95.0% (95% CI: 83.5% – 98.6%)	
Negative Agreement		62/62 = 100% (95% CI: 94.2% - 100.0%)	

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to corroborate the LoD. Extractions were performed using the Qiagen EZ1 DSP virus kit on the Qiagen EZ1 Advanced benchtop automated extraction instrument according to the manufacturer's instructions. The SARS-CoV-2 DETECTR™ Reagent Kit was used with the Thermo Scientific ABI 7500 Fast Dx Real-Time PCR System Software Version 1.4.1. The results are summarized in Table 12.

Table 12: 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	Nasopharyngeal Swab	5.4×10^5 NDU/mL	N/A
MERS-CoV		N/A	ND

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

Symbols Used In Packaging

REF	Reference Number
IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
In Vitro Test	In Vitro Test
For In Vitro Diagnostic Use	For In Vitro Diagnostic Use
AMP TRAY	AMP Tray
ACT TRAY	ACT Tray
Rx ONLY	For Prescription Use Only
	Warning
	Systemic Health Effects
	Caution
	Consult Instructions for Use
	Temperature Limit
	Sufficient for
	Use By
	Manufacturer

References

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

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

Contact Information, Ordering, and Product Support

Information and product support can be obtained from:

Contact: Mammoth Biosciences, Inc. Customer Support

Email: support@mammothbiosci.com

Phone: +1-650-294-8583

Product support information

- Technical support
- Order and web support

Product documentation

- Fact Sheet for Healthcare Providers
- Fact Sheet for Patients
- Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.