For use under an Emergency Use Authorization (EUA) Only

For *in vitro* diagnostic (IVD) use

Rx Only

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Introduction

This Emergency Use Authorization (EUA) package insert must be read carefully prior to use. EUA package insert instructions must be followed accordingly. Reliability of EUA assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Intended Use

Quick SARS-CoV-2 rRT-PCR Kit is a real-time RT-PCR test intended for the qualitative detection of nucleic acids from SARS-CoV-2 in upper respiratory specimens (such as nasal, nasopharyngeal, mid-turbinate or oropharyngeal swabs), and lower respiratory specimens (such as sputum, tracheal aspirates, and bronchoalveolar lavage) from patients 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, to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper and lower respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

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

The *Quick* SARS-CoV-2 rRT-PCR Kit is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time RT-PCR assays. The *Quick* SARS-CoV-2 rRT-PCR Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

Summary and Explanation of Test

Nucleic acid extraction and purification is performed using the *Quick*-DNA/RNA Viral MagBead extraction kit on the automated KingFisher™ Flex Purification System or with a manual procedure. The *Quick* SARS-CoV-2 rRT-PCR Kit is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test on the Bio-Rad CFX96 Touch Real-Time PCR Detection System using the Bio-Rad CFX Maestro™ 1.1 Version 4.1.2433.1219 software (or higher). The SARS-CoV-2 primer and probe sets are designed to detect RNA from the SARS-CoV-2 N gene in upper and lower respiratory samples from patients who are suspected of COVID-19 by their healthcare provider.

Principles of the Procedure

Nucleic acid from patient samples are extracted and purified as described in the procedural steps. Selective amplification of target nucleic acid from the sample is achieved by reverse transcription of the SARS-CoV-2 RNA as well as the host specific RNase P RNA and subsequent PCR amplification using the target-specific forward and reverse primers.

The *Quick* SARS-CoV-2 rRT-PCR Kit detects three SARS-CoV-2 specific target sequences (Target 1, Target 2, and Target 3) from the Nucleocapsid gene (N) of the virus and one host specific target sequence from the RNase P gene. The primers and probes used in the rRT-PCR assay are based on the CDC published primer and probe sequences with some modification. In addition to the RNase P gene, the test is using a positive control and a negative template control that are taken through all procedural steps, including the extraction, and that monitor integrity of reagents and correct performance of the testing procedure.

Reagents and materials

Materials Provided

Components manufactured and supplied with the ***Quick*** **SARS-CoV-2 rRT-PCR kit:**

Component Name	Description	Concentration	Volume	Quantity
CV Positive Control (R3011-3-100)	SARS-CoV-2 positive control	25 copies per reaction *in vitro* transcribed SARS-CoV-2 N gene fragment spiked into 1 ng/µl human cell RNA	100 µl	1
CV Mix 1 (R3011-1-1)	Cocktail for one-step rRT-PCR detection of SARS-CoV-2. Includes: reverse transcriptase, dNTPs, MgCl2, salts and additives, Taq polymerase, RNase inhibitors, and the SARS-CoV-2 primers and probes	2X	1 ml	1
CV Mix 2 (R3011-2-1)	Cocktail for one-step rRT-PCR detection of Human RNase P. Includes: reverse transcriptase, dNTPs, MgCl2, salts and additives, Taq polymerase, Rnase inhibitors, and the RNase P primers and probes	2X	1 ml	1
No Template Control (NTC) (R3011-4-1)	Nuclease-free water for the No Template Control	N/A	1 ml	3

Note - Integrity of kit components is guaranteed for up to the claimed expiration date on the kit under proper storage conditions. Reagents are routinely tested on a lot-to-lot basis to ensure they provide maximal performance and reliability.

Reagent Storage and Handling

The *Quick* SARS-CoV-2 rRT-PCR kit is to be shipped on dry ice.

If received in a condition other than label indicates, or that are damaged, contact Zymo Research Corp. directly.

Upon receipt all components of the kit should be stored at ≤-70 °C.

Reagents are stable for up to five (5) freeze-thaw cycles.

Reagents and Materials Required (NOT provided):

Product Name	Catalog No.	Manufacturer
DNA/RNA Shield™ Swab Collection Kit	R1124, R1107, R1109	Zymo Research
DNA/RNA Shield™ Saliva Collection Kit **For sputum collection**	R1210	Zymo Research
Quick-DNA/RNA Viral MagBead	R2140, R2141	Zymo Research
Proteinase K w/ Storage Buffer Set	D3001-2-20	Zymo Research
Beta-mercaptoethanol	Non-specific	Non-specific
95% or 190 Proof Ethanol	Non-specific	Non-specific
99.5% Isopropanol	Non-Specific	Non-Specific
Magnetic rack for 96-well PCR plate (Manual)	Non-specific	Non-specific
CFX96 Touch Real-Time PCR Detection System (with optics capable of detecting HEX and Quasar 670 fluorophores) with CFX	1855195	Bio-Rad Bio-Rad
KingFisher™ Flex Purification System	5400630-KF	ThermoFisher Scientific
1.5 ml microcentrifuge tubes (DNase/RNase-Free)	Non-specific	Non-specific
Microcentrifuge	Non-specific	Non-specific
Mini Plate Spinner	Non-specific	Non-specific
Thermal incubator	Non-specific	Non-specific
Hard Shell PCR Plate, 96-well, thin wall	HSP9601	Bio-Rad
Microseal 'B' seal	MSB1001	Bio-Rad
Aerosol barrier pipette tips (Nuclease-Free)	Non-specific	Non-specific
Micropipettes (2, 10, 200, 1000 µl)	Non-specific	Non-specific
Vortex mixer	Non-specific	Non-specific
Biological safety cabinet	Non-specific	Non-specific
Disposable gloves, powder-free	Non-specific	Non-specific
Non-frost-free freezer (-20°C)	Non-specific	Non-specific
BioShake	1808-0506-1021	Qinstruments

General Laboratory Warnings and Precautions

This kit is for use under Emergency Use Authorization Only.

This assay is for *in vitro* diagnostic use and is for prescription use only.

• Wear gloves when handling specimens or reagents.

• Do not pipette by mouth.

• Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where these materials are handled.

• Clean and disinfect spills of specimens by including the use of soap and water (i.e., 20% aqueous solution of Sodium Dodecyl Sulfate disinfectant (SDS))

• Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and federal regulations.

The following warning apply:


GHS07

Acute Tox. 4 H302 Harmful if swallowed.

Skin Irrit. 2 H315 Causes skin irritation.

Eye Irrit. 2B H320 Causes eye irritation.

Important information regarding the safe handling, transport, and disposal of this product is contained in the Safety Data Sheet. Safety Data Sheets are available from Zymo Research Corp. Inquire directly.

Special Precautions

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

Only personnel proficient in handling infectious materials and the use of the *Quick* SARS-CoV-2 rRT-PCR kit and the Bio-Rad CFX96 Touch Real-Time PCR Detection System together with the Bio-Rad CFX Maestro™ 1.1 Version 4.1.2433.1219 software should perform this procedure.

Handling Precautions for Specimens

All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.3

The *Quick* SARS-CoV-2 rRT-PCR kit is only for use with specimen that have been handled and stored as described in the 'Specimen Collection, Storage, and Transport to the Test Site' section.

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. Refer to CLSI MM13-A5 as an appropriate resource.

Compliance with good laboratory practices is essential to minimize the risk of cross-contamination between samples and the inadvertent introduction of RNases into samples.

Proper aseptic technique should always be used when working with RNA.

Amplification technologies such as PCR are sensitive to accidental introduction of product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the reagents used become contaminated by accidental introduction of even a few molecules of amplification product. Measures to reduce the risk of contamination in the laboratory include physically separating the activities involved in performing PCR in compliance with good laboratory practices.

Work Areas

The KingFisher™ Flex Purification System and the Bio-Rad CFX96 Touch Real-Time PCR Detection System instruments should be operated in separate dedicated areas. The use of 2 dedicated areas (Sample Preparation Area and Amplification Area) within the laboratory is recommended when performing the *Quick* SARS-CoV-2 rRT-PCR kit.

The Sample Preparation Area is dedicated to processing samples and to adding processed samples and controls to the 96-Well PCR plate. All reagents used in the Sample Preparation Area should remain in this dedicated area at all times. Do not bring amplification product into the Sample Preparation Area.

The Amplification Area is dedicated to the amplification and detection of amplified product. Laboratory coats and equipment used in the Amplification Area must remain in this area and not be moved to the Sample Preparation Area.

Work area and instrument platforms must be considered potential sources of contamination. Change gloves after contact with potential contaminants (specimens, eluates, and/or amplified product) before handling unopened reagents, negative control, positive controls, or specimens.

Decontaminate and dispose of all potentially biohazardous materials in accordance with local, state, and federal regulations.

Prevention of Nucleic Acid Contamination

Nucleic acid contamination is minimized through:

- Reverse transcription, PCR amplification, and oligonucleotide hybridization occur in a sealed 96-Well Reaction Plate.
- Detection is carried out automatically without the need to open the 96-Well Reaction Plate.
- Pipettes with aerosol barrier tips are used for all pipetting, which are discarded after use.
- Separate, dedicated areas are used to perform the *Quick* SARS-CoV-2 rRT-PCR Kit. Refer to the **Special Precautions** section of this package insert.

Specimen Collection, Storage, and Transport

Specimen Collection and Storage

Human upper and lower respiratory specimens collected into DNA/RNA Shield™ Swab Collection Kits and/or sputa specimens collected into DNA/RNA Shield™ Saliva Collection Kits may be used with the *Quick* SARS-CoV-2 rRT-PCR kit.

Note: The DNA/RNA Shield™ Saliva Collection Kit is used only for the collection of sputum. It is not to be used with saliva specimens.

The swabs provided in the swab collection kits are with a synthetic tip with a nylon, and a plastic shaft (please see swabs recommended by FDA). Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended. 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

Specimen Transport

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 SARS-CoV-2 specimens.

The following transport and storage conditions were validated for samples collected in the DNA/RNA Shield™ Saliva Collection Kit or the DNA/RNA Shield™ Swab Collection Kit.

Transport: Specimens stored in DNA/RNA Shield™ can be shipped at ambient temperature but must not exceed shipping times of 56 hours.

Alternatively, specimen transportation guidelines from CDC for SARS-CoV-2 specimens can be followed. (https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html

Storage: Upon receipt, specimens in transport media can be stored at ambient temperature not to exceed 4 days.

Procedures

Quick SARS-CoV-2 rRT-PCR Kit Procedure

This insert contains instruction for the *Quick* SARS-CoV-2 rRT-PCR Kit.

Kit Protocol

Laboratory personnel should be trained to operate the Bio-Rad CFX96 Touch Real-Time PCR Detection System and, for automated sample preparation, the KingFisher™ Flex Purification System. Operators should have a thorough knowledge of the software run on the respective instruments and must follow good laboratory practices.

Bio-Rad CFX96 Touch Real-Time PCR Detection System must be linked together with the Bio-Rad CFX Maestro™ 1.1 Version 4.1.2433.1219 software and be installed prior to performing the assay. For a detailed description of how to use the application refer to the software guide: https://www.bio-rad.com/en-us/product/cfx-maestro-software-for-cfx-real-time-pcr-instruments?ID=OKZP7E15

For a detailed description of how to use the CFX96 Touch Real-Time PCR Detection System, refer to the instrument guide. (https://www.bio-rad.com/webroot/web/pdf/lsr/literature/10000068706.pdf).

For a detailed description of how to use the KingFisher™ Flex Purification System, refer to the instrument guide.(https://assets.thermofisher.com/TFSAssets/LSG/manuals/KingFisher_Flex_User_Manual_54006 30_5400640.pdf).

Sample Preparation and RNA Extraction Protocols – *Quick*-DNA/RNA™ Viral MagBead

All steps below are to be performed in the '**Sample Preparation Area**'

Manual RNA Extraction Protocol

1. **Reagent Preparation**:

   1.1. Add beta-mercaptoethanol (BME) to the **Viral DNA/RNA Buffer** to a final dilution of 0.5% (v/v).

   Example: 0.5 ml BME per 100 ml **Viral DNA/RNA Buffer** (D7020-1-100).

   1.2. Reconstitute lyophilized **Proteinase K** to a concentration of 20 mg/ml prior to use.

   Example: Add 210 µl of Proteinase K Storage Buffer to reconstitute the lyophilized 5 mg Proteinase K or add 1,040 µl of Proteinase K Storage Buffer to reconstitute the lyophilized 20 mg Proteinase K. **Store at -20 ± 5 ˚C after use.**

   1.3. Add Isopropanol to **MagBead DNA/RNA Wash 1** concentrate to a final dilution of 40% (v/v).

   Example: 20 ml Isopropanol per 30 ml MagBead **DNA/RNA Wash 1** concentrate (R2130-1-30) or 80 ml of Isopropanol per 120 ml MagBead DNA/RNA Wash 1 concentrate (R2130-1-120).

   1.4. Add Isopropanol to **MagBead DNA/RNA Wash 2** concentrate to a final dilution of 60% (v/v).

   Example: 30 ml Isopropanol per 20 ml MagBead DNA/RNA Wash 2 concentrate (R2130-1-20) or 120 ml of Isopropanol per 80 ml **MagBead DNA/RNA Wash 2** concentrate (R2130-2-80).

2. **Sample Preparation**:

   Perform all steps at room temperature (20 ± 5 °C)

   Important: The **No Template Contro**l (**NTC**) should be run through the entire sample preparation process, from sample extraction to rRT-PCR.

   2.1. Wear two layers of gloves before proceeding. Inside the biosafety cabinet, take the **DNA/RNA Shield™ Collection Tube** out of the biohazard bag.

2.2. Perform the following tasks outside of the biosafety cabinet:

2.2.1. Calculate and aliquot the amount of **Proteinase K** (from **Step 1.2**) needed for this batch of samples, **including NTC**. **Example**: 3 µl of **Proteinase K** for the **NTC**, 10 µl of **Proteinase K** for each swab sample, and/or 40 µl of **Proteinase K** for each sputum sample. **Store the Proteinase K at -20 ± 5 ˚C.**

2.3. Place the sample tubes on a rack. Spray the outside of the tubes with 70% Ethanol and wait 10 minutes for the tubes to dry.

2.4. While waiting for the tubes to dry, discard the outer layer gloves into the biohazard bin outside of the biosafety cabinet and put on a new pair of gloves.

2.5. Once the tubes are dry, label the cap and/or the side of the tube sequentially starting with the number "1" up to the maximum number of samples. Place tubes back on the rack after labeling.

2.6. Hold the top of tube and flick downward to pull the liquid to the bottom of the tube.

2.7. If processing a **swab sample**, follow these instructions:

2.7.1. Unscrew the tube cap to open the tube. Hold the open tube and cap with the non-pipetting hand. Add 10 µl **Proteinase K** (aliquoted from **Step 2.2.1**) to the sample.

2.7.2. Close and cap the tube securely, invert 3 times, then mix well by vortexing at max speed for 15 seconds.

2.7.3. Incubate the sample mixture at room temperature for 15 minutes.

2.8. If processing a **sputum sample**, follow these instructions:

2.8.1. Unscrew the tube cap to open the tube. Hold the open tube and cap with the non-pipetting hand. Add 40 µl **Proteinase K** (aliquoted from **Step 2.2.1**) to the sample.

2.8.2. Close and cap the tube securely, invert 3 times, then mix well by vortexing at max speed for 15 seconds.

2.8.3. Incubate the sample mixture at room temperature for 15 minutes.

2.9. For the **No Template Control** (**NTC**):

2.9.1. Transfer 300 µl of **NTC** into a new 1.5 ml microcentrifuge tube and label the microcentrifuge tube appropriately. Add 3 µl **Proteinase K** (aliquoted from **Step 2.2.1**) to the NTC.

2.9.2. Close the tube cap securely, invert 3 times, then mix well by vortexing at max speed for 15 seconds.

2.9.3. Incubate the **NTC** mixture at room temperature for 15 minutes.

2.10. After incubating, hold the top of tube and flick downward to pull the liquid to the bottom of the tube.

2.11. Transfer 300 µl of the Proteinase K-treated sample into a new 1.5 ml microcentrifuge tube and label the microcentrifuge tube with the sample number from **Step 2.5**.

2.12. Add 600 µl **Viral DNA/RNA Buffer** (from **Step 1.1**) to the **NTC** and sample microcentrifuge tubes.

2.13. Vortex the bottle of **MagBinding Beads** for 15 seconds at max speed.

2.14. Add 20 µl **MagBinding Beads** to the sample and **NTC**. After every 5 samples, cap and re-vortex the bottle containing MagBinding Beads for 15 seconds to keep the beads in suspension.

2.15. Bring the capped microcentrifuge tube outside the biosafety cabinet. Place the tube onto the microcentrifuge tube carousel. Turn-on the BioShake for 10 minutes at 1,800 RPM at 25°C, or room temperature, to keep the **MagBinding Beads** suspended in solution.

2.16. Take the microcentrifuge tube off the BioShake. Place the tubes into the benchtop microcentrifuge. Centrifuge by short pulsing for 1 second at 50 RCF to pull-down the liquid.

2.17. Place the microcentrifuge tube on a magnetic rack and incubate for 30 seconds to pellet the **MagBinding Beads**. Open the flip-cap. Hold the bottom of the tube while opening to secure the tube and avoid splashing.

2.18. Without touching MagBinding Bead biohazard liquid pellet, slowly aspirate and discard the clear supernatant into the waste bottle.

2.19. Add 500 µl **MagBead DNA/RNA Wash 1** (from **Step 1.3**) to the microcentrifuge tube.

2.20. Vortex for 10 seconds at max speed or shake using the BioShake for 30 seconds at 1,800 RPM. Centrifuge by short pulsing for 1 second at 50 RCF to pull-down the liquid.

2.21. Place the tube onto a magnetic rack and incubate for 1 minute to pellet the MagBinding Beads. Open the flip-cap. Hold the bottom of the tube while opening to secure the tube and avoid splashing.

2.22. Without touching **MagBinding Beads** pellet, slowly aspirate and discard the clear supernatant.

2.23. Repeat **Step 2.19 – 2.22** using the buffers below in place of **MagBead DNA/RNA Wash 1**. Samples should be transferred to a new 1.5 ml microcentrifuge tube after resuspension with the final Ethanol wash in **Step 2.23.3**.

2.23.1. **500 µl MagBead DNA/RNA Wash 2** (from **Step 1.4**)

2.23.2. **900 µl Ethanol (95-100%)**

2.23.3. **500 µl Ethanol (95-100%)**

2.24. After removing the Ethanol supernatant (from **Step 2.23.3**), use a p10 pipette to completely aspirate any residual Ethanol.

2.25. Keep the flip-cap open and air-dry the **MagBinding Beads** for 15 minutes on a microcentrifuge rack.

2.26. Add 50 µl **DNAse/RNAse-Free Water** to the **MagBinding Beads**. Mix well by vortexing for 30 seconds at max speed. Close the flip-cap.

2.27. Centrifuge by short pulsing for 1 second at 50 RCF to pull-down the liquid. Then vortex again at Speed 6 for 15 seconds. Hold the tube firmly while vortexing to avoid having the **MagBinding Beads** splash onto the flip-cap.

2.28. Incubate for 10 minutes at room temperature on a microcentrifuge rack.

2.29. Centrifuge by short pulsing for 1 second at 50 RCF to pull-down the liquid.

2.30. Transfer the tube to a magnetic rack. Incubate for 30 seconds to pellet the **MagBinding Beads**.

2.31. Tilt the magnetic rack 45 degrees to allow the magnetic beads to move up the microcentrifuge tube wall for easy eluate aspiration.

2.32. Without touching the **MagBinding Bead** pellet, aspirate as much of the eluate as possible and transfer to a new Hard-Shell 96-Well PCR plate for amplification reaction set-up.

2.33. Using a sealing film seal the Hard-Shell 96-Well PCR plate containing the purified RNA samples.

Note: The Elution Plate from step 2.33 now contains the **purified RNA samples** to be run through the rRT-PCR assay (described in '**Reaction Plate Set-up**'). The samples can be immediately used to set up the rRT-PCR Reaction plate in the Sample Preparation Work Area or stored at ≤ -70 °C. This Elution plate is **NOT** to be used directly for the rRT-PCR. Please perform '**Reaction Plate Set-up'** on a **NEW** 96-well optically-clear plate.

All steps below are to be performed in the '**Sample Preparation Area**'

Automated RNA Extraction Protocol

1. **Buffer Preparation:**

1.1. Add beta-mercaptoethanol (BME) to the **Viral DNA/RNA Buffer** to a final dilution of 0.5% (v/v).

Example: 0.5 ml BME per 100 ml **Viral DNA/RNA Buffer** (D7020-1-100).

1.2. Vortex the bottle containing **MagBinding Beads** for 30 seconds at max speed. Then add MagBinding Beads to the **Viral DNA/RNA Buffer** (containing BME from **Step 1.1**) to a final dilution of 3.3% (v/v).

Example: 3.4 mL **MagBinding Beads** per 100 mL **Viral DNA/RNA Buffer**.

1.3. Reconstitute lyophilized **Proteinase K** to a concentration of 20 mg/ml prior to use.

Example: Add 210 µl of **Proteinase K Storage Buffer** to reconstitute the lyophilized 5 mg **Proteinase K** or add 1,040 µl of **Proteinase K Storage Buffer** to reconstitute the lyophilized 20 mg Proteinase K. **Store at -20 ± 5 ˚C after use.**

1.4. Add Isopropanol to **MagBead DNA/RNA Wash 1** concentrate to a final dilution of 40% (v/v).

Example: 20 ml Isopropanol per 30 ml **MagBead DNA/RNA Wash 1** concentrate (R2130-1-30) or 80 ml of Isopropanol per 120 ml **MagBead DNA/RNA Wash 1** concentrate (R2130-1-120).

1.5. Add Isopropanol to **MagBead DNA/RNA Wash 2** concentrate to a final dilution of 60% (v/v).

Example: 30 ml Isopropanol per 20 ml **MagBead DNA/RNA Wash 2** concentrate (R2130-1-20) or 120 ml of Isopropanol per 80 ml **MagBead DNA/RNA Wash 2** concentrate (R2130-2-80).

2. **Sample Preparation:**

Perform all steps at room temperature (20 ± 5 °C)

Important: The **No Template Contro**l (**NTC**) should be run through the entire sample preparation process, from sample extraction to rRT-PCR.

2.1. Wear two layers of gloves before proceeding.

2.2. Inside the biosafety cabinet, take the **DNA/RNA Shield™ Collection Tube** out of the biohazard bag.

2.3. Perform the following tasks outside of the biosafety cabinet:

Calculate and aliquot the amount of **Proteinase K** (from **Step 1.3**) needed for this batch of samples. **Example**: 3 µl of **Proteinase K** for the **NTC**, 10 µl of **Proteinase K** for each swab sample and/or 40 µl of **Proteinase K** for each sputum sample. Store the **Proteinase K** stock at **-20 ± 5 °C**. Give the aliquoted **Proteinase K** to the person handling the sample in the biosafety cabinet.

2.4. Place the sample tubes on a rack. Spray the outside of the tubes with 70% Ethanol and wait 10 minutes for the tubes to dry.

2.5. While waiting for the tubes to dry, discard the outer layer gloves into the biohazard trash bin outside of the biosafety cabinet and put on a new pair of gloves.

2.6. Once the tubes are dry, label the cap and/or the side of the tube sequentially starting with the number "1" up to the maximum number of samples. Place tubes back on the rack after labeling.

2.7. Hold the top of tube and flick the tube to pull-down the liquid inside.

2.8. If processing a **swab sample**, follow these instructions:

2.8.1. Unscrew the tube cap to open the tube. Add 10 µl Proteinase K (aliquoted from **Step 2.3**) to the sample.

2.8.2. Close the tube cap securely, invert 3 times, then mix well by vortexing at max speed for 15 seconds.

2.8.3. Incubate the sample mixture at room temperature for 15 minutes.

2.9. If processing a **sputum sample**, follow these instructions:

2.9.1. Unscrew the tube cap to open the tube. Add 40 µl Proteinase K (aliquoted from **Step 2.3**) to the sample.

2.9.2. Close the tube cap securely, invert 3 times, then mix well by vortexing at max speed for 15 seconds.

2.9.3. Incubate the sample mixture at room temperature for 15 minutes.

2.9.4. Discard the outer layer gloves into the biohazard trash bin outside of the biosafety cabinet and put on a new pair of gloves.

2.9.5. While waiting for the samples to incubate, prepare the KingFisher™ Plates.

2.10. For the **No Template Control** (**NTC**):

2.10.1. Transfer 300 µl of **NTC** into each of two (2) new 1.5 ml microcentrifuge tube and label the microcentrifuge tube appropriately. Add 3 µl **Proteinase K** (aliquoted from **Step 2.3**) to each **NTC**.

2.10.2. Close the tube cap securely, invert 3 times, then mix well by vortexing at max speed for 15 seconds.

2.10.3. Incubate the **NTC** mixture at room temperature for 15 minutes.

3. **KingFisher™ Plates Preparation:**

3.1. Prepare all KingFisher Plates <u>outside</u> of the biosafety cabinet.

3.2. **Sample Plate Preparation**:

3.2.1. Vortex the container of **Viral DNA/RNA Buffer** (added with BME and **MagBinding Beads** from **Step 1.2**) for 10 seconds at maximum speed. Aliquot 620 µl into a new KingFisher Deepwell 96 Plate, V-bottom. Fill the well-positions up to the number of samples being processed (maximum 90). Below is a typical sample plate schematic:

	1	2	3	4	5	6	7	8	9	10	11	12
A	x	S7	S15	S23	S31	S39	x	S53	S61	S69	S77	S85
B	NTC	S8	S16	S24	S32	S40	NTC	S54	S62	S70	S78	S86
C	S1	S9	S17	S25	S33	S41	S47	S55	S63	S71	S79	S87
D	S2	S10	S18	S26	S34	S42	S48	S56	S64	S72	S80	S88
E	S3	S11	S19	S27	S35	S43	S49	S57	S65	S73	S81	S89
F	S4	S12	S20	S28	S36	S44	S50	S58	S66	S74	S82	S90
G	S5	S13	S21	S29	S37	S45	S51	S59	S67	S75	S83	S91
H	S6	S14	S22	S30	S38	S46	S52	S60	S68	S76	S84	S92

X = leave empty
NTC = No Template Control
S1 = Sample 1

3.2.2. Label the side of the deepwell plate “Sample Plate”

3.3. **Wash 1 Plate Preparation**:

3.3.1. Aliquot 500 µl of **MagBead DNA/RNA Wash 1** (added with Isopropanol from **Step 1.4**) into a new KingFisher™ Deepwell 96 Plate, V bottom. Fill the well-positions mirroring the diagram on **Step 3.2.1**.

3.3.2. Label the side of the deepwell plate "**Wash 1**".

3.4. **Wash 2 Plate Preparation**:

3.4.1. Aliquot 500 µl of **MagBead DNA/RNA Wash 2** (added with Isopropanol from **Step 1.5**) into a new KingFisher™ Deepwell 96 Plate, V bottom. Fill the well-positions mirroring the diagram on **Step 3.2.1**.

3.4.2. Label the side of the deepwell plate "**Wash 2**".

3.5. **Ethanol (EtOH) 1 Plate Preparation**:

3.5.1. Aliquot 900 µl of 95% Ethanol into a new **KingFisher™ Deepwell 96 Plate, V bottom**. Fill the well-positions mirroring the diagram on **Step 3.2.1**.

3.5.2. Label the side of the deepwell plate "EtOH 1".

3.6. **EtOH 2 Tip Plate Preparation**:

3.6.1. Add 500 µl of 95% Ethanol into a new **KingFisher™ Deepwell 96 Plate, V bottom**. Fill the well-positions mirroring the diagram on **Step 3.2.1**.

3.6.2. Slowly load the **KingFisher™ 96 tip comb** to the plate. Do not cause the Ethanol to splash out of the well.

3.6.3. Label the side of the deepwell plate "**EtOH 2 Tip**".

3.7. **Elution Plate Preparation**:

3.7.1. Add 60 µl of **DNase/RNase-Free Water** into a new **KingFisher™ 96 KF microplate**. Fill the well-positions mirroring the diagram on **Step 3.2.1**.

3.7.2. Label the side of the plate "**Elution**".

4. **Transferring Samples into the Sample Plate:**

4.1. Bring the prepared Sample Plate (from **Step 3.2**) into the biosafety cabinet.

4.2. Inside the biosafety cabinet, hold the sample tube from the top and flick to pull-down the liquid.

4.3. Transfer 300 µL of **Proteinase K-treated sample in DNA/RNA Shield™** (from **Step 2.8** or **Step 2.9**) and **NTC** into the Sample Plate following plate diagram in **Step 3.2.1**. Prevent cross contamination by dispensing the sample after the pipette tip is half-way into the well. DO NOT let liquid from the pipette tip touch another well while entering or removing the tip from the well.

4.4. After all the samples have been transferred bring the Sample Plate outside of the biosafety cabinet.

5. **Operating the KingFisher™ Flex and Loading Plates:**

5.1. Outside the biosafety cabinet, discard the outer layer gloves into the biohazard bin, and put on a new pair.

5.2. Ensure the Kingfisher™ Flex machine is on. If it is off, turn it on *via* the power switch on the left-hand side. The screen will power on and the turntable will rotate. Wait 30 seconds for the machine to fully turn on.

5.3. Open the plastic shield sliding door.

5.4. Press the "**Right**" arrow on the machine to highlight the center icon. The menu highlight will turn **ORANGE**.

5.5. Press the "**Down**" arrow to highlight the "**RNA**" menu then press "**OK**".

5.6. Highlight the "**SARS-CoV-2 RNA V7**" program. Ensure the **SARS-CoV-2 RNA V7 program** is downloaded from the Zymo Research Website.

5.7. Press the green "**Start**" button to initiate the program. The screen will now indicate where to load the Kingfisher™ plates on the turntable.

5.8. Load the prepared Elution Plate (from **Step 3.7**) onto the turntable position "**1**". Load the Elution Plate so that the "**A1**" well is at the A1 position indicated on the turntable. Ensure the plate sits flat on the turntable.

5.9. Press "**Start**". The turntable will rotate to an empty position. The screen will indicate which plate (from **Step 3.3** to **3.6**) to load next and onto which turntable position. Load the plate in a similar manner to **Step 5.8**. Repeat until all the plates have been loaded onto the deck. The Sample Plate is the last plate to be loaded.

5.10. Once the Sample plate is loaded, close plastic shield door and press "**Start**" to begin the RNA extraction program.

5.11. The program takes about 50 minutes to complete.

5.12. After program is complete, the screen will ask to remove the plates from the turntable.

5.13. Open the plastic shield sliding door and carefully remove the Elution Plate from the turntable.

5.14. Place the Elution Plate on the bench away from the Kingfisher™ Flex and seal the plate to prevent contamination. If not analyzed immediately with rRT-PCR, store the RNA samples at ≤ -70°C until needed.

5.15. Press "**Start**" to rotate the turntable and remove the remaining plates one-by-one.

5.16. Discard liquid from the Sample Plate according to institutional practices.

5.17. Discard liquid from the rest of the plates into liquid waste container and discard the plates into regular trash bin.

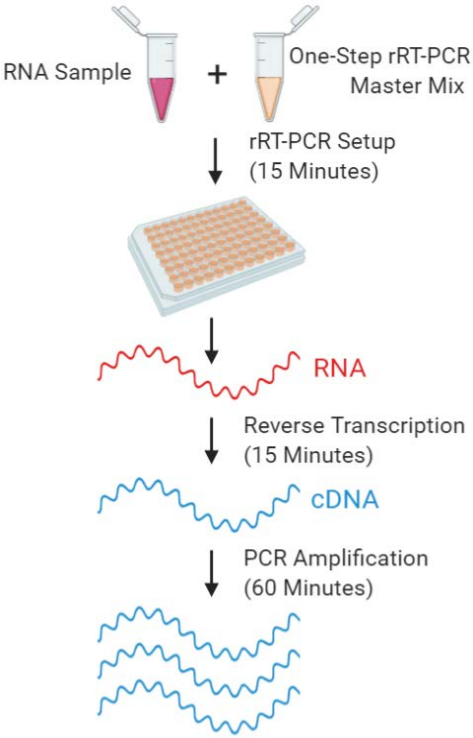
Note: The Elution plate from step 5.14 now contains the **purified RNA samples** to be run through the rRT-PCR assay (described in '**Reaction Plate Set-up**'). The samples can be immediately used to set up the rRT-PCR Reaction plate in the Sample Preparation Work Area or stored at ≤ -70 °C. This Elution plate is **NOT** to be used directly for the rRT-PCR. Please perform '**Reaction Plate Set-up'** on a **NEW** 96-well optically-clear plate.

Amplification Reaction Set-up Procedure

Overview:

The assay can be used with purified RNA extracted from the manual and automated extraction protocols above.

As illustrated in the workflow figure below, purified RNA samples are directly mixed with the CV Mix 1 and 2 and incubated in the Real-Time PCR System. Results are ready to be analyzed after the reverse transcription and qPCR steps are completed.



I. rRT-PCR Reaction Setup:

Steps of rRT-PCR Reaction setup are to be performed in the '**Sample Preparation Area**'

Before starting, thaw frozen reagents on ice, mix 10 times by inversion, centrifuge briefly, and place back on ice.

Avoid exposing the CV Mix 1 and 2 reactions to direct light and keep the 96-well PCR plate on ice during preparation.

To prevent contamination, handle all reagents carefully and aliquot the CV Mix 1 and 2 in the 96-well PCR plate before handling any test samples or included controls.

1. Add 10 µl of **CV Mix 1** to each well selected for the detection of SARS-CoV-2.
2. Add 10 µl of **CV Mix 2** to each well selected for the detection of human RNase P.
3. Add 10 µl of every sample to be tested or 10 µl of the **NTC** to a well containing **CV Mix 1** and a well containing **CV Mix 2**.
4. Add 10 µl of **CV Positive Control** to a well containing **CV Mix 1** and a well containing **CV Mix 2** in each plate.
5. Firmly seal the 96-well PCR plate with Microseal 'B' sealing film.
6. Briefly vortex the 96-well PCR plate to mix and centrifuge to eliminate bubbles and bring any droplets to the bottom of the well.
7. Place the 96-well PCR plate on ice.

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC	S7	S15	S23	S31	S39	PC	S7	S15	S23	S31	S39
B	NTC	S8	S16	S24	S32	S40	NTC	S8	S16	S24	S32	S40
C	S1	S9	S17	S25	S33	S41	S1	S9	S17	S25	S33	S41
D	S2	S10	S18	S26	S34	S42	S2	S10	S18	S26	S34	S42
E	S3	S11	S19	S27	S35	S43	S3	S11	S19	S27	S35	S43
F	S4	S12	S20	S28	S36	S44	S4	S12	S20	S28	S36	S44
G	S5	S13	S21	S29	S37	S45	S5	S13	S21	S29	S37	S45
H	S6	S14	S22	S30	S38	S46	S6	S14	S22	S30	S38	S46

White Wells = CV Mix 1
Grey Wells = CV Mix 2
PC = Positive Control
NTC = No Template Control
S# = Test Sample

II. Real-Time PCR Machine Set Up:

Steps of '**Real-Time PCR Machine Set Up**' are to be performed in the '**Amplification Area**'

1. Using the Real-Time PCR software (Bio-Rad CFX Maestro™ 1.1 Version 4.1.2433.1219 software for the CFX96 Touch™ Real-Time PCR Detection System), create the following PCR program using the following parameters:

Step	Temperature	Time (min:sec)	
1	55°C	15:00	
2	95°C	10:00	
3	95°C	0:05	Steps 3-5, repeat 45 cycles
4	57°C	0:30	
5	**Plate Read**		
6	**END**		

2. Assign each well the corresponding sample names, targets, and fluorophores. **CV Mix 1** and **CV Mix 2** contain 2 fluorophores.

CV Mix	SARS-CoV-2 Targets	Fluorophores	Ex.	Em.
1	1, 2, 3	HEX™	538 nm	555 nm
2	RP	Quasar® 670	647 nm	666 nm

3. Enable all the light filters required to detect both fluorophores.

4. Load the 96-well PCR plate into the real-time PCR machine and start the following PCR program.

III. Data Analysis (Bio-Rad CFX Maestro™ 1.1 Version 4.1.2433.1219 software):

a. **Pre-Analytical Steps** of '**Real-Time PCR Machine Set Up**' are to be performed in the '**Amplification Area**'.

1. Visually inspect the plate for any issues that occurred during PCR (e.g. evaporation due to improper plate sealing) and take note of any problems.

2. Adjust the Fluorescence Baseline Threshold Value to 50 RFU for each fluorophore.

3. Under **"Settings**", click on **"Baseline Settings"**, and select **"Apply Fluorescence Drift Correction"**.

4. Note and record any samples, **PC**, or **NTC** with amplification in the HEX or Quasar 670 channel before 10 cycles. This step is necessary to identify samples with Ct values < 10 which may be excluded by adjustments made in **Step 5**.

5. Exclude the first 10 cycles of the PCR by going to **"Settings"**, clicking on **"Cycles to Analyze"**, and selecting **"Analyze Data from Cycle."** Manually enter the range as 10 to 45.

b. **Quality Control**

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. Visually inspect the signals for the controls and verify their validity

Positive Control: after rRT-PCR, the Positive Control sample will show fluorescent signal for both the HEX and Quasar 670 channels, which detect the presence of SARS-CoV-2 and RNase P, respectively. Signals generated from the Positive Control sample will be considered valid if detected at or below 40 amplification cycles when using the recommended systems settings. If signals for the Positive Control sample are detected after 40 amplification cycles, the control must be replaced with a new aliquot. If this problem is not resolved, the whole kit must be replaced with a new one.

No Template Control: The No Template Control sample will show no fluorescent signal for targets in the HEX channel or RNase P in the Quasar 670 channel. The No Template Control sample will be considered valid if no amplification occurs in either the HEX or Quasar 670 channels. If amplification occurs in either channel, contamination of extraction and/or rRT-PCR reagents may have occurred and reagents must be replaced.

Table 1. Expected Performance of Controls

Control Type	External Control Name	SARS-CoV-2 N	IC	Expected Ct Values
Negative Control	NTC	-	-	No Ct values in any
Positive Control	PC	+	+	Ct of ≤ 40 Ct for all N targets; and Ct ≤ 30 for RNAse P

c. **Interpretation of Results**

Assessment of clinical specimen test results must be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the positive or negative controls is invalid, the patient results cannot be interpreted, and all patient specimens need to be retested after the root cause has been identified and eliminated.

Amplification signals (cycle threshold; Ct) for each of the fluorescence channels (HEX and Quasar 670) are analyzed and the sample is classified as either SARS-CoV-2 Negative, SARS-CoV-2 Positive, or Invalid, specifically:

Visually inspect the amplification curves for RNase P target. Any sample with no RNase P amplification or RNase P signal greater or equal to cycle 40 is invalid and must be repeated

Extraction Internal Control: The extraction internal control must generate a fluorescence signal for RNase P (Quasar 670 channel) in each clinical sample and in the Positive Control samples. RNase P and SARS-CoV-2 signal intensities are not necessarily correlated in positive samples; therefore, a SARS-CoV-2 signal can be detected even in samples with extremely small content of host RNA. Specifically, amplification signals are considered valid if the signal is detected ≤40 Ct. If the extraction internal control does not show amplification or amplifies outside the cycle threshold as specified above, the sample result are considered invalid and the sample must be re-extracted and re-tested.

- Samples with a Ct value for the Extraction Internal Control target ≥ 40 are considered invalid. The sample must be re-extracted and re-tested. If the problem persists, a new sample must be obtained.
- Samples with a Ct of < 40 for the Extraction Internal Control Target and no amplification of the viral targets (signal in the Quasar 670, but not HEX channel) will be classified as SARS-CoV-2 Negative.
- Samples with a Ct of < 40 for the Extraction Internal Control Target and any amplification of the viral targets (signal in both the HEX and Quasar 670 channels) will be classified as SARS-CoV-2 Positive. Results are considered presumptive for SARS-CoV-2 since a positive result may also arise from infection by another Sarbecovirus, including SARS-CoV.

Visually inspect one-by-one the amplification curve for each sample that exhibits SARS-CoV-2 signal. It is important to inspect each sample with SARS-CoV-2 signal individually. The presence of signal from other samples may automatically adjust the scale of the Y axis to a higher RFU making it impossible to analyze the curve of samples with late and/or weak SARS-CoV-2 signal in detail.

Any sample with amplification curves ≤ 40 Ct is interpreted as positive. Note any sample displaying aberrant amplification curves. Refer to the figures **Examples of Normal and Aberrant Amplification Curves** for examples and detailed explanations of aberrant amplification curves.

Table 2. Result Interpretation for Patient Specimens

Host Target RNase P Quasar 670 channel	Viral Target Target 1, 2, and 3 HEX channel	Results	Action
Ct value below 40	No amplification	SARS-CoV-2 Negative	Deliver Results
Ct value below 40	Amplification ≤ 40 Ct	SARS-CoV-2 Positive**	Deliver Results
Signal undetected or Ct 40 or above	No amplification	Invalid	Re-extract and re-test the sample*

* If the sample is repeatedly invalid a new sample shall be obtained.

** A positive result using the *Quick* SARS-CoV-2 rRT-PCR Kit is considered presumptive for SARS-CoV-2 because it may result from infection by another Sarbecovirus, such as SARS-CoV. Follow-up testing may be performed if differentiation between targets is desired.

Examples of Normal and Aberrant Amplification Curves:

Note: As indicated in section III of the kit protocol, the first 10 cycles of PCR have been excluded from the graphs and the baseline threshold for both fluorophores has been adjusted to 50 RFU.

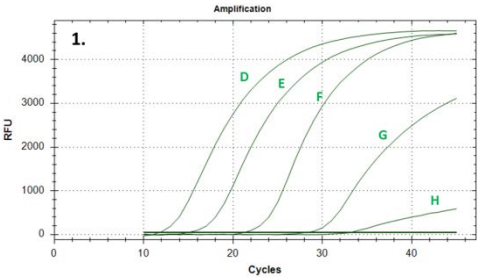


Figure 1. Curves D, E, F, G, and H (Green Letters) are examples of normal amplifications for SARS-CoV-2 target. H represent the SARS-CoV-2 signal in the CV Positive Control.

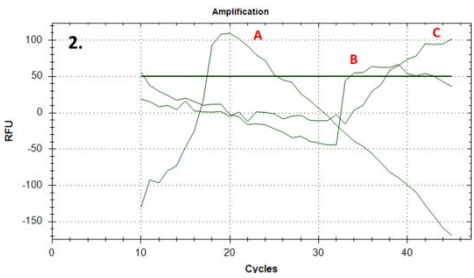


Figure 2. Curves A, B, and C (Red Letters) are examples of aberrant amplifications for the SARS-CoV-2 target. The Y axis scale has been adjusted (magnified) to visualize details of the curves.

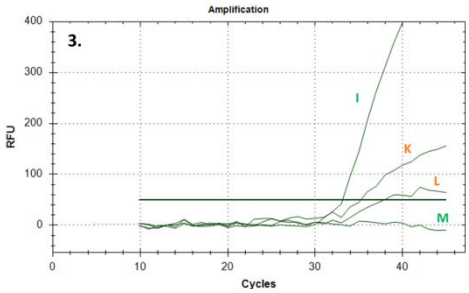


Figure 3. Curve I is considered a positive signal for SARS-CoV-2 target. Curves K and L can be either weak positive signals or aberrant signals for the SARS-CoV-2 target; in these two cases the rRT-PCR reaction should be repeated to confirm the results. Curve M is a negative signal (SARS-CoV-2 Negative). The scale of the Y axis has been adjusted (magnified) to 400 RFU in order to visualize the differences between the signals.

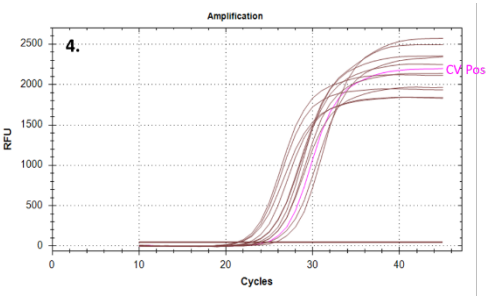


Figure 4. Examples of samples with normal amplification curves for RNase P target are shown in brown. Signal for CV Positive Control is indicated in pink.

Post-Processing Procedures

1. At the end of the run, remove the 96-well plate from the CFX96 Touch Real-Time PCR Detection System and dispose of according to instrument guide.
2. Place the 96-well plate in a sealable plastic bag and dispose according to the CFX96 Touch Real-Time PCR Detection System Guide along with the gloves used to handle the plate.
3. Clean the CFX96 Touch Real-Time PCR Detection System according to guide recommendations.

Troubleshooting Guide

Problem	Possible Causes and Suggested Actions
Reduction in volume observed in the wells after rRT-PCR.	• **Cause:** The PCR reaction evaporated because the PCR plate was not sealed properly. • **Action:** Record the wells with less volume and proceed with normal analysis.
Aberrant amplification after excluding the first 10 PCR cycles from analysis. Please see examples of aberrant and normal amplification curves on page 21.	• **Cause:** Presence of air bubbles in the reaction, poor quality sample, incorrect set up, or compromised reagents. • **Action:** Record the wells and fluorophores that show aberrant amplification. Samples that exhibit aberrant amplification should be repeated in order to determine a conclusive result. Re-extracting the sample or using all new reagents may be necessary if re-testing the sample does not produce a clear signal.
SARS-Cov-2 signal in CV Positive Control detected after 40 Cycles.	• **Cause:** Incorrect Set-Up or the *Quick* SARS-CoV-2 reagents may have been compromised. • **Action:** Re-run the test. If the problem persists, carry out the test again with all new reagents.
RNase P signal in CV Positive Control detected after 30 Cycles.	• **Cause:** Incorrect Set-Up or the *Quick* SARS-CoV-2 reagents may have been compromised. • **Action:** Rerun the test. If the problem persists, carry out the test again with all new reagents.

Limitations of Procedures

For use under an Emergency Use Authorization only

For *in vitro* diagnostic Use

For prescription use only

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

Optimum performance of this kit requires appropriate specimen collection, storage, and transport to the test site (refer to Specimen Collection, Storage, and Transport in this package insert).

Detection of SARS-CoV-2 RNA may be affected by sample collection methods, patient factors (e.g. presence of symptoms), and/or stage of infection.

False-negative results may arise from improper collection, shipping, and/ or storage of specimen.

Performance of the test was established in sputum. A shift in Ct was observed for all targets at high mucin concentrations. Therefore, high mucin concentrations at or above 0.1% (≥1mg/ml) may result in invalid results.

The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated.

As with any molecular test, mutations within the target regions detected by the *Quick* SARS-CoV-2 rRT-PCR kit could affect primer and/or probe binding resulting in failure to detect the presence of virus.

The performance of this SARS-CoV-2 assay was established using sputum. Nasal, nasopharyngeal, oropharyngeal and mid-turbinate swabs, BAL and tracheal aspirates are also considered acceptable specimen types for use with the SARS-CoV-2 Assay but performance has not been established. Testing of nasal and mid-turbinate nasal swabs (self-collected at a healthcare site or collected by a healthcare provider) is limited to patients with symptoms of COVID-19. Results should be interpreted by a trained professional in conjunction with the patient's history and clinical signs and symptoms, and epidemiological risk factors.

Negative results do not preclude infection with the SARS-CoV-2 virus and should not be the sole basis of a patient treatment/management or public health decision. Follow up testing should be performed according to the current CDC recommendations.

- Members of the Infectious disease laboratory will be trained to perform this assay and competency will be assessed and documented per CAP regulations.
- 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.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

Conditions of Authorization for the Laboratory

The *Quick* SARS-CoV-2 Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd.

However, to assist clinical laboratories using the *Quick* SARS-CoV-2 ("your product" in the conditions below), the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[1] using your product will include with result reports of your product, 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 your product will use your product as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.

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

D. Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Zymo Research (EUAREPORTING@zymoresearch.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

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

G. You, authorized distributors, and authorized laboratories using your product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1] The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests" as "authorized laboratories."

PERFORMANCE CHARACTERISTICS

Limit of Detection (LoD) - Analytical Sensitivity:

The LoD of the *Quick* SARS-CoV-2 rRT-PCR Kit utilizing the entire test system from sample preparation to detection, has been determined. To determine a preliminary LoD, sputum was used as a clinical matrix containing spiked-in whole genome RNA extracted from SARS-CoV-2, strain Muc-IMB-1. The SARS-CoV-2 whole genome RNA was quantified using RT-qPCR for use in validation studies. The preliminary LoD study was performed using the automated extraction method on sputum samples containing 10-fold dilutions of viral RNA from 0 to 8.33 x 10^4 GEC/ml. The preliminary LoD was determined to be 83 GEC/ml (5 GEC/rxn), the lowest concentration for which 5/5 independent replicates tested positive.

Table 3. Preliminary LoD study in sputum specimens using automated extraction

Concentration in Dilution Tested [*GEC/ml*]	Concentration in Dilution Tested [*GEC/rxn*]	Replicate 1 C_t	Replicate 2 C_t	Replicate 3 C_t	Replicate 4 C_t	Replicate 5 C_t	Call Rate	Average Ct	Lowest Concentration with Uniform Positivity	Preliminary Limit of Detection (LoD)
8.33 x 10^4	*5,000*	*27.34*	*27.53*	*27.61*	*27.48*	*27.36*	*5/5*	*27.46*	*83 GEC/ml (5 GEC/rxn)*	*83 GEC/ml (5 GEC/rxn)*
8.33 x 10^3	*500*	*31.02*	*30.90*	*31.16*	*31.11*	*30.84*	*5/5*	*31.00*		
8.33 x 10^2	*50*	*34.19*	*34.66*	*34.89*	*34.73*	*33.86*	*5/5*	*34.47*		
8.3 x 10^1	*5*	*36.89*	*36.32*	*37.65*	*38.73*	*38.46*	*5/5*	*37.61*		
8.3 x 10^0	*0.5*	*NA**	*NA*	*NA*	*NA*	*NA*	*0/5*	*-*		

*NA: No Amplification

The LoD of the kit was confirmed for both the manual and automated extraction methods using sputa as a clinical matrix. Whole genome RNA from SARS-CoV-2 was spiked into negative sputa samples and 20 replicates were independently processed for LoD determination. The lowest concentration at which all 5 replicates were positive in the preliminary LoD (i.e. 83 GEC/mL) was used as a starting point for the confirmatory LoD study. Testing of 83 GEC/mL could not confirm the tentative LoD. Therefore, concentrations above 83 GEC/mL (increasing by factor 2) were tested until ≥ 19/20 replicates tested positive. The final LoD for both the manual and automated extraction methods was determined to be 250 GEC/ml (15 GEC/rxn).

Table 4. Confirmatory LoD for Sputum Specimens

Automated Extraction						Manual Extraction			
Replicate	250 GEC/ml (15 GEC/rxn)	167 GEC/ml (10 GEC/rxn)	83 GEC/ml (5 GEC/rxn)	Lowest Concentration with at least 19/20 (95%) Positive	Confirmatory Limit of Detection (LoD)	Replicate	250 GEC/ml (15 GEC/rxn)	Lowest Concentration with at least 19/20 (95%) Positive	Confirmatory Limit of Detection (LoD)
	Ct	Ct	Ct				Ct		
1	33.46	34.96	33.95	250 GEC/ml (15 GEC/rxn)	250 GEC/ml (15 GEC/rxn)	1	35.32	250 GEC/ml (15 GEC/rxn)	250 GEC/ml (15 GEC/rxn)
2	33.58	33.26	34.62			2	35.90		
3	33.31	33.30	33.47			3	35.84		
4	34.14	35.11	NA *			4	34.81		
5	33.52	NA	34.21			5	NA		
6	34.18	33.55	35.11			6	34.95		
7	33.59	34.52	NA			7	34.79		
8	34.02	34.06	35.46			8	36.99		
9	33.65	34.68	36.74			9	36.64		
10	33.69	34.96	35.38			10	34.57		
11	34.11	33.12	NA			11	35.71		
12	34.15	NA	35.23			12	36.36		
13	33.05	34.25	35.25			13	33.86		
14	34.51	36.09	34.87			14	34.95		
15	33.55	35.56	34.45			15	36.20		
16	33.40	33.18	35.65			16	36.20		
17	34.63	35.51	NA			17	34.46		
18	33.02	NA	37.81			18	38.16		
19	33.64	35.20	NA			19	35.47		
20	34.55	33.38	34.84			20	33.19		
Call Rate	20/20	17/20	15/20			Call Rate	19/20		

*NA: No Amplification

Table 5. Confirmatory LoD Study – Summary

Target Level	Method	Valid results	SARS-CoV-2 N (all targets) Positive			Internal Control Positive		
			n	Average Ct	Detection Rate	n	Average Ct	Detection Rate
250 GEC/ml (15 GEC/rxn)	Automated	20	*20*	*33.79*	*100%*	*20*	*28.29*	*100%*
167 GEC/ml (10 GEC/rxn)	Automated	20	*17*	*34.39*	*85%*	*20*	*28.17*	*100%*
83 GEC/ml (5 GEC/rxn)	Automated	20	*15*	*35.14*	*75%*	*20*	*28.20*	*100%*
250 GEC/ml (15 GEC/rxn)	Manual	20	*19*	*35.49*	*95%*	*19*	*27.16*	*95%*

Analytical Reactivity (Inclusivity)

In silico inclusivity analysis of the oligo sets for SARS-CoV-2 was performed using multiple sequence alignment of the individual SARS-CoV-2 primers and probes against all SARS-CoV-2 N gene sequences found in the NCBI database (1,354 in total as of April 24, 2020). One primer for SARS-CoV-2 showed 100% homology to all sequences, while all other primers and probes were found to have single nucleotide mismatches within a small number (≤ 0.81%) of the N gene sequences analyzed. None of the mismatches occurred within the last five 3' nucleotides of any of the primers, reducing the potential detrimental effect of those mismatches on PCR efficiency. None of the N gene sequences analyzed were found to have nucleotide mismatches within more than one Target region. Failure of one SARS-CoV-2 Target to efficiently amplify poses a low risk for a false negative result since amplification of the two additional virus Targets is unaffected. The specificity of the oligo sets for SARS-CoV-2 was predicted to detect SARS-CoV-2 and possibly SARS-CoV. Based on *in silico* analysis, the primers and probe for one of the targets were found to have 81-90% homology with SARS-CoV, which may result in pan-sarbecovirus detection of the subgenus containing SARS-CoV-2 and SARS-CoV.

Analytical Specificity (Cross Reactivity)

Cross reactivity studies were performed using the NCBI Basic Alignment Search Tool (BLAST) to identify the largest regions of homology between any of the primers and probes to the genomes indicated. Homology ≥ 80% was found for the following organisms:

1. *Streptococcus pneumoniae*
2. *Mycoplasma pneumoniae*
3. *Legionella longbeachae*
4. *Neisseria meningitidis*

In silico analysis for possible cross reactivity with organisms listed in **Table 6** was conducted and showed homology of some primers and probes with the closely related SARS-CoV; however, SARS-CoV, is not currently prevalent and poses minimal detection risk. Furthermore, the reaction mix includes primer/probe pairs specific for SARS-CoV-2, which ensures specificity for SARS-CoV-2. As for endemic coronaviruses (229E, NL63, HKU1, and OC43) or MERS-CoV little cross reactivity was found.

Table 6. *Quick* SARS-CoV-2 rRT-PCR Kit Analytical Specificity Microorganisms

Microorganisms from the Same Genetic Family	High Priority Organisms
Human coronavirus 229E	Human adenovirus 1
Human coronavirus OC43	Human metapneumovirus
Human coronavirus HKU1	Human parainfluenza virus 1-4
Human coronavirus NL63	Influenza A virus
SARS coronavirus	Influenza B virus
MERS coronavirus	Enterovirus D68
	Respiratory syncytial virus
	Rhinovirus A
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococus pneumoniae
	Streptococcus pyogenes
	Bordetella pertussis
	Mycoplasma pneumoniae
	Influenza C virus
	Parechovirus
	Candida albicans
	Corynebacterium diphtheriae
	Legionella longbeachae
	Bacillus anthrasis (Anthrax)
	Moraxella cararrhalis
	Neisseria elongata
	Neisseria meningitidis
	Pseudomonas aeruginosa
	Staphylococcus epidermidis
	Streptococcus salivarius
	Leptospira interrogans
	Chlamydia psittaci
	Coxiella burnetti (Q-Fever)
	Staphylococcus aureus

Microbial Interference Studies

Wet bench cross-reactivity studies were performed on the 4 microorganisms identified by *in silico* analysis as having ≥80% homology with one or more primers or probes used in this device. In addition, *Pseudomonas aeruginosa, Candida albicans,* and pooled nasal wash were also tested. Individual microorganisms were tested at a concentration of 1 x 10^6 cells/sample which were negative or low positive for SARS-CoV-2 whole genome RNA (3X LoD, or 750 GEC/ml). Cross-reactivity was tested using 3 independent replicates of each sample were independently extracted using the manual method. No rRT-PCR signal was detected in any of the SARS-CoV-2 negative samples tested, indicating that no cross-reactivity of the SARS-CoV-2 primers and probes used in this assay occurs with these specific microorganisms or a microbe-rich specimen like pooled nasal wash. In addition, the average Ct value for each sample was within 2 Ct values of the no organism 3X LoD control, demonstrating that no interference occurred from any of the microorganisms or pooled nasal wash for SARS-CoV-2 detection.

Table 7. Microbial interference testing

Organism	SARS-CoV-2	Replicate 1 Ct	Replicate 2 Ct	Replicate 3 Ct	Average Ct
None	750 GEC/ml (3X LoD)	*33.01*	*33.63*	*33.57*	*33.40*
Pooled Nasal Wash	751 GEC/ml (3X LoD)	*34.60*	*33.23*	*33.10*	*33.64*
Pseudomonas aeruginosa	750 GEC/ml (3X LoD)	*33.82*	*33.01*	*33.33*	*33.39*
Candida albicans	750 GEC/ml (3X LoD)	*34.26*	*34.54*	*34.15*	*34.32*
Streptococcus pneumoniae	750 GEC/ml (3X LoD)	*34.68*	*33.60*	*33.20*	*33.83*
Mycoplasma pneumoniae	750 GEC/ml (3X LoD)	*33.97*	*33.51*	*33.96*	*33.82*
Legionella longbeachae	750 GEC/ml (3X LoD)	*33.28*	*33.21*	*34.13*	*33.54*
Neisseria meningitidis	750 GEC/ml (3X LoD)	*33.80*	*33.73*	*34.86*	*34.13*
None	Negative	*NA**	*NA*	*NA*	*NA*
Pooled Nasal Wash	Negative	*NA*	*NA*	*NA*	*NA*
Pseudomonas aeruginosa	Negative	*NA*	*NA*	*NA*	*NA*
Candida albicans	Negative	*NA*	*NA*	*NA*	*NA*
Streptococcus pneumoniae	Negative	*NA*	*NA*	*NA*	*NA*
Mycoplasma pneumoniae	Negative	*NA*	*NA*	*NA*	*NA*
Legionella longbeachae	Negative	*NA*	*NA*	*NA*	*NA*
Neisseria meningitidis	Negative	*NA*	*NA*	*NA*	*NA*

* NA: No Amplification

Endogenous Interference Substances Studies

Interfering substances which may be present in respiratory specimens were evaluated for the ability to generate false *positive* results in sputum specimens negative for SARS-CoV-2. Interfering substances were added to sputum specimens at the indicated concentration (see **Table 8**) and three replicates were independently extracted using the automated method. No rRT-PCR signal for SARS-CoV-2 was detected in any of the samples, indicating that none of the substances tested contributed to a false positive result at the indicated concentration.

Table 8. Interfering substances: Evaluation of the potential to cause false *positive* results in SARS-CoV-2 negative sputum specimens

Potential Interfering Substance	Concentration (% v/v or w/v)	Results (Detected X/3)
Mucin: bovine submaxillary gland, type I-S*	0.1%	0/3
Mucin: bovine submaxillary gland, type I-S	0.01%	0/3
Mucin: bovine submaxillary gland, type I-S	0.001%	0/3
Blood (human)	10%	0/3
Nasal spray (Afrin)	10%	0/3
Nasal spray (CVS Nasal Spray)	10%	0/3
Nasal corticosteroid (Rhinocort)	10%	0/3
Nasal corticosteroid (Flonase)	10%	0/3
Nasal gel (Zicam)	10%	0/3
Homeopathic allergy relief medicine (Alkolol)	10%	0/3
Throat lozenge (Cepacol)	10%	0/3
Oral anesthetic and analgesic (Chloroseptic spray)	10%	0/3
Antibiotic, nasal ointment (Mupirocin)	10%	0/3
Antibacterial, systemic (Tobramycin)	10%	0/3
Antiviral (Oseltamivir)	10%	0/3
Antiviral (Zanamivir)	10%	0/3

*Performance of the test was established in sputum. A shift in Ct was observed for all targets at high mucin concentrations. Therefore, high mucin concentrations at or above 0.1% (≥1mg/ml) may result in invalid results.

Interfering Substances: SARS-CoV-2

Interfering substances which may be present in respiratory specimens were evaluated for the ability to generate false *negative* results in sputum specimens positive for SARS-CoV-2. Contrived low positive samples were generated by spiking in whole genome SARS-CoV-2 RNA at 3X LoD (750 GEC/ml) into sputum specimens. Interfering substances were added to three replicates of low positive sputum specimens at the indicated concentration (see **Table 9**) and independently extracted in using the automated method. rRT-PCR signal for SARS-CoV-2 was detected in all samples. We have demonstrated that SARS-CoV-2 RNA can be reproducibly detected in a mucus-rich specimen (sputum); both SARS-CoV-2 and RNase P were detected in sputum samples with up to 0.1% (1 mg/ml) mucin, which is a component of mucus (see Limitations section for more details).

Table 9. Interfering substances: Evaluation of the potential to cause false negative results in SARS-CoV-2 3X LoD sputum specimens

Potential Interfering Substance	Concentration (% v/v or w/v)	Results (Detected X/3)
Mucin: bovine submaxillary gland, type I-S*	0.1%	3/3
Mucin: bovine submaxillary gland, type I-S	0.01%	3/3
Mucin: bovine submaxillary gland, type I-S	0.001%	3/3
Blood (human)	10%	3/3
Nasal spray (Afrin)	10%	3/3
Nasal spray (CVS Nasal Spray)	10%	3/3
Nasal corticosteroid (Rhinocort)	10%	3/3
Nasal corticosteroid (Flonase)	10%	3/3
Nasal gel (Zicam)	10%	3/3
Homeopathic allergy relief medicine (Alcolol)	10%	3/3
Throat lozenge (Cepacol)	10%	3/3
Oral anesthetic and analgesic (Chloroseptic spray)	10%	3/3
Antibiotic, nasal ointment (Mupirocin)	10%	3/3
Antibacterial, systemic (Tobramycin)	10%	3/3
Antiviral (Oseltamivir)	10%	3/3
Antiviral (Zanamivir)	10%	3/3

*Performance of the test was established in sputum. A shift in Ct was observed for all targets at high mucin concentrations. Therefore, high mucin concentrations at or above 0.1% (≥1mg/ml) may result in invalid results.

Clinical Performance Evaluation

A clinical evaluation was performed using 30 contrived positive and 30 non-reactive, or negative, specimens in a randomized blinded fashion. Negative specimens were collected from patients experiencing one or more symptoms of respiratory infection under supervision of a healthcare provider, including 21 sputum and 9 oropharyngeal swab samples using the DNA/RNA Shield™ Saliva Collection Kit and the DNA/RNA Shield™ Swab Collection Kit. Additionally, 30 individual negative sputum samples were collected using the DNA/RNA Shield™ Saliva Collection Kit and spiked with whole genome SARS-CoV-2 RNA. Twenty of the contrived positive specimens were generated to contain 2x LoD of SARS-CoV-2 RNA (500 GEC/ml), with the remaining 10 specimens spanning a range of viral RNA concentrations up to 10,000X LoD (2.5 x 10^6 GEC/ml). All samples were processed independently using the automated extraction method.

Results from the clinical evaluation demonstrate that the device was able to detect SARS-CoV-2 in 100% of the contrived positive samples and none of the negative samples.

Table 10. Evaluation with Contrived Specimens

Specimens	Sample Concentration	n	Target 1-3		IC	
			Positive (n)	Average Ct	Positive (n)	Average Ct
Positive spiked sputum	500 GEC/ml (30 GEC/rxn) 2 X LoD	20	20	33.87	20	25.20
	1,250 GEC/ml (75 GEC/rxn) 5 X LoD	2	2	33.25	2	25.15
	2,500 GEC/ml (150 GEC/rxn) 10 X LoD	2	2	31.50	2	25.45
	100xLoD	2	2	28.24	2	25.14
	1,000xLoD	2	2	25.26	2	24.97
	10,000xLoD	2	2	21.96	2	24.46
Negative	N/A	30	0	-	30	24.62

Positive Percent Agreement (PPA): 30/30 = 100% (95% CI: 88.7% - 100%)
Negative Percent Agreement (NPA): 30/30 = 100% (95% CI: 88.7% - 100%)

Symbols Legend


Collect sample by

Catalog number


Manufacturer


Storage instructions


Harmful

Lot number


Do not reuse

Sterilized by ethylene oxide


Keep away from sunlight

For prescription use only


See instruction manual

Contains sufficient for <n>tests

Package contains

Reagent

Positive Control

IN VITRO DIAGNOSTIC MEDICAL DEVICE

Technical Support

For technical support, call Zymo Research Corp. Technical Support at 1-949-679-1190 ext. 3, email tech@zymoresearch.com

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