# DETECTR BOOST™ SARS-CoV-2 Reagent Kit



## INSTRUCTIONS FOR USE

For in vitro diagnostic (IVD) use

For Prescription (Rx) use only

For Emergency Use Authorization (EUA) only

**CATALOG NUMBER:** DETECTRA-1KT, DETECTRB-1KT, DETECTRC-1KT

**COMPANY:** Mammoth Biosciences, Inc.

# Table of Contents

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## Intended Use

The DETECTR BOOST™ SARS-CoV-2 Reagent Kit is a CRISPR-based, reverse transcription and isothermal amplification test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal, anterior nasal, mid-turbinate nasal or oropharyngeal swab 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 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 coinfection 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 DETECTR BOOST™ SARS-CoV-2 Reagent Kit is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of nucleic acid amplification, CRISPR detection and in vitro diagnostic procedures. The DETECTR BOOST™ SARS-CoV-2 Reagent Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.



## Summary and Explanation of the Test

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, and then renamed to SARS coronavirus 2 (SARS-CoV-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 309 million people and causing the deaths of over 5.4 million worldwide as of January 10, 2022 (1). Although most infections are asymptomatic or associated only with mild illness, 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. Older adults are more likely to get severely ill from COVID-19. More than 81% of COVID-19 deaths occur in people over age 65. The number of deaths among people over age 65 is 80 times higher than the number of deaths among people aged 18-29 (2). Asymptomatic infection and transmission have also been described. In the

United States, there have been over 61 million reported cases and over 837 thousand deaths as of January 10, 2022 (3).

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

## Principle of the Procedure

The Mammoth Biosciences DETECTR BOOST™ SARS-CoV-2 Reagent Kit uses CRISPR-Cas enzymatic detection for SARS-CoV-2 with a reverse transcription and isothermal amplification from patients suspected of COVID-19 by their healthcare provider. The isothermal amplification primers target a sequence in the SARS-CoV-2 N-gene. The CRISPR enzyme uses a guide RNA that targets the SARS-CoV-2 N-gene to detect the isothermal amplification amplicons.

The DETECTR BOOST™ SARS-CoV-2 Reagent Kit includes the following materials: Lysis/Binding Solution, Wash Solution 1, Elution Buffer, RNA Binding Beads, Carrier RNA, Lysis/Binding Enhancer, RT-LAMP Activator, RT-LAMP Master Mix, DETECTR™ Master Mix, SARS-CoV-2 Positive Control, and SARS-CoV-2 Negative Control. First, SARS-CoV-2 nucleic acid is extracted from specimens following inactivation, lysis and RNA binding to magnetic beads. These steps involve only the removal of waste after lysing and washing, such that the sample RNA remains in the same microplate well throughout the extraction process. Next, the RT-LAMP Activator and RT-LAMP Master Mix is added to the well containing sample RNA and incubated at 57ºC for 30 minutes. Finally, the DETECTR™ Master Mix is added directly to the entire RT-LAMP reaction in the same well and incubated at 37ºC for 10 minutes. The DETECTR™ Master Mix contains the CRISPR–Cas12 enzyme that cleaves a reporter DNA linker releasing a fluorophore from its quencher molecule if the SARS-CoV-2 N-gene target region has been amplified and detected. The rise of fluorescence signal indicates a positive detection of SARS-CoV-2.

The assay is run on the Agilent BRAVO BenchCel DB liquid handling platform.



## Reagents and Equipment

### Reagents and Materials Provided

Each DETECTR BOOST™ SARS-CoV-2 Reagent Kit, shown in Figures 1 through 4, can process 768 tests and consists of three packaged sub-kits stored at different temperatures. The reagents in each kit are listed in Table 1. The reagent kit volumes include the necessary volume overage needed for using the Agilent BRAVO BenchCel DB liquid handling platform to process 768 tests.


*Figure 1: DETECTR BOOST™ SARS-CoV-2 Reagent Kit*


*Figure 2: DETECTR BOOST™ SARS-CoV-2 Kit A*




*Figure 3: DETECTR BOOST™ SARS-CoV-2 Kit B*


*Figure 4: DETECTR BOOST™ SARS-CoV-2 Kit C*

*Table 1: DETECTR BOOST™ SARS-CoV-2 Reagents*

| Catalog Number | Reagent Name | Reagent Composition | Volume Per Kit | #/Kit | Storage | Hazard |
|---|---|---|---|---|---|---|
| DETECTRA-1KT | DETECTR BOOST™ SARS-COV-2 KIT A | | | | 20 °C to 25 °C |  |
| QMAM007-1VL | Lysis/Binding Solution | 55-80% Thiocyanic acid, compound with guanidine (1:1) | 8[illegible] mL | 1 bottle | 20 °C to 25 °C |  |
| QMAM008-1VL | Wash Solution 1 | 25-40% Thiocyanic acid, c[illegible]pound with guanidine (1[illegible]) | 63.8 mL | 1 bottle | 20 °C to 25 °C |  |
| QMAM009-1VL | Elution Buffer | pH buffered wate[illegible] | 31.9 mL | 1 bottle | 20 °C to 25 °C | |
| DETECTRB-1KT | D[illegible]ECTR BOOST™ [illegible]ARS-COV-2 KIT B | | | | 2 °C to 8 °C | |
| QMAM006-1VL | RNA Binding Beads | Silica m[illegible]gnetic beads, [illegible]um Azide <0.1% | 3.6 mL | 1 bottle | 2 °C to 8 °C | |
| DETECTRC-1KT | DETECTR BOOST™ SARS-COV-2 KIT C | | | | -20 °C to 0 °C |  |
| QMAM100-1EA | RT-LAMP Master Mix | 46% Deoxynucleoside triphosphate (dNTPs), 0.05% RT-LAMP amplification enzymes, 0.05% RNase inhibitor, 0.2% pH | 9.6 mL | 1 plate 96 well | -20 °C to 0 °C | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | buffers, <0.1% Magnesium and potassium salts, <0.1% surfactant | | | | |
| QMAM200-1EA | RT-LAMP Activator | 5% pH buffers, 1% salts of acetic acid, 0.25% surfactant, <0.1% EDTA, < 0.1% DNA primers | 31.9 mL | 1 bottle | -20 °C to 0 °C |  |
| QMAM300-1EA | DETECTR™ Master Mix | <0.1% CRISPR-CAS12 enzyme, 9% Glycerol, <0.1% Magnesium and potassium salts, 0.2% pH buffers, <0.1% surfactant <0.1% MES (4-Morpholineethanesulfonic acid) | [illegible] mL | 1 bottle | -20 °C to 0 °C |  |
| QMAM004-1VL | Carrier RNA | RNA in pH buffered [illegible] | 1.3 mL | 1 vial | -20 °C to 0 °C | |
| QMAM005-1VL | Lysis/Binding Enhancer | 40-70% Glycerol, [illegible]% Proteinase K | 27.9 mL | 1 bottle | -20 °C to 0 °C |  |
| QMAM010-1VL | SARS-CoV-2 Negative Control | Dulbecco's Phosphate Buffered Saline (DPBS) | 0.92 mL | 1 vial | -20 °C to 0 °C | |
| QMAM011-1VL | SARS-CoV-2 Positive Control | SeraCare AccuPlex SARS-CoV-2 target diluted to 7,500 copies/mL in DPBS | 0.92 mL | 1 vial | -20 °C to 0 °C | |

## Materials Required (but not provided)

Table 2 lists the user provided reagents needed to process 768 tests using the DETECTR BOOST™ SARS-CoV-2 Reagent Kit.

*Table 2: User Supplied Reagents*

| Reagent Name | Quantity Needed per Kit | Supplier / part number |
|---|---|---|
| Isopropanol, 99.5%, Molecular Biology grade | 200 mL per kit | Millipore Sigma / I9516 or equivalent |
| Mineral Oil, Molecular Biology grade | 30 mL per kit | Millipore Sigma / M5904 or equivalent |



## Reagent Storage Requirements

Table 3 lists the storage requirements for the reagents in the DETECTR BOOST™ SARS-CoV-2 Reagent Kit.

*Table 3: Storage for DETECTR BOOST™ SARS-CoV-2 Reagent Kit*

| Material | Vendor/Part Number | Storage Condition |
|---|---|---|
| DETECTR BOOST™ SARS-CoV-2 KIT A | DETECTRA-1KT | 20 °C to 25 °C |
| DETECTR BOOST™ SARS-CoV-2 KIT B | DETECTRB-1KT | 2 °C to 8 °C |
| DETECTR BOOST™ SARS-CoV-2 KIT C | DETECTRC-1KT | -20 °C to 0 °C |

## Equipment and Consumables Required

The assay is run on an Agilent laboratory BRAVO BenchCel DB liquid handling platform configured as specified in Table 4 using labware consumables as specified in Table 5.

*Table 4: Agilent BRAVO BenchCel DB based liquid handling platform*

| Item | Vendor/Part Number |
|---|---|
| BRAVO BenchCel DB liquid handling platform | Agilent / G5578GA running VWorks 13.1.6 or higher |
| Cytation 5 multi-mode plate reader | Biotek / CYT5F with Gen5 software version 3.10 or higher |
| BenchCel Integration Kit for use with Cytation 5 | Biotek / 2000001 |
| Assay Method Scripts | Mammoth / Lysis _Detection_v[illegible].vzp or higher running on Agilent VWorks and DETECTX_3.0.xpt or higher running on BioTek Gen5 |

*Table 5: Labware consumables for Agilent BRAVO BenchCel DB liquid handling platform*

| Item | Vendor/Part Number |
|---|---|
| Reservoir, single cavity, polypropylene, 300 mL, 96 pyramids base geometry, 44 mm height | Agilent / 201244-100 |
| 96 well microplate, polypropylene, U-bottom, 300 µL working volume, black | Greiner BIO-ONE / 650209 |
| 170 µL Automation Tips for Agilent Robotic Platforms: Racked, Non-Sterile, Filtered, 96 tips per rack | Thomas Scientific / 20A00M139 |
| Lids, universal, clear, polystyrene | Agilent / 200856-100 |
| Adhesive Film for PCR Plates | VWR / 60941-078 or equivalent |

## Calibration and Qualification

Any equipment used with the DETECTR BOOST™ SARS-CoV-2 Reagent Kit should be calibrated and qualified to perform according to the manufacturer's specifications. It is recommended that laboratories execute equipment qualification for the BRAVO BenchCel DB based liquid handling platform with Agilent's CrossLab Compliance Services.

## Sample Collection, Transport, and Storage

### Sample Collection

Nasopharyngeal, anterior nasal, oropharyngeal and mid-turbinate nasal swab specimens should be collected by a trained health care provider.

Collect specimens according to standard collection techniques using flocked or polyester-tipped swabs and insert into 3 mL of saline.

Refer to the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19) (https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html) or the FDA FAQs on Diagnostic Testing for SARS-CoV-2 (https://www.fda.gov/medical-devices/emergency-situations-medical-devices/faqs-testing-sars-cov-2).

### Sample Transport and Storage

Store specimens at 2-8 °C for up to 72 hours after collection. If a delay in testing or shipping is expected, store specimens at -70 °C or below.

Do not test frozen specimens.



## Warning and Precautions

- For in vitro diagnostic use (IVD).
- For use under an Emergency Use Authorization (EUA) only.
- For prescription use only.
- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.
- 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 only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

- 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/lab-biosafety-guidelines.html. All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition - CDC and in the CLSI Document M29-A4. Only personnel proficient in handling infectious materials should perform this procedure.
- 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.
- Use personal protective equipment such as (but not limited to) gloves, eye protection, and lab coats when handling reagents while performing this assay and handling materials including samples, reagents, pipettes, and other equipment and reagents.
- Laboratories within the United States and its territories are required to report all 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.



## Limitations of the Assay

- Testing frozen specimens can lead to erroneous results. Frozen specimens should not be tested with the DETECTR BOOST™ SARS-CoV-2 Reagent Kit.
- Invalid results may occur in the presence of *Pseudomonas aeruginosa* at ≥2.02 x $10^8$ CFU/mL.
- The performance of the DETECTR BOOST™ SARS-CoV-2 Reagent Kit has been validated with nasopharyngeal swab specimens in saline. It has not been validated for other specimen types.

- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- As with any molecular test, mutations within the target regions of SARS-CoV-2 could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutics or immunosuppressant drugs have not been evaluated.
- A false negative result may occur if a specimen is improperly collected, transported or handled.
- 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.
- Performance has not been established in asymptomatic individuals.

## Conditions of Authorization for Labs

Conditions of Authorization the DETECTR BOOST™ SARS-CoV-2 Reagent Kit[1] 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 DETECTR BOOST™ SARS-CoV-2 Reagent Kit[1] the relevant Conditions of Authorization are listed below:

A. 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.

B. Authorized laboratories using this product must use this product as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use this product are not permitted.



[1] "This product" refers to the DETECTR BOOST™ SARS-CoV-2 Reagent 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."

C. Authorized laboratories that receive this product must notify the relevant public health authorities of their intent to run this product prior to initiating testing.

D. 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.

E. 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 Mammoth Biosciences, Inc. Technical Support (via email: support@mammoth.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.

F. All laboratory personnel using this product must be appropriately trained in CRISPR detection techniques, the specific processes and instruments used in DETECTR BOOST SARS-CoV-2 Reagent Kit[1] and use appropriate laboratory and personal protective equipment when handling this kit, and use this product in accordance with the authorized labeling.

G. Mammoth Biosciences, Inc. 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.



## Quality Control

Each reagent kit contains sufficient material to perform 8 SARS-CoV-2 Positive Control reactions for SARS-CoV-2 N gene and 8 SARS-CoV-2 Negative Control reactions for SARS-CoV-2 N gene. Each microplate must have a positive control and negative control with the positive control in well A1 and negative control in well B1. The maximum fluorescence signals from the N gene reaction for the positive control and negative control are used to determine positivity and to determine if the samples assayed in the microplate can be considered valid.

Each specimen sample is assayed in parallel for the presence of RNase P as an internal control on a separate fluorescent channel.

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.

# Instructions for Use/Test Procedure

## Reagent Preparation for Assay Procedure, Automated

Once opened or mixed and between uses, all reagents should be stored covered to minimize evaporation. Do not re-freeze reagents.

Remove and thaw at room temperature all reagents from Kit C which are stored at -20 °C to 0 °C.

The RT-LAMP Activator, RT-LAMP Master Mix, DETECTR™ Master Mix, SARS-CoV-2 Positive Control and SARS-CoV-2 Negative Control should be stored covered at 2 to 8 °C or on ice to minimize evaporation between uses.

The reagent volumes provided in each kit, with the exception of RT-LAMP Master Mix, includes at least 20 mL of extra volume to compensate for unusable volume required when reservoirs are used on laboratory fluid handling platforms.



Lysis Buffer Preparation

1. Completely thaw the Carrier RNA reagent.
2. Invert the Carrier RNA reagent tube 10 times to mix.
3. Label a reagent reservoir with "Lysis Buffer" and the date and time of preparation.
4. Combine the entire contents of the Carrier RNA reagent tube into the Lysis/Binding Solution reagent bottle. Cap and vortex the Lysis/Binding Solution reagent bottle for 15 to 20 seconds to mix the Carrier RNA reagent into the Lysis/Binding Solution reagent.
5. Transfer the entire contents of the prepared Lysis Buffer reagent from the Lysis/Binding Solution reagent bottle into the labeled reagent reservoir.
6. The Lysis Buffer reagent should be stored covered at room temperature to minimize evaporation when not used.

Bead Solution Preparation

1. Completely thaw the Lysis/Binding Enhancer reagent.
2. Label a reagent reservoir with "Bead Solution" and the date and time of preparation.
3. Vortex the Lysis/Binding Enhancer reagent bottle for 10 to 15 seconds to mix.
4. Vortex the RNA Binding Beads bottle for at least 30 seconds. Check the beads for full resuspension into the liquid. If not resuspended, continue Vortex mixing until fully resuspended. The bottle may need to be applied to the Vortex mixer on its side or upside down to resuspend the beads.
5. Immediately after vortex mixing, pour the beads into the Lysis/Binding Enhancer bottle. Use a pipette to transfer any residual bead solution so that the entire contents of the RNA Binding Beads bottle is added to the Lysis/Binding Enhancer reagent bottle. Cap and invert the Lysis/Binding Enhancer reagent bottle with the RNA Binding Beads 10 times to mix. Transfer the entire mixed contents into the labeled reagent reservoir.

6. The Bead Solution should be stored covered at 2 to 8 °C to minimize evaporation when not used.

Wash Solution Preparation

1. Label a reagent reservoir with "Wash Solution" and the date and time of preparation.
2. Invert the Wash Solution 1 reagent bottle 10 times to mix.
3. Transfer the entire contents of the Wash Solution 1 reagent bottle into the labeled reagent reservoir.
4. The Wash Solution should be stored covered at room temperature to minimize evaporation when not used.

RT-LAMP Activator Preparation

1. Completely thaw the RT-LAMP Activator reagent.
2. Label a reagent reservoir with "RT-LAMP Activator" and the date and time of preparation.
3. Invert the RT-LAMP Activator reagent bottle 10 times to mix.
4. Transfer the entire contents of the RT-LAMP Activator reagent bottle into the labeled reagent reservoir.
5. The RT-LAMP Activator should be stored covered at 2 to 8 °C or on ice to minimize evaporation between uses.

Elution Buffer Preparation

1. Label a reagent reservoir with "Elution Buffer" and the date and time of preparation.
2. Invert the Elution Buffer reagent bottle 10 times to mix.
3. Transfer the entire contents of the Elution Buffer reagent bottle into the labeled reagent reservoir.
4. The Elution Buffer should be stored covered at room temperature to minimize evaporation when not used.



Mineral Oil

1. Label a reagent reservoir with "Mineral Oil" and the date and time of preparation.
2. Transfer 30 mL of mineral oil into the labeled reagent reservoir.
3. The mineral oil can be stored covered at room temperature to protect against contamination when not used.

DETECTR™ Master Mix Preparation

1. Completely thaw the DETECTR™ Master Mix reagent.
2. Label a reagent reservoir with "DETECTR™ Master Mix" and the date and time of

preparation

3. Invert the DETECTR™ Master Mix reagent bottle 10 times to mix.
4. Transfer the entire contents of the DETECTR™ Master Mix reagent bottle into the labeled reagent reservoir.
5. The DETECTR™ Master Mix should be stored covered at 2 to 8 °C or on ice to minimize evaporation between uses.

RT-LAMP Master Mix Preparation

1. The RT-LAMP Master Mix is provided in a 96 well plate with enough reagent in each well to process 8 tests. RT-LAMP Master Mix will be used directly from the 96 well plate.
2. Label the plate with the date and time removed from -20 °C storage.
3. Completely thaw the RT-LAMP Master Mix reagent plate's liquid.
4. Spin the RT-LAMP Master Mix plate on a centrifuge to move all the liquid to the well bottom.
5. Carefully remove the plate's sealing film.
6. Cover the plate with a lid, Agilent p/n 200856-100, to prevent liquid evaporation. The instrument will automatically remove and replace the plate's lid.
7. The RT-LAMP Master Mix should be stored covered at 2 to 8 °C or on ice to minimize evaporation between uses.

Isopropanol Preparation

1. Label a reagent reservoir with "Isopropanol" and the date and time of preparation.
2. Transfer 200 mL of Isopropanol into the labeled reagent reservoir.
3. The Isopropanol can be stored covered at room temperature to protect against evaporation when not used.



## Assay Procedure, Automated, Agilent BRAVO

The DETECTR BOOST™ SARS-CoV-2 Reagent Kit is run on the Agilent BRAVO BenchCel DB liquid handling platform as shown in Figure 5 by following the workflow as described in Figure 6.

## General Handling

- Regularly clean and DNA and RNase decontaminate all work surfaces, pipets, centrifuges, and other equipment prior to use. Viable decontamination agents include 10% dilution of sodium hypochlorite and commercially available reagents such as DNAzapTM or RNase AWAY®.
- Always wear powder-free latex, vinyl, or nitrile gloves while handling reagents, tubes and RNA samples.

- Change gloves frequently and keep reagents covered when not in use.
- During the procedure, avoid delays and keep cold storage reagents at 2 °C to 8 °C or on ice when possible to avoid degradation and evaporation of working reagents.

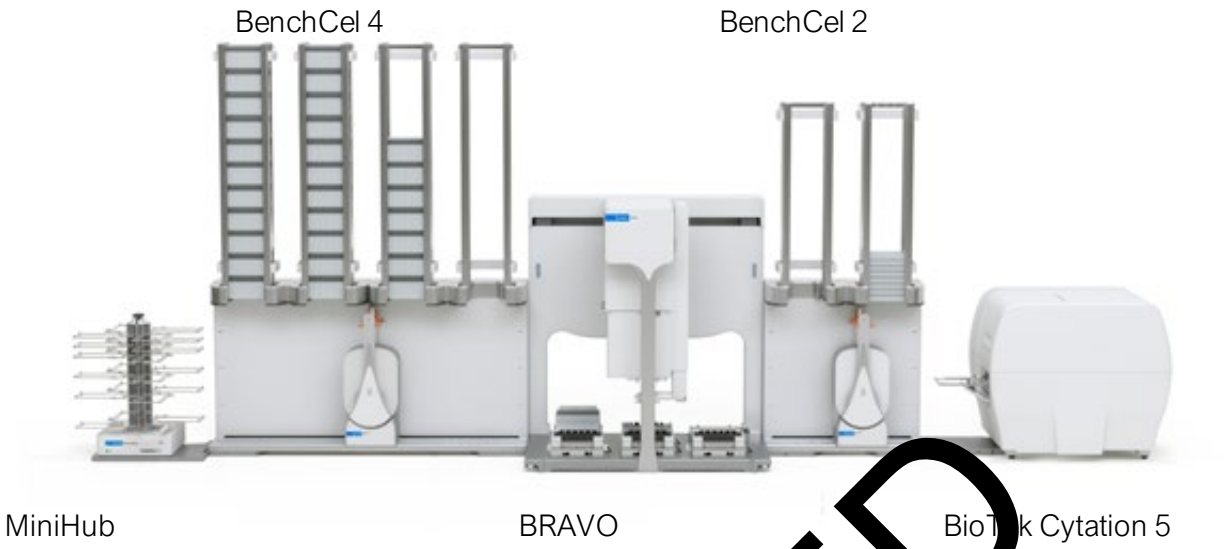



*Figure 5: Agilent BRAVO BenchCel DB liquid handling platform with BioTek Cytation 5 multi-mode plate reader*



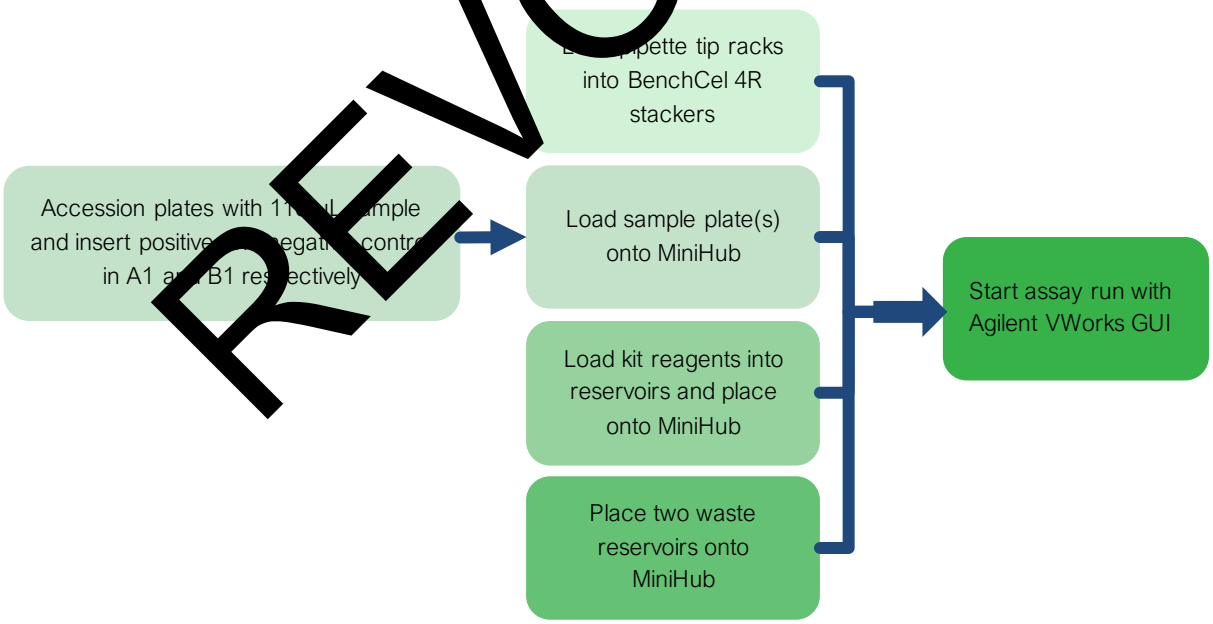



*Figure 6: Workflow to execute the DETECTR BOOST™ SARS-CoV-2 Reagent Kit using the Agilent BRAVO BenchCel DB liquid handling platform*

## Step 1. Load Pipette Tip Racks

1.a. Load 7 tip boxes for each sample plate to be processed. Fill the right most stacker (stacker 4) first. Stacker 4 can hold a maximum of 11 tip boxes. Stackers 1, 2 and 3 can hold 15 tip boxes. See Figure 7.

1.b. Ensure that the BenchCel2's moveable stacker rails are closed. The BenchCel2 stackers will be empty before the assay run.

NOTE:

- The BenchCel4 does not have moveable rails.
- The tip box label must face backwards if using Thomas Scientific tips from GEB (p/n 20A00M139).
- 56 tip boxes will be used if 8 sample plates are processed in a run.




*Figure 7: BenchCel4 Stackers use pipette tips starting from right to left. Stacker 4, which holds a maximum of 11 boxes, is used first.*

## Step 2. Sample Plate Accession

2.a. Insert 110 µL of SARS-CoV-2 Positive Control in well A1 of the 96 well microplate.

2.b. Insert 110 µL of SARS-CoV-2 Negative Control in well B1 of the 96 well microplate.

2.c. Insert 110 µL of sample to be tested into each of the remaining 94 wells of the microplate.

NOTE:

- It is not required to load a sample into every available well of the microplate.
- The assay workflow can be run with as few as 1 sample plate and up to 8 sample plates.

## Step 3. Load MiniHub

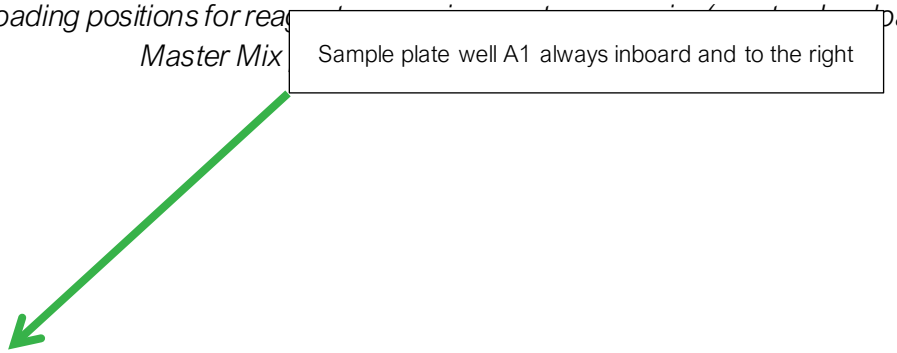
3.a. The MiniHub contains 24 positions for reservoirs or sample plates in a 4-cassette carousel with 6 slots in each cassette. The reagent reservoirs, waste reservoirs and sample plates are loaded into the MiniHub in the positions identified in Figure 8 and shown in Figure 9.

3.b. The sample plate well A1 must be positioned to the right side, inboard corner of the carousel. Use the lowest number sample plate position available when loading a sample plate. In other words, use sample plate 1 position first, then 2, then 3 and so on. The MiniHub can be loaded with as few as 1 sample plate and as many as 8 sample plates. It is recommended that the sample plates have a barcode that can be read by the platform to facilitate sample to result data tracking from the Cycler on 5.

3.c. Two empty reservoirs collect liquid lysate and wash waste.

| | Cassette 1 | Cassette 2 | Cassette 3 | Cassette 4 |
|---|---|---|---|---|
| Slot 6 | EMPTY | EMPTY | EMPTY | EMPTY |
| Slot 5 | Sample Plate 5 | Sample Plate 6 | Sample Plate 7 | Sample Plate 8 |
| Slot 4 | Sample Plate 1 | Sample Plate 2 | Sample Plate 3 | Sample Plate 4 |
| Slot 3 | Isopropanol | Wash Solution | RT-LAMP Master Mix | EMPTY |
| Slot 2 | Lysis Buffer | Waste | RT-LAMP Activator | DETECTR™ Master Mix |
| Slot 1 | Bead Solution | Waste | Elution Buffer | Mineral Oil |

*Figure 8: MiniHub loading positions for reagent reservoirs, waste reservoirs, sample plates (empty and loaded), Master Mix*



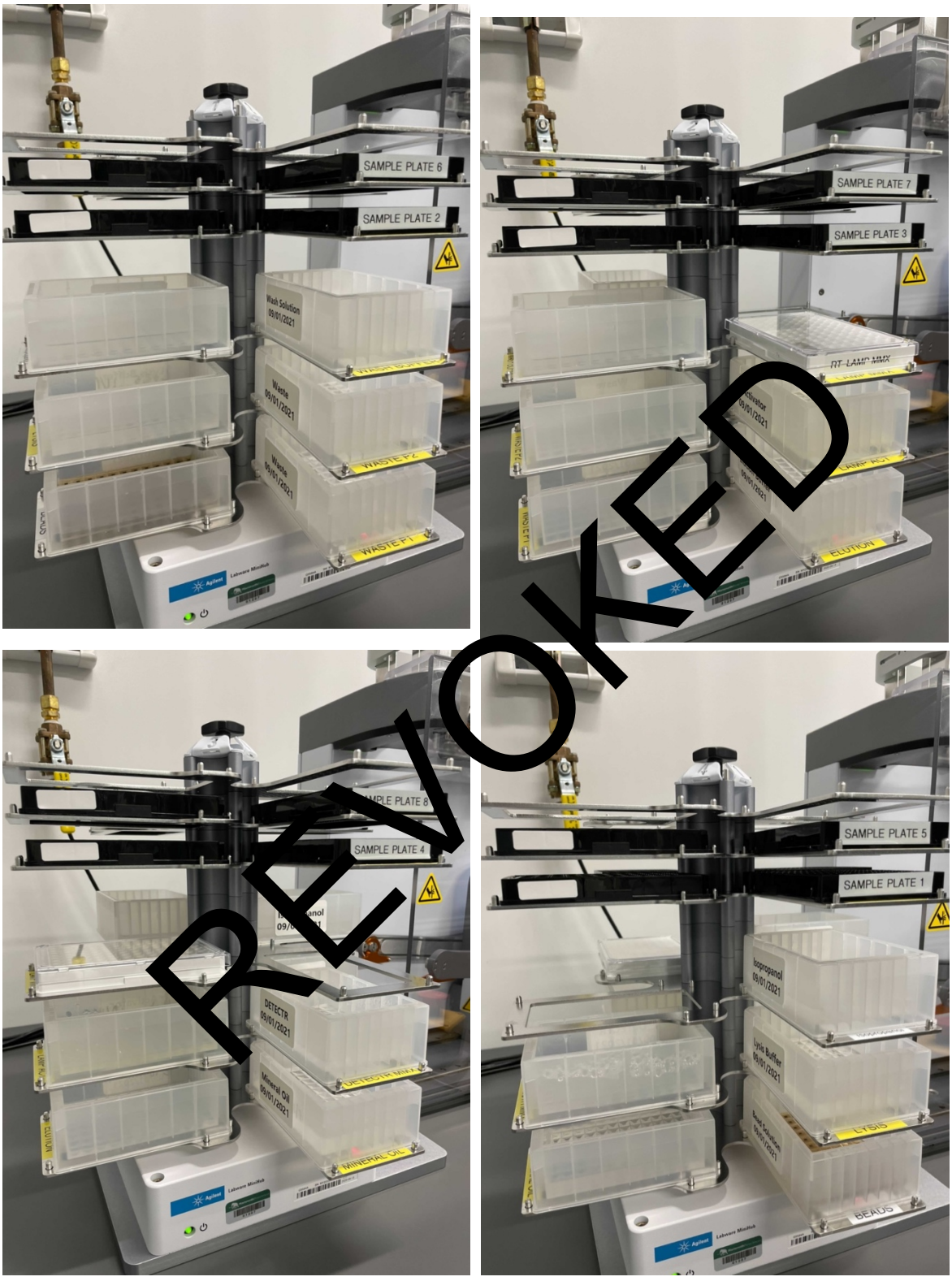



*Figure 9: MiniHub Cassettes 1 to 4 loaded with sample plates, reagent reservoirs and waste reservoirs*

Step 4. Start BRAVO BenchCel DB Workstation

4.a. Open the DETECTR VWorks GUI.

4.b. Select Initialize Devices.

4.c. Input the number of sample plates to be processed from 1 to 8.

4.d. Select the tip type: 96 GEB 250uL Tips.

4.e. Selecting Start Protocol will start the automated workflow.

## Step 5. Results

5.a. After a sample plate completes scanning in the plate reader it will be placed into the BenchCel2.

5.b. Using Biotek's Gen5 application, open the data file output folder within the path on the BRAVO's computer: C://VWorks Workplace/Mammoth/Gen5 Gen5 Output

5.c. Select the output file to be analyzed.

5.d. Gen5 will automatically analyze the results. If the signals from the control wells (A1 and B1) are invalid then the results for the entire plate will be invalid.

5.e. A report for each plate can be generated by navigating within the Gen5 software to Plate -> Export. The report will be saved at location C: -> Documents -> upload-test.

## Step 6. Clean up

6.a. The used pipette tip boxes will be placed in the BenchCel2 (first 11 tip boxes) and the BenchCel4. Remove used tip boxes from the BenchCel stackers and discard.

6.b. The processed sample plates will be placed into the BenchCel2. Remove the processed sample plates from the BenchCel2 for discard. There may be viable amplicon in the plate. It is recommended to seal the plate with a film such as VWR Part No. 60941-078 or equivalent before discarding. It is not recommended that the processed sample be retained as the DETECTR™ reagent may cleave the amplicon such that repeat amplification is unreliable.

6.c. The reagent reservoirs, filled waste reservoirs, and the RT-LAMP Master Mix plate will be on the Mini Hub at the loaded positions. If the reagents are not fully consumed, then load additional sample plate(s) until a maximum of 8 sample plates.

NOTE: If there will be a one-hour time gap before using the reagents again then it is suggested to cover the reagents to minimize evaporation.

6.d. The RT-LAMP Activator, RT-LAMP Master Mix, DETECTR™ Master Mix, SARS-CoV-2 Positive Control and SARS-CoV-2 Negative Control can be stored at 2 to 8 °C or on ice to minimize evaporation between uses. These reagents can be covered in between uses.

# Results Interpretation

The Biotek reader will automatically label a patient specimen result as positive or negative or invalid using the rules described below and in Table 6. The controls used in the DETECTR BOOST™ SARS-CoV-2 Reagent Kit are:

SARS-CoV-2 Positive Control: A positive SARS-CoV-2 N gene template control goes through the entire assay process and is tested in one well of each microplate of specimen samples. This positive control confirms reagent and instrument function.

SARS-CoV-2 Negative Control: A negative SARS-CoV-2 N gene template control goes through the entire assay process and is tested in one well of each microplate of specimen samples.

Internal Control: An RNase P target (RP) is assayed with every specimen sample. Each plate well containing a specimen sample is assayed on different optical channels for both SARS-CoV-2 N gene and RP. The RP controls for the presence of human material from the swab and that inhibition of the assay process due to a substance in the specimen has not occurred.

The positive and negative controls are examined prior to interpretation of patient specimen results. If the positive and negative controls are not valid, the patient specimen results cannot be interpreted.

## Positive and Negative Control Interpretation

The maximum fluorescence signals from the positive control and negative control are used to determine positivity and to determine if the samples in the microplate can be considered valid.

Validity Equation:

*Negative Control N Gene Signal < 0.4 × ((Positive Control N Gene Signal – Negative Control N Gene Signal) × 0.33)*

Failure of the Validity Equation requires all samples in that plate to be invalidated and be repeat tested. The Biotek reader will automatically label all results as invalid for the specimen samples in that plate if the validity equation fails.

## Examination and Interpretation of Patient Specimen Results

Assessment of patient specimen test results in the microplate are performed after the positive and negative controls in that microplate have been examined and determined to be valid.

A positive result for SARS-CoV-2 occurs when the maximum fluorescence N signal from a well with a sample to be tested is greater than the cut-point as defined in Table 6 or the slope of the fluorescence N signal is greater than 10.

A negative result for SARS-CoV-2 occurs when the maximum fluorescence signal from a well with a sample to be tested is less than the cut-point or the slope of the fluorescence N signal is 10 or less and RP signal is detected above the cut-point as defined in Table 6.

*Table 6: Criteria for determining assay results*

| Plate Positive and Negative Control Interpretation | Signals from Specimen Samples (N gene) | Signals from Sample Internal Control (RP) | Specimen Sample Interpretation | Action |
|---|---|---|---|---|
| Valid | Max fluorescence N gene signal > cut-point* or N gene signal slope > 10 | RP detected** | Positive | Report as SARS-CoV-2 Detected |
| | Max fluorescence N signal > cut-point* or N signal slope > 10 | RP not detected** | | |
| | Max florescence N gene signal ≤ cut-point* and N gene signal slope ≤ 10 | RP detected** | Negati | Report as SARS-CoV-2 Not Detected |
| | Max florescence N gene signal ≤ cut-point* and N gene signal slope ≤ 10 | RP not detected** | Invalid | Repeat test patient specimen sample |
| Invalid | N/A | N/A | Invalid | Repeat testing of positive and negative controls and patient specimen samples for that plate |

*Cut-point = (Positive Control Signal –Negative Control Signal) × 2 for Biotek Cytation 5

**RNaseP (RP) detected when RP signal > SARS-CoV-2 Positive Control RP signal + 3000 RFU.



## Retesting

If the positive and negative controls are not valid for a microplate, then the testing of controls and specimens must be repeated.

If a patient specimen sample interpretation is invalid then the testing of that specimen must be repeated.

## Test Result Reporting

The DETECTR BOOST™ patient specimen test results must be reported to healthcare providers and relevant public health authorities in accordance with local, state, and federal requirements, using appropriate LOINC and SNOMED codes, as defined by the Laboratory In Vitro Diagnostics (LIVD) Test Code Mapping for SARS-CoV-2 Tests provided by the Centers for Disease Control and Prevention (CDC). The Instructions For Use (IFU) instructs users to collect the core diagnostic data elements as defined by the Department of Health and Human Services (HHS), along with technical specifications for implementation for lab-based tests.

# Maintenance

All of the equipment used by the lab with the DETECTR BOOST™ SARS-CoV-2 Reagent Kit should be maintained according to manufacturer's instructions and the lab's standard operating procedures.

# Procedural Notes

## Amplicon Contamination Control Recommendations

It is recommended to decontaminate surfaces and equipment daily with 2% (v/v) bleach or another chemical validated to eradicate DNA amplicons.

# Performance Characteristics

## Analytical Sensitivity (LoD)

To determine the limit of detection (LoD), a heat inactivated sample of SARS-CoV-2 from strain 2019-nCoV/USA-WA1/2020 procured from ATCC, catalogue number, VR-1986HK, was obtained and quantified using digital droplet PCR. Dilutions of the heat inactivated SARS-CoV-2 virus were made in saline and a human matrix from a confirmed SARS-CoV-2 negative nasopharyngeal swab.

The preliminary LoD was assessed with 3 replicates using the Agilent BRAVO based liquid handling platform.

*Table 7: Preliminary LoD*

| Virus Copies/mL | Replicates Tested | Positive Results |
|---|---|---|
| 2500 | 3 | 3 |
| 1000 | 3 | 3 |
| 600 | 3 | 3 |
| 400 | 3 | 3 |
| 300 | 3 | 3 |
| 150 | 3 | 3 |
| 100 | 3 | 2 |
| 50 | 3 | 2 |
| 0 | 3 | 0 |

The LoD was confirmed with 20 replicates at the expected LoD.

*Table 8: Confirmed LoD*

| Virus Copies/mL | Replicates Tested | Positive Results |
|---|---|---|
| 300 | 20 | 20 |
| 150 | 20 | 20 |
| 100 | 20 | 20 |
| 50 | 20 | 12 |

The LoD in nasopharyngeal matrix is 100 copies/mL with the automated Agilent BRAVO based liquid handling platform.

## Inclusivity

An *in silico* inclusivity analysis was performed on January 06, 2022 to establish the inclusivity of the DETECTR BOOST™ SARS-CoV-2 Reagent Kit on available SARS-CoV-2 sequences using the ROSALIND platform to interrogate the GISAID database. The ROSALIND bioinformatics platform evaluates the sequence position and frequency in which mismatches occur, including single nucleotide variant (SNV) mismatches, in recently sequenced genomes to assign a severity score from 0-5, with 5 being the most severe. As of January 06, 2022, the GISAID database contained a total of 6,199,850 high-quality and full-length sequences. Of the analyzed genomes, ROSALIND reported a total of thirty-three (33) incidents within the last 90 days with various degrees of predicted severity by ROSALIND based on predicted change in theoretical hybridization temperature.

- Thirty (30) of these incidents were the result of a single SNV within one of the six primer regions (F3, B3, LF, LB, FIP, or BIP)
- Three (3) of these incidents had a SNV within the gRNA region

Of these thirty-three incidents, only one (1) had a Rosalind severity score of four or five. A summary of the results of this analysis for each assay component is presented in Table 9.

*Table 9: Summary of mutations that overlap assay components. Results are broken down by number of mismatches (mm) to each assay component.*

| | Component | Incidents identified by ROSALIND | Incidents with ROSALIND Severity Score of 4 or 5 | Incidents with 2 mm | Incidents with 3' end mm |
|---|---|---|---|---|---|
| LAMP primers | F3 | 3 | 0 | 0 | 0 |
| | B3 | 0 | 0 | 0 | 0 |
| | FIP | 7 | 0 | 0 | 0 |
| | BIP | 6 | 0 | 0 | 0 |
| | LF | 2 | 0 | 0 | 0 |
| | LB | 6 | 0 | 0 | 0 |
| CRISPR | gRNA | 3 | 1 | 0 | 0 |

One SNV in a primer region is unlikely to affect the sensitivity of the assay unless it is at the 3' end of the primer sequence. No incidents were reported with a 3' end mismatch in one of the LAMP primers.

A sequence containing a SNV in the gRNA region may also affect sensitivity. One (1) incident with a severity score of four (4) was reported with a SNV in the gRNA targeting region. The corresponding mutation of this incident was present in 0.0155% of all GISAID sequences within the last ninety (90) days.

No incidents were reported with two SNVs within a primer sequence or the gRNA sequence.

Risk assessment of variants of interest (Alpha, Beta, Gamma, Delta, Omicron):

To understand the impact of the new SARS-CoV-2 variants that have arisen around the world, we performed an analysis of the ROSALIND incidents reported for Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529, BA.1, or BA.2) lineages which is summarized in Table 10, 11, 12, 13 and 14, respectively. Our analysis of the SARS-CoV-2 variants shows that observed mismatches are unlikely to impact the performance of the DETECTR BOOST™ SARS-CoV-2 Reagent Kit.



*Table 10: Summary of mutations in Alpha (B.1.1.7) strains from GISAID, 90 days prior to analysis, that overlap assay components*

| | Component | Incidents identified by ROSALIND | Incidents with ROSALIND Severity Score of 4 or 5 | Incidents with 2 mm | Incidents with 3' end mm |
|---|---|---|---|---|---|
| LAMP primers | F3 | 3 | 0 | 0 | 0 |
| | B3 | 6 | 0 | 0 | 0 |
| | FIP | 7 | 0 | 0 | 0 |
| | BIP | 6 | 0 | 0 | 0 |
| | LF | | 0 | 0 | 0 |
| | LB | 6 | 0 | 0 | 0 |
| CRISPR | gRNA | | 1 | 0 | 0 |

*Table 11: Summary of mutations in Beta (B.1.351) strains from GISAID, last 90 days prior to analysis, that overlap assay components.*

| | Component | Incidents identified by ROSALIND | Incidents with ROSALIND Severity Score of 4 or 5 | Incidents with 2 mm | Incidents with 3' end mm |
|---|---|---|---|---|---|
| LAMP primers | F3 | 3 | 0 | 0 | 0 |
| | B3 | 5 | 0 | 0 | 0 |
| | FIP | 7 | 0 | 0 | 0 |
| | BIP | 5 | 0 | 0 | 0 |
| | LF | 2 | 0 | 0 | 0 |
| | LB | 6 | 0 | 0 | 0 |
| CRISPR | gRNA | 3 | 1 | 0 | 0 |

*Table 12: Summary of mutations in Gamma (P.1) strains from GISAID, last 90 days prior to analysis, that overlap assay components.*

| | Component | Incidents identified by ROSALIND | Incidents with ROSALIND Severity Score of 4 or 5 | Incidents with 2 mm | Incidents with 3' end mm |
|---|---|---|---|---|---|
| LAMP primers | F3 | 3 | 0 | 0 | 0 |
| | B3 | 5 | 0 | 0 | 0 |
| | FIP | 7 | 0 | 0 | 0 |
| | BIP | 5 | 0 | 0 | 0 |
| | LF | 2 | 0 | 0 | 0 |
| | LB | 6 | 0 | 0 | 0 |
| CRISPR | gRNA | 3 | 1 | 0 | 0 |

*Table 13: Summary of mutations in Delta (B.1.617.2) strains from GISAID, last 90 days prior to analysis, that overlap assay components*

| | Component | Incidents identified by ROSALIND | Incidents with ROSALIND Severity Score of 4 or 5 | Incidents with 2 mm | Incidents with 3' end mm |
|---|---|---|---|---|---|
| LAMP primers | F3 | 3 | 0 | 0 | 0 |
| | B3 | 5 | 0 | 0 | 0 |
| | FIP | 7 | 0 | 0 | 0 |
| | BIP | 6 | 0 | 0 | 0 |
| | LF | 2 | 0 | 0 | 0 |
| | LB | 6 | 0 | 0 | 0 |
| CRISPR | gRNA | 3 | 1 | 0 | 0 |

*Table 14: Summary of mutations in Omicron (B.1.1.529, BA.1, & BA.2) strains from GISAID, last 90 days prior to analysis, that overlap assay components*

| | Component | Incidents identified by ROSALIND | Incidents with ROSALIND Severity Score of 4 or 5 | Incidents with 2 mm | Incidents with 3' end mm |
|---|---|---|---|---|---|
| LAMP primers | F3 | 3 | 0 | 0 | 0 |
| | B3 | 3 | 0 | 0 | 0 |
| | FIP | 6 | 0 | 0 | 0 |
| | BIP | 3 | 0 | 0 | 0 |
| | LF | 2 | 0 | 0 | 0 |
| | LB | 5 | 0 | 0 | 0 |
| CRISPR | gRNA | 3 | 1 | 0 | 0 |



## Analytical Specificity

Cross reactivity was validated by:

***In silico* Analysis:** *In silico* BLASTn analysis queries of the DETECTR BOOST™ SARS-CoV-2 Reagent Kit's RT-LAMP N-gene primers and DETECTR™ gRNAs against public domain nucleotide sequences in NCBI (National Center for Biotechnology Information) nucleotide collection (nt) using default parameters for the viruses and bacteria listed in Table 15.

*Table 15: List of Organisms to be tested and analyzed for cross-reactivity*

| Other high priority pathogens from the same genetic family | High priority organisms likely present in a respiratory specimen |
|---|---|
| Human coronavirus 229E | Adenovirus (Type 3, Type 4, Type 7a) |
| Human coronavirus OC43 | Human Metapneumovirus (hMPV) |
| Human coronavirus HKU1 | Parainfluenza virus 1-4 |
| Human coronavirus NL63 | Influenza A & B |
| SARS-coronavirus | Enterovirus (Type 68, 2007 Isolate) |
| MERS-coronavirus | Respiratory syncytial virus |
| | Rhinovirus |
| | *Chlamydophila pneumoniae* |
| | *Haemophilus influenzae* |
| | *Legionella pneumophila* |
| | *Mycobacterium tuberculosis* |
| | *Streptococcus pneumoniae* |
| | *Streptococcus pyogenes* |
| | *Bordetella pertussis* |
| | *Mycoplasma pneumoniae* |
| | *Pneumocystis jirovecii* |
| | *Candida albicans* |
| | *Pseudomonas aeruginosa* |
| | *Staphylococcus epidermis* |
| | *Streptococcus salivarius* |



N gene RT-LAMP primers and DETECTR™ gRNA:

- RT-LAMP 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.
- RT-LAMP B3 → 81.8% identity to a sequence in the *Homo sapiens* genome.
- RT-LAMP FIP (F2-F1c) → 100% homology to SARS-CoV. No significant homology to other organisms of interest.
- RT-LAMP BIP (B2-B1c) → 100% homology for the B1c portion of the BIP primer to SARS-CoV. No significant homology to other organisms of interest.
- RT-LAMP LF → homology to SARS-CoV (94%), *Chlamydia pneumoniae* (84%), *Streptococcus pyogenes* (84%), and *Homo sapiens* genome (89%).
- RT-LAMP LB →No significant homology to other organisms of interest.
- N-gene DETECTR™ gRNA → 80% homology to a sequence in the *Homo sapiens* genome however the required proto-spacer adjacent motif (PAM) to the target

sequence is not present. The Cas12 enzyme will not activate due to the lack of PAM adjacent to the target sequence.

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 with its gRNA. With respect to SARS-CoV, 3 N gene primers (BIP, FIP, LF) have high homology (94% - 100%) to SARS coronavirus, cross-reactivity would not be expected given the lack of sequence homology in the other 3 primers and the N-gene gRNA.

**Wet-Testing:** The organisms analyzed *in silico* were also tested with the DETECTR BOOST™ SARS-CoV-2 Reagent Kit at concentrations as specified in Tables 16 and 17. All of the organisms were added to a SARS-CoV-2 negative human nasopharyngeal matrix in saline.



*Table 16: Viral Culture in Nasopharyngeal Matrix in Saline*

| Virus | Concentration ($TCID_{50}$/mL) | Samples Detected /Samples Tested |
|---|---|---|
| Adenovirus Type 3 | $1.9x10^6$ | 0/3 |
| Adenovirus Type 4 | $2.09x10^5$ | 0/3 |
| Adenovirus Type 7 | $5.75x10^6$ | 0/3 |
| Human Coronavirus HKU1 | Ct range 25-28 | 0/4* |
| Human Coronavirus 229E | $6.3 x10^5$ | 0/3 |
| Human Coronavirus NL63 | $8.5x10^4$ | 0/3 |
| Human Coronavirus OC43 | $1.9 x10^6$ | 0/5** |
| Enterovirus Type 68 (2007 Isolate) | $7.55x10^5$ | 0/3 |
| Human Metapneumovirus (hMPV) 16 Type A1 | $6.3x10^5$ | 0/3 |
| Human Metapneumovirus (hMPV) 27 Type A2 | $1.78x10^5$ | 0/3 |
| Human Metapneumovirus (hMPV) 3 Type B1 | $7.75x10^3$ | 0/3 |
| Human Metapneumovirus (hMPV) 4 Type B2 | $5.25x10^5$ | 0/3 |
| Influenza A H1N1 (New Cal/20/99) | $7.55x10^5$ | 0/3 |
| Influenza B (Florida/02/06) | $7.05x10^4$ | 0/3 |
| MERS-CoV (Strain: Florida/USA-2_Saudi Arabia_2014) | $1.78x10^5$ | 0/3 |
| Parainfluenza Virus Type 1 | $5.25x10^5$ | 0/3 |
| Parainfluenza Virus Type 2 | $2.51x10^5$ | 0/3 |
| Parainfluenza Virus Type 3 | $1.41x10^7$ | 0/3 |

| | | |
|---|---|---|
| Parainfluenza Virus Type 4A | $2.81x10^4$ | 0/3 |
| Parainfluenza Virus Type 4B | $2.51x10^5$ | 0/3 |
| Respiratory Syncytial Virus Type A (RSV-A) | $1.78x10^5$ | 0/3 |
| Respiratory Syncytial Virus Type B (RSV-B) | $2.29x10^6$ | 0/3 |
| Rhinovirus Type 1A | $1.78x10^5$ | 0/3 |
| SARS CoV (NATtrolTM Coronavirus SARS Stock) | Ct 27.62 | 0/3 |

*Human Coronavirus HKU stock was depleted after 1 test. Additional stock was ordered and tested 3 additional times for 4 total replicates.

**Human Coronavirus OC43 testing had 1 invalid result in the first 3 replicates. Three additional replicates were tested with no invalids for 5 total replicates.

*Table 17: Bacterial and Fungi Culture in Nasopharyngeal Matrix in Saline*

| Organism | Final concentration | Samples Detected /Samples Tested |
|---|---|---|
| *Bordetella pertussis* | 1.51 X 10[illegible] CFU/mL | 0/3 |
| *Candida albicans* | 1.06 X 10[illegible] CFU/mL | 0/3 |
| *Chlamydophila pneumoniae* | ≥$10^3$ TCID50/mL | 0/3 |
| *Haemophilus influenzae* type b | 1.28 X $10^7$ CFU/mL | 0/3 |
| *Haemophilus parainfluenzae* | 3.29 X $10^6$ CFU/mL | 0/3 |
| *Legionella pneumophila* | 3.34 X $10^8$ CFU/mL | 0/3 |
| *Mycobacterium tuberculosis* | 1.47 X $10^6$ CFU/mL | 0/3 |
| *Mycoplasma pneumoniae* | 7.44 X $10^6$ CFU/mL | 0/3 |
| *Pneumocystis jirovecii* [b] | 1.49 X $10^7$ CFU/mL | 0/3 |
| *Pseudomonas aeruginosa* [c] | 2.02 X $10^8$ CFU/mL | 0/3 |
| *Staphylococcus epidermidis* | 2.18 X $10^8$ CFU/mL | 0/3 |
| *Streptococcus pneumoniae* | 9.79 X $10^6$ CFU/mL | 0/3 |
| *Streptococcus pyogenes* | 6.26 X $10^7$ CFU/mL | 0/3 |
| *Streptococcus salivarius* | 9.86 X $10^6$ CFU/mL | 0/3 |



a. Reported as ≥$10^3$ TCID50 on the certificate of analysis from the vendor.
b. *Pneumocystis jirovecii* was tested using a recombinant fungus.
c. *Pseudomonas aeruginosa* was tested twice, the first time two invalid results were observed suggesting organism related inhibition of RNase P control amplification. The second time the bacteria was diluted 1:10 and the results are reported here.

## Interfering Substances

A panel of potential endogenous and exogenous interferents were tested by spiking at the specified concentrations listed Table 18 into SARS-CoV-2 negative nasopharyngeal matrix in saline and then the heat inactivated SARS-CoV-2 was added at 300 copies/mL (3x LoD) and tested with the DETECTR BOOST™ SARS-CoV-2 Reagent Kit .

*Table 18: Potential Interfering Substances in Nasopharyngeal Matrix*

| Interfering Substances for Nasopharyngeal Swab samples | Test Concentration | Samples Detected / Samples Tested Positive SARS-CoV-2 RNA present at 3x LoD |
|---|---|---|
| Mucin | 0.5% (w/v) | [illegible] |
| Whole Blood | 1% (v/v) | 3/3 |
| NeoSynephrine Cold and Sinus Extra Strength Spray | 20% (v/v) | [illegible] |
| Afrin | 20% (v/v) | 3/3 |
| Zicam Allergy Relief | 20% (v/v) | 3/3 |
| Flonase (Fluticasone) | 0.04 mg/m | 3/3 |
| Dexamethasone | 0.5 mg/mL | 3/3 |
| Mupirocin | 10 [illegible]/mL | 3/3 |
| Zanamivir (Relenza) | 0.3 mg/mL | 3/3 |
| Tamiflu (Oseltamivir phosphate) | 0.01 mg/mL | 3/3 |
| Tobramcyin | 2.5 mg/mL | 3/3 |



## Clinical Evaluation Study

A total of one hundred twenty six (126) nasopharyngeal (NP) swab specimens in saline were collected and tested retrospectively with the DETECTR BOOST™ SARS-CoV-2 Reagent Kit and a highly sensitive EUA authorized RT-PCR comparator assay. All NP swabs were placed immediately into 3mL of saline upon collection.

The 126 NP swab specimens were thawed and divided into aliquots. The aliquots were frozen at -80 °C and shipped on dry ice to two independent labs. One independent lab executed the EUA RT-PCR comparator method, and a second independent lab executed the test using the DETECTR BOOST™ SARS-CoV-2 Reagent Kit. Each aliquot was tested for the study the day it was thawed.

Results from 46 individuals who tested positive by the comparator assay and 80 individuals who tested negative by the comparator assay are provided in Table 19. The positive percent agreement (PPA) between the two assays was 95.7% (44/46) and the negative percent agreement (NPA) was 100% (80/80). The binomial proportion confidence interval provided in Table 19 was calculated using the Wilson score method.

*Table 19: Evaluation with NP Specimens*

| | | Comparator EUA RT-PCR Assay | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| DETECTR BOOST™ SARS-CoV-2 Reagent Kit | Positive | 44 | 0 | 44 |
| | Negative | 2 | 80 | 82 |
| | Total | 46 | 80 | 126 |
| Positive Percent Agreement (PPA) | | 95.7% (95% CI: 85.5%-98.8%) | | |
| Negative Percent Agreement (NPA) | | 100% (95% CI: 95.4%-100.0%) | | |



## Fresh versus Frozen Study on Contrived Specimens

A Fresh versus Frozen study was performed on contrived specimens to evaluate if freezing samples affects the accuracy of test results.

ATCC-VR-1986 HK inactivated SARS-CoV-2 was spiked into negative NP clinical matrix at 2x LoD concentration for 30 specimens and 5x LoD concentration for 10 specimens. Ten negative specimens contained only NP clinical matrix. Specimens were frozen at <-70°C and thawed twice and tested with the DETECTR BOOST SARS-CoV-2 Reagent Kit. These data demonstrate that frozen specimens should not be tested with the DETECTR BOOST™ SARS-CoV-2 Reagent Kit (Table 20).

*Table 20: Summary of Contrived Specimen Results after Two Freeze/Thaw Cycles*

| | | DETECTR BOOST Timepoint 0 # detected/# tested | DETECTR BOOST After 2 freeze/thaw cycles # detected/# tested |
|---|---|---|---|
| 5x LoD | 500 copies/mL | 10/10 | 2/10 |
| 2x LoD | 200 copies/mL | 30/30 | 3/30 |
| negative | 0 copies/mL | 0/10 | 0/10 |

## Contact Information, Ordering, Product and Technical Support

Information and product support can be obtained from:

Contact: Mammoth Biosciences, Inc. Customer Support

Email: support@mammoth.bio

Phone: +1-650-294-8583

### Product support information

- Technical support help
- Order and web support

### Product documentation

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

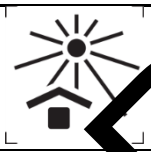
**Note:** For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.



## References


1. WHO Coronavirus (COVID-19) Dashboard: https://covid19.who.int/
2. CDC People with Certain Medical Conditions: https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/people-with-medical-conditions.html
3. CDC COVID Data Tracker: https://covid.cdc.gov/covid-data-tracker/#datatracker-home

## Symbols Used In Packaging

| Symbol | Definition |
|---|---|
|  | Catalog number |
|  | Batch Code |
|  | Use-by Date |
|  | GHS pictogram- Exclamation Mark |
|  | GHS pictogram- Corrosion |
|  | GHS pictogram- Health Hazard |
|  | For Prescription Use Only |
|  | In vitro diagnostic medical device |
|  | Temperature limitation |
|  | Consult instructions for use |
|  | Keep away from sunlight |
|  | Positive control |
|  | Negative control |