Biomeme

# Biomeme SARS-CoV-2 Real-Time RT-PCR Test

Instructions for Use, v3

**For Use Under an Emergency Use Authorization (EUA) Only**

Rx Only | For *In Vitro* Diagnostic Use

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# Brief Overview

The Biomeme SARS-CoV-2 Real-Time RT-PCR Test for use on the Biomeme Franklin™ Real-Time PCR System is a qualitative multiplex, *in vitro* diagnostic (IVD) assay. It is only for use under the **Emergency Use Authorization (EUA)** and is intended for the detection of RNA from SARS-CoV-2.

**SAFETY WARNING**
When working with our products, always wear appropriate personal protective equipment (PPE) (e.g. lab coat, disposable gloves with adequate chemical resistance, mouth/face protection, goggles, etc.) For more information, please review the product's safety data sheet(s) (SDS).

# Intended Use

The Biomeme SARS-CoV-2 Real-Time RT-PCR Test is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal, nasal, and oropharyngeal swab specimens, and nasopharyngeal wash/aspirate or nasal aspirate specimens collected 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 which is generally detected in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

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

The Biomeme SARS-CoV-2 Real-Time RT-PCR Test is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The Biomeme SARS-CoV-2 Real-Time RT-PCR Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

The following indications are authorized under the Pooling and Serial Testing Amendment (https://www.fda.gov/media/147737/download) for use in laboratories certified under CLIA to perform high complexity tests: Qualitative detection of RNA from SARS-CoV-2 in pooled samples containing up to 10 individual human anterior nasal swabs placed in a single vial containing transport media after being collected by a healthcare provider (HCP) or self-collected under the supervision of an HCP from individuals without symptoms or other reasons to suspect COVID-19, when tested as part of a serial testing program including testing at least once per week, and Qualitative detection of RNA from SARS-CoV-2 in pooled samples containing aliquots of transport media from up to 10 individual human anterior nasal swab specimens that were collected by a healthcare provider (HCP) or self-collected under the supervision of an HCP from individuals without symptoms or other reasons to suspect COVID-19 and placed in individual vials containing transport media, when tested as part of a serial testing program including testing at least once per week.

These indications are authorized with the following validated protocol: Sample Pooling Protocol for SARS-CoV-2 Testing. Negative results from pooled testing should not be treated as definitive. If a patient's clinical signs and symptoms are inconsistent with a negative result or results are necessary for patient management, then the patient should be considered for individual testing. Specimens included in pools with a positive or invalid result must be reported as presumptive positive or tested individually prior to reporting a result.

# Summary and Explanation

An outbreak of respiratory illness of unknown etiology in Wuhan City, Hubei Province, China was initially reported to the World Health Organization (WHO) on December 31, 2019.[1] Chinese authorities identified a novel coronavirus (2019-nCoV), which has resulted in thousands of confirmed human infections in multiple provinces throughout China and exported cases in several Southeast Asian countries and more recently the United States. Cases of severe illness and some deaths have been reported. The International Committee for Taxonomy of Viruses (ICTV) renamed the virus SARS-CoV-2.[2]

[1] Centers for Disease Control and Prevention. https://www.cdc.gov/coronavirus/2019-ncov/index.html.
[2] bioRxiv. https://www.biorxiv.org/content/10.1101/2020.02.07.937862v1.

The Biomeme SARS-CoV-2 Real-Time RT-PCR Test is a molecular *in vitro* diagnostic test that is based on widely used nucleic acid amplification technology. The Biomeme SARS-CoV-2 Real-Time RT-PCR Test contains primers and probes and internal controls used in RT-PCR for the *in vitro* qualitative detection of SARS-CoV-2 RNA in upper respiratory specimens.

# Principle of the Procedure

The Biomeme SARS-CoV-2 Real-Time RT-PCR Test utilizes Biomeme's **M1 Sample Prep Cartridge** for RNA extraction, Biomeme's **SARS-CoV-2 Go-Strips** assay, and Biomeme's portable **Franklin™ Real-Time qPCR Thermocycler**. Franklin's companion mobile app, **Biomeme Go**, scans tests, runs PCR experiments online or offline, and is used to quickly interpret your test results while conveniently syncing data to the **Biomeme Cloud**.

Biomeme's M1 Sample Prep Cartridges require no lab equipment, refrigeration, electricity, incubation, alcohol precipitation or phenol chloroform extraction. Instead, they utilize a filtration-based method in which nucleic acids selectively bind to the silica membrane inside Biomeme's proprietary M1 Sample Prep columns. Subsequent washes through a sequence of specially formulated buffers yields purified nucleic acids upon elution in minutes. High throughput sample preparation can also be conducted with a Kingfisher Flex purification system.

The Biomeme SARS-CoV-2 Real-Time RT-PCR Test detects two different SARS-CoV-2 genes and is multiplexed together with Biomeme's RNA Process Control (RPC) for RNA extraction and RT-PCR (MS2 bacteriophage) in 0.1 mL low-profile, thin-walled, optically clear 3-well strips (**Go-Strips**). Each reaction well of the 3-well Go-Strip already contains lyophilized master mix, enzymes, and multiplexed primer/probes for the following triplex reaction:

- **Orf1ab** - Open reading frame 1ab gene
- **S** - Spike gene
- **RPC** - RNA Process Control (MS2 bacteriophage)

Go-Strips are designed for the Biomeme Franklin™ mobile handheld qPCR thermocycler. The Biomeme SARS-CoV-2 Real-Time RT-PCR Test is also available in a 96-well **Go-Plate** format for direct use on the Bio-Rad CFX96 or QuantStudio5 using the "fast" block (see Appendix 1 & 2).

# Test Contents

The materials available for the Biomeme SARS-CoV-2 Real-Time RT-PCR Test can be found in Table 1 below. Equipment, software, and other materials that are required to run and analyze test results but not provided can be found in Table 2.

## Table 1: Biomeme SARS-CoV-2 Real-Time RT-PCR Test - Consumables

| Source: REF# | Component | Description |
|---|---|---|
| Biomeme: 3000567 | 200µL Transfer Pipette Pack | Pack of disposable transfer pipettes to transfer VTM into Extraction Kit |
| Biomeme: 3000536<br>or<br>Biomeme: 3000574 | Biomeme M1 Sample Prep Cartridge Kit for RNA 2.0<br>or<br>Biomeme M1 Sample Prep Cartridge Kit for RNA 2.0 NC | RNA Extraction Kit containing cartridges, syringes, and binding column tips. |
| Biomeme: 3000011 | 20µL Fixed Volume Pipette Kit | 20µL pipette to transfer purified RNA into Biomeme Go-Strips |
| Biomeme: 3000572 | Pipette Tips | Boxes of 96 pipette tips to transfer purified RNA into Biomeme Go-Strips |
| Biomeme: 3000150 | 2mL Self-Standing Tubes Pack | Pack of tubes for storing purified samples |
| Biomeme: 3000555 | Biomeme SARS-CoV-2 Go-Strips* Kit | Pre-aliquoted 3-well PCR strips. Each well contains a 20µL lyophilized triplex reaction.<br><br>The kit also includes Biomeme's lyophilized RNA Extraction and RT-PCR Process Control pellets ("RPC" - MS2 bacteriophage). |
| Biomeme: 3000591 | Biomeme SARS-CoV-2 Dry Swab Pooling Kit | Sterile swabs<br>Conical swab collection tube<br>Preservation buffer |

***Note**: Contains Bovine Serum Albumin of USA origin. Certified BSE free.*

Each item above can be purchased individually.

## Additional Form Factors

SARS-CoV-2 Assays also come in one additional form factor:

- 3000562: Biomeme SARS-CoV-2 Go-Plates (96 rxns at 20 uL)

## Table 2: Biomeme SARS-CoV-2 Real-Time RT-PCR Test – Equipment, Software, and Other Materials

The following equipment and software are required to run the test and analyze results. While Go-Strips are designed for the Biomeme Franklin™ mobile handheld qPCR thermocycler, the Biomeme SARS-CoV-2 Real-Time RT-PCR Test is also available in a 96-well **Go-Plate** format for direct use on the Bio-Rad CFX96 or QuantStudio 5 using the "fast" block (see Appendix 1 & 2). Both the Go-Strip and Go-Plate form factors are validated for use on these alternative instruments.

| Source: REF# | Component | Description |
|---|---|---|
| Biomeme: 1000003<br>or<br>Biomeme: 1000018 | Biomeme Franklin three9 Real-Time PCR Thermocycler (Black)<br>or<br>Biomeme Franklin three9 Real-Time PCR Thermocycler (White) | Real-Time PCR Thermocycler |
| Biomeme: 1000013<br>or<br>Biomeme: 1000012 | Android Smartphone w/ Biomeme Go or Go Dx Mobile App<br>or<br>Rugged Android Smartphone w/ Biomeme Go or Go Dx Mobile App | Controller for Biomeme Franklin Thermocycler |
| Biomeme: 2000006 | Biomeme Cloud | PCR Data Management Software |
| Biomeme: 3000563 | Biomeme Sample Prep Tray | Tray to streamline preparation and extraction of DNA or RNA from your samples |
| Biomeme: 3000577 | Biomeme DNA/RNA Preservation Buffer | Used to collect and maintain samples during transport and before molecular analysis. |

| Source: REF# | Component | Description |
|---|---|---|
| **External Controls*** | | |
| No Template Control (NTC) | Molecular Grade Water | Monitors contamination that could produce false positive results |
| Positive Control (PC) | BEI NR-52285: Viral Genomic RNA (from SARS-Related Coronavirus 2, Isolate USA-WSA1/2020<br>or<br>Exact Diagnostics COV019: SARS-CoV-2 Standard | Control that is not exposed to the experimental treatment and is known to produce a positive result |

***Note:** External controls other than Biomeme's exogenous RNA Process Control (MS2 Bacteriophage) are not provided with the Biomeme SARS-CoV-2 Real-Time RT-PCR Test. Quality control requirements should be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard quality control procedures.*

***Note**: Biomeme's Safety Data Sheets (SDS) are available at help.biomeme.com under 'Product Document Library'.*

***Note:** Samples should be collected in accordance with CDC Guidelines.*

***Note:** The Biomeme SARS-CoV-2 Real-Time RT-PCR Test can also be used with the Bio-Rad CFX Maestro 1.1 version 4.1.2433.1219 (REF 1855195) and the Applied Biosystems Quant Studio 5 Design and Analysis Software v1.4.3 (REF A34322).*

# Warnings & Precautions

As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination. The Biomeme SARS-CoV-2 Real-Time RT-PCR Test workflow should be performed by qualified and trained staff to avoid the risk of erroneous results.

- For *in vitro* diagnostic use only.
- For Emergency Use Authorization only.

- For Prescription Use only.
- The Biomeme SARS-CoV-2 Real-Time RT-PCR Test has not been FDA cleared or approved; the test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by authorized laboratories; use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
- The Biomeme SARS-CoV-2 Real-Time RT-PCR Test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The Biomeme SARS-CoV-2 Real-Time RT-PCR Test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.
- Specimens should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures. Refer to **Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2**.
- Follow necessary precautions when handling specimens. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples. Refer to **Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition - CDC**.
- Always use pipette tips with aerosol barriers. Tips that are used must be sterile and free from DNases and RNases.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Modifications to assay reagents, assay protocol, or instrumentation are not permitted, and are in violation of the product Emergency Use Authorization.
- Do not use the kit after the indicated expiry date.
- Dispose of waste in compliance with local, state, and federal regulations.
- Safety Data Sheets (SDS) are available upon request.

- Positive results are indicative of the presence of SARS-CoV-2 RNA.
- Handle all samples and controls as if they are capable of transmitting infectious agents.
- Individual specimens with low viral loads may not be detected due to the decreased sensitivity or increased interference when tested with pooled testing.
- Only non-flocked swabs should be used for dry swab pooling.

# Sample Collection, Handling, and Storage

Proper specimen collection, storage, and transport are critical to the performance of this test. Inadequate specimen collection, improper specimen handling and/or transport may yield a false result. Sample handling and storage should be consistent with CDC guidelines. The Biomeme SARS-CoV-2 Real-Time RT-PCR Test has been validated for use with BD Universal Viral Collection Kit and the Biomeme DNA/RNA Preservation Buffer. Samples collected in the BD Universal Viral Collection Kit should be handled and stored according to the manufacturer's instructions. Samples collected in Biomeme DNA/RNA Preservation Buffer can be stored at room temperature (15–30 °C) for up to 14 days until sample extraction is performed using Biomeme's M1 Sample Prep Cartridges or the Kingfisher Flex purification system.

**SAFETY WARNING**
Handle all samples and controls as if they are capable of transmitting infectious agents. Refer to the **CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19)**.

# Instructions for Use

## Minimize the Risk of Contamination

- For components that are bulk packaged, such as empty tubes or void filling caps for the Go-Strips, always pour out onto a clean surface a few from the bag rather than reaching your fingers into the bag.
- Do not reuse consumables. They are for one-time use only.

- Always use caution when transferring specimens from primary containers to secondary tube(s).
- Always use a new pipette tip for each specimen.
- Precautions must be taken to prevent cross contamination of samples.

## RNA Extraction Using M1 Sample Prep Cartridge

After collecting your sample, use Biomeme's M1 Sample Prep Cartridge (REF# **3000536**) to purify your RNA. Samples are lysed by mixing in Biomeme's Lysis Buffer (BLB). The lysed sample is then passed through the M1 Sample Prep column by use of the provided 1mL luer lock syringe, binding RNA to the silica membrane inside of the column. Subsequent washes remove unwanted material and salts. As a result, purified nucleic acids are eluted off the column into the provided buffer.

Buffers come pre-aliquoted in the provided sample prep cartridges for ease-of-use and the extraction method is designed to be completed in 6 simple steps. But, before beginning the sample extraction process, please take a moment to read these important tips:

- **Clean your work area between each RNA extraction** to avoid contamination between samples. It is recommended that you utilize separate work areas for sample preparation, nucleic acid extraction, and PCR amplification.
- **The extraction cartridge can be labeled** on the side with the sample ID using a Sharpie® or similar style marker.
- **Puncture 2 holes in each section of the M1 Sample Prep Cartridge** as you move through each step to minimize liquid splatter (except Air Dry step).
- **Pump slowly**, except during the Air-Dry step where rapid pumping is required, to not only minimize liquid splatter but to also improve binding to the sample prep column.
- **Additional pumps in each cartridge section** beyond the specified number will not adversely affect extraction performance.
- **Prior to removing the syringe barb tip from each cartridge section** rock the syringe completely forward and backward to enlarge the holes in the foil covering.

## Prepare RNA Process Control

The Biomeme SARS-CoV-2 Real-Time RT-PCR Test includes an RNA Process Control (RPC) detection assay and lyophilized RPC Pellets (MS2 bacteriophage).

1. Remove and open the 2mL screw cap tube containing your RPC pellet.
2. Open the 5mL screw cap tube containing your RPC buffer.
3. Using a 1mL transfer pipette, pull 0.5 - 0.75mL of RPC buffer and add it to the RPC pellet in the 2mL tube.
4. Pipette up and down with the transfer pipette to mix.
5. Transfer the entire volume back into the 5mL RPC buffer tube, again pipetting up and down to mix.

   ***Note***: *Check the box on the tube to indicate the RPC Buffer now contains the RPC material.*

6. Your RPC is now ready to add to your upcoming sample extractions (this will equal ~400 pfu per 20µL PCR reaction).

The resuspended RPC has a maximum shelf life of **2 days** when stored at room temperature. For longer term storage, aliquot out and freeze at -20°C for up to three months.

## Add Your Sample

1. Open your M1 Sample Prep Cartridge pouch and remove the contents.
2. Secure the sample prep column to the syringe and puncture the red section of your sample prep cartridge twice. Temporarily set aside the syringe- place the 1mL luer lock syringe with column attached on a tube rack such that the tip of the column is not touching any surface.
3. Using a 200µL transfer pipette (REF# 3000567), or your own 200µL pipette, transfer 200µL of media from the collection tube containing your sample and add it into the red section of your sample prep cartridge.
4. Discard your transfer pipette and incubate at room temperature (15–25°C) for 10 minutes. You can move to adding your RNA Process Control (RPC) while you wait.

## Add RNA Process Control (RPC)

1. Attach a pipette tip to your 20µL fixed volume pipette and transfer 20µL of RPC buffer containing the RPC into the punctured red section of your sample prep cartridge.
2. After the sample has finished incubating for at least 10 minutes at room temperature (15–25°C) inside the red section of the cartridge, proceed to Lysis & Binding.

***Note***: *If you intend to extract and test multiple samples (e.g., 7 samples + NTC, PC) you can label and line up 7 of the M1 Sample Prep cartridges. Then for each cartridge: use a clean pipette tip to puncture the red section of a cartridge, add your sample to the cartridge, add your RPC to the cartridge, and then set the cartridge aside to incubate. As each cartridge is finished incubating, you can proceed to Lysis & Binding below.*

## Lysis & Binding (10 Pumps)

1. Place the syringe with the attached sample prep column back into the **red** section of the sample prep cartridge and draw Biomeme Lysis Buffer (BLB) fluid all the way up the syringe and pump all the way back out. Repeat for a total of **10 pumps**.

2. Push all fluid in the syringe into the red section of the sample prep cartridge prior to beginning the next step. **Do not transfer any liquid from one section of the sample prep cartridge to the next. This applies to each remaining step of the sample extraction protocol**.

   ***Note***: *If the column starts to clog, you will experience an increase in pressure. Do not press harder as this will cause additional clogging. Instead, pull the syringe up slightly (but not all the way out of the cartridge) to reduce the pressure and gently pull back the plunger, wait a few seconds, and slowly push the plunger back down. Some of the liquid should discharge at the open end of the syringe. Repeat this process until all liquid has been discharged from the column then proceed to the next step.*

## Protein Wash (2 Pumps)

1. Move the 1mL syringe with the attached sample prep column into the **red-orange** section of the sample prep cartridge (Biomeme Protein Wash - BPW) and pierce through the foil. Remember to pierce 2 holes per section of the cartridge to minimize liquid splatter, except during the Air Dry step.

2. Draw the BPW fluid all the way up the syringe and pump all the way back out. Repeat for a total of **2 pumps** assuring that no buffer remains in the syringe before beginning the next step.

## Salt Wash (1 Pump)

1. Move the 1mL syringe with the attached sample prep column to the **orange** section of the sample prep cartridge (Biomeme Wash Buffer - BWB) and pierce through the foil twice.

2. Draw the BWB fluid all the way up the syringe and pump all the way back out **once** assuring that no buffer remains in the syringe before beginning the next step.

## Drying Wash (1 Pump)

1. Move the 1mL syringe with the attached sample prep column to the **yellow** section of the Sample Prep Cartridge (Biomeme Drying Wash - BDW) and pierce through the foil twice.
2. Draw the BDW fluid all the way up the syringe and pump all the way back out **once** assuring that no buffer remains in the syringe before beginning the next step.

## Air Dry (20+ Pumps)

1. Move the 1mL syringe with the attached sample prep column to the **blue** section of the Sample Prep Cartridge and pierce through the foil once.
2. Draw air up through the syringe and quickly pump back out. Repeat pumping vigorously **20 or more times** until the sample prep column appears dry and does not spray fluid droplets. Ensure that the sample prep column is completely dry before continuing to the elution step.

## Elution (5 Pumps)

1. Move the 1mL syringe with the attached sample prep column to the **green** section of the Sample Prep Cartridge (Biomeme Elution Buffer - BEB) and pierce through the foil twice.
2. Elute by drawing the BEB fluid all the way up through the syringe and slowly pump back out for a total of **5 pumps**.

## Transfer Extracted RNA to Storage Tube

1. After completing the 5th pump, draw up the entire fluid (about 850µL) into the syringe from the green section and transfer it to a 2mL self-standing tube (REF# **3000150**).
2. Cap the tube and dispose of the M1 Sample Prep Cartridge and syringe with binding column.

**SAFETY WARNING**
Always dispose of potentially biohazardous solutions according to your local, regional or national waste-disposal guidelines. DO NOT add bleach or acidic solutions directly to the liquids contained in Biomeme's M1 Sample Prep cartridges. The BLB and BPW buffers contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with a suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

### Repeat Extractions and Transfer Extracted RNA to Storage Tubes

1. Repeat these Sample Collection & Extraction steps with new clinical specimens to optimize throughput of the Biomeme Franklin™ thermocycler (e.g., for up to 7 total samples plus 1 NTC and 1 PC).
2. Proceed to **Loading Pure Sample into Go-Strips**.

## Preparation of Positive Control Samples

The Biomeme SARS-CoV-2 Real-Time RT-PCR Test has been validated with two external positive controls that are to be prepared as described below. Only one positive control is needed for each assay.

### Viral Genomic RNA (from SARS-Related Coronavirus 2, Isolate USA-WSA1/2020 (BEI REF NR-52285)

1. Resuspend the RPC included in the SARS-CoV-2 Go-Strip Kit according to Biomeme's instructions
2. Dilute the stock of BEI viral genomic RNA down to 1000 genome equivalent per µL (GE/µL)
    a. For example: Stock concentration at 4.8E4 GE/µL

   b. Add 2.08 µL of stock into 97.91 µL of TE = 1000 GE/µL
3. Add 20 µL of resuspended RPC into Biomeme M1 RNA sample prep kit for cartridges
4. Add 200 µL of negative NP swab into prep
5. Add 7.65 µL of viral genomic RNA at 1000 GE/µL into prep
6. Follow Biomeme's assay instructions and isolate total RNA
7. Transfer the elution into a 1.75 mL Eppendorf tube. This will be the positive control.
8. Aliquot out into 20 µL volume
9. Add 20 µL of positive control into one well of the SARS-CoV-2 Go-Strip

### Exact Diagnostics SARS-CoV-2 (REF COV019)

1. Resuspend the RPC included in the SARS-CoV-2 Go-Strip Kit according to Biomeme's instructions
2. Add 20 µL of resuspended RPC into Biomeme M1 RNA sample prep kit for cartridges
3. Add 200 µL of negative NP swab into prep
4. Add 10 µL of EXACT diagnostic standard at 200 copies per µL into prep
5. Follow Biomeme's assay instructions and isolate total RNA
6. Transfer the elution into a 1.75 mL Eppendorf tube. This will be the positive control.
7. Aliquot out into 20 µL volume
8. Add 20 µL of positive control into one well of the SARS-CoV-2 Go-Strip

## Loading Pure Sample into Go-Strips

**ATTENTION**
Contents of the Go-Strip may shift during transport. When starting to work with any Go-Strip, make sure the cake of the lyophilized reagent rests at the bottom of the Go-Strip wells. Tap the bottom of the sealed Go-Strip gently but firmly against a solid surface before removing the foil seal and adding your sample.

Open the contents of a Biomeme SARS-CoV-2 Go-Strips (REF# **3000555**). Do not immediately discard the Go-Strips pouch as you'll need to scan the QR code in a later step.

1. Start with a single Go-Strip and remove the foil covering.
2. Attach a pipette tip to a 20µL fixed volume pipette (REF# **3000011**) or prepare your own 20µL pipette.

   ***Note**: The strip connections between the tubes of your Go-Strip will face the back of the thermocycler once inserted. When resuspending your reactions and transferring your extracted RNA into the different reaction wells, replicate this orientation to ensure accurate result interpretation (e.g. transfer sample 1 into the far left reaction well of your first Go-Strip, sample 2 into the middle reaction well of your first Go-Strip, and sample 3 into the far right reaction well of your first Go-Strip).*

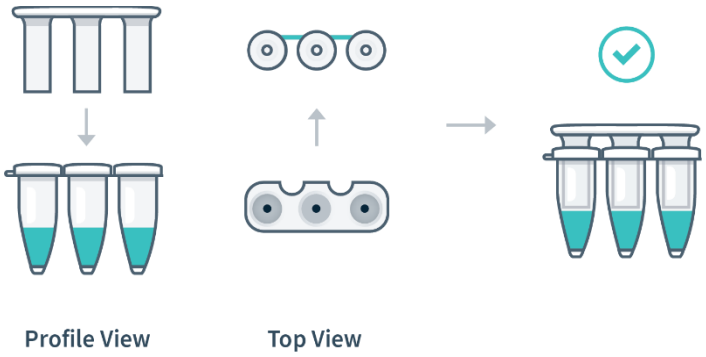
3. Additionally, when mixing your samples try to avoid introducing bubbles.

***Note**: If bubbles have been introduced, remove them from the lower portion of the PCR tubes by gently tapping the Go-Strips against your work surface before capping. Bubbles may remain at the top of the tube, but bubbles at the bottom are not acceptable.*

4. Unscrew the cap of your first purified sample in the 2mL tube and transfer 20µL of the extracted RNA into the **first** reaction well of your Go-Strip. Pipette up and down 3-5 times to mix your PCR reaction.

*Note: To help avoid creating excess bubbles and foam, always keep the pipette tip under the liquid. Additionally, only draw up approximately 50% of the solution into the pipette tip while mixing to further help avoid creating bubbles.*

5. Discard your pipette tip and repeat the process of transferring your samples only for the remaining 2 reaction wells. Once all wells of a single Go-Strip are filled, make sure to place a void filling cap into the Go-Strip to minimize any risk of contamination. Align the Go-Strip and void filling cap so that the strip connections are visible through the cap cutouts as shown in the illustration below:



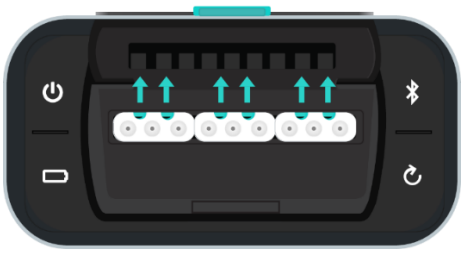

6. The void filling caps may feel slightly loose, this is normal. The thermocycler lid will secure the caps into place when closed, sealing each PCR reaction. **DO NOT** attempt to push the cap down.

***Note***: *If utilizing a No Template Control (NTC) and/or external Positive Control (PC), add in a similar manner to other samples. It is recommended that the NTC be added first (Well 1) and the PC last (Well 9) after the addition of samples.*

## Placing Go-Strips into Franklin™ Thermocycler

1. Open the lid of the thermocycler (REF# 1000003 or REF# 1000018) by pressing the latch on top of the unit.

2. Starting from the left position place the Go-Strips, with caps inserted, into each 3-well slot. Once again, make sure the strip connections are visible through the void filling cap cutouts and are facing the back of the thermocycler as shown in the illustration below.



### PCR Layout Example (for one full Franklin™ run) - without External Controls

| | Go-Strip 1 (left) | | | Go-Strip 2 (middle) | | | Go-Strip 3 (right) | | |
|---|---|---|---|---|---|---|---|---|---|
| **Well** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 |

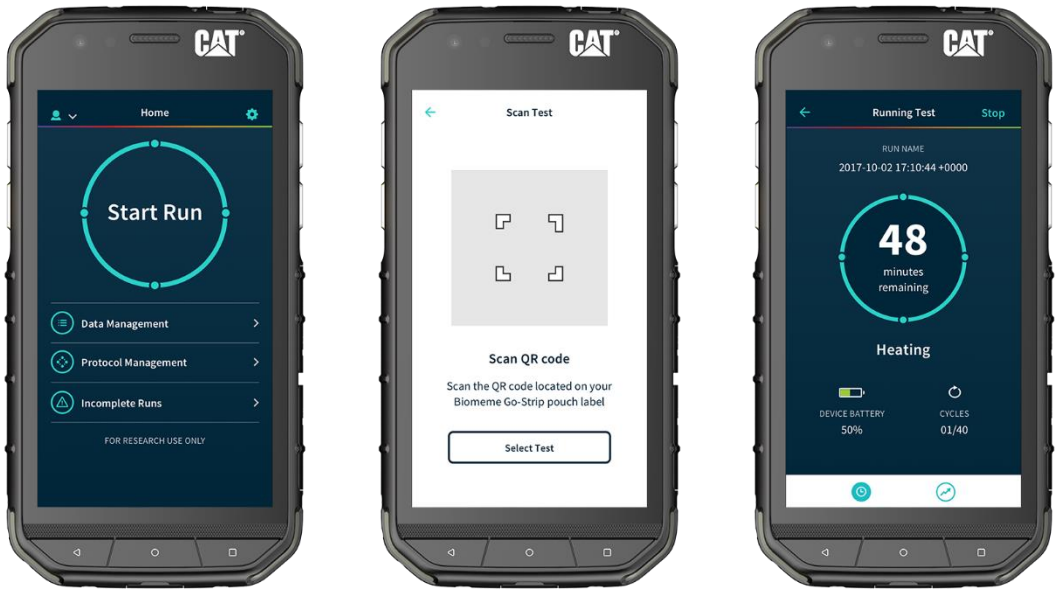
### PCR Layout Example (for one full Franklin™ run) - with External Controls

| | Go-Strip 1 (left) | | | Go-Strip 2 (middle) | | | Go-Strip 3 (right) | | |
|---|---|---|---|---|---|---|---|---|---|
| **Well** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| | NTC | S1 | S2 | S3 | S4 | S5 | S6 | S7 | PC |

3. Close the thermocycler lid securely.

***Note:*** *After your run has completed, be careful when removing your Go-Strips and void filling caps.* ***DO NOT*** *remove only the void filling cap to avoid liquid splatter and PCR amplicon contamination.*

## Launch Biomeme Go or Go Dx App on Smartphone




Biomeme Go (REF# 1000013 or REF# 1000012) is an intuitive smartphone app that pairs wirelessly with the Franklin™ real-time PCR thermocycler. It is compatible with Android 6.0.0+. The easy-to-use interface allows you to run, monitor, and analyze your tests online or offline and quickly interpret your results. Follow the simple steps outlined below to begin your test.

1. Launch the Biomeme Go or Go Dx app on your smartphone by tapping the icon on your phone's home screen if you haven't already and log in.
2. From the main dashboard of Biomeme App, tap **Start Run**.
3. Use the camera on your smartphone to scan the **QR code** printed on the Go-Strips pouch you opened earlier.

   ***Note:*** *The first time you launch the QR code scanner, you may be asked to give permission to access the camera on your device. You will only have to grant permission once.*

4. **Confirm** you have scanned the correct test.

5. **Confirm** the test protocol is as follows:

| **Name** | SARS-CoV-2 |
|---|---|
| **RT** | 55°C 120 sec |
| **Initial Denature** | 95°C 60 sec |
| **Cycles** | 45 |
| **Cycling Denature** | 95°C 3 sec |
| **Anneal** | 60°C 30 sec |

6. Select the quantity of 3-well Go-Strips to run simultaneously in your thermocycler by adjusting the **+ (Add)** and **- (Subtract)** buttons, then tap **Confirm**. The maximum number of Go-Strips per test run is 3 (9 reactions).

7. Choose to **Scan** or **Generate** your Sample ID. You can change the sample ID on the next screen if you would like.

8. Review your Sample IDs and tap **Continue** once you're ready to proceed.

9. Select which folder you'd like to save your run into. If you haven't created a folder, click **Add Folder** located towards the top right corner and create one.

10. Once you've selected the folder to save your run into, you can optionally change the **Run Name**, update your **GPS Coordinates** and/or add **Location** tags. If you wish, you can also add a note to the run by selecting the **Note** icon in the upper right corner.

11. Tap **Confirm** to proceed to **Run Setup**.

12. If you haven't done so already, power on your thermocycler by pressing and holding the **Power** button on the top of your device and tap **Continue** back in the Biomeme Go or Go Dx app.

13. Select your preferred connection method:

    a. Connect via **Bluetooth**:

i. Press the Bluetooth button on top of your device and tap **Confirm**.

ii. Tap **Scan** and wait a few seconds for your thermocycler to be found.

*__Note__: the first time you try to scan for devices, you may be asked to give the Biomeme Go or Go Dx app permission to turn on Bluetooth. Please make sure that the "Location" service is enabled in your phone settings. The latest version of Bluetooth requires that location discovery is enabled to properly pair devices.*

iii. Once the thermocycler is found, select it and tap **Confirm** to pair your devices.

b. Connect via **USB**:

i. Insert the long USB cable into the back of the thermocycler (note the correct orientation of the cable plug shown in the app).

ii. Insert the short USB cable into the phone. Then connect the two cables together (note the correct orientation of the cable plug shown in the app).

iii. Tap **OK** in the pop-up screen.

iv. Wait for confirmation in the app that your connection was successful.

14. Confirm the subsequent tutorial screens to ensure your Go-Strips are loaded properly and close the lid on your thermocycler before starting your run.

15. Tap the **Start Test** button to begin your test!

## Monitor Your PCR Run in Real Time

1. During the PCR run you can monitor the progress of your PCR, including the real-time PCR amplification plots by swiping left.

2. Once the PCR run is completed the thermocycler will download the run results to the smartphone controller.

*__Note__: You don't need to worry about your smartphone screen turning off or going to sleep. The experiment will continue to run. If the app freezes or crashes, the experiment will also continue to run and your data can be found in the Incomplete Runs section of the app once you've reloaded the*

*Biomeme Go or Go Dx app and reconnected to the thermocycler. For more information on recovering and reattaching test data, please see help.biomeme.com.*

## Sample Pooling Protocol for SARS-CoV-2 Testing

Pools of up to 10 dry swab samples or up to 10 pooled media samples may be tested using the Biomeme SARS-CoV-2 Real-Time RT-PCR Test.

### Dry Sample Pooling Methods

1. Combine up to 10 individual human anterior nasal swabs using non-flocked swabs collected by a health care provider (HCP) or self-collected under supervision of an HCP into a 50 mL conical tube.
2. Add 6mL of Biomeme DNA/RNA preservation buffer to the conical tube.
3. Close the tube cap and vortex for 10 seconds and let sit for 10 minutes.
4. Follow M1 sample prep or Kingfisher sample prep steps to isolate RNA.

### Media Pooling Methods

1. Collect individual nasal swab specimens into 3mL of Biomeme DNA/RNA preservation buffer (REF# 3000577).
2. Using a 2mL Self-Standing Tube (REF# 3000150) or equivalent combine 100uL from each patient sample. A maximum of 10 patient samples may be combined (1000uL total).
3. Close the tube cap and vortex for 10 seconds.
4. Follow M1 sample prep or Kingfisher sample prep steps to isolate RNA.

### Pool Results Reporting and Follow-up Testing

Interpretation of pool results is the same as for individual results as described in the Interpretating Results section.

- If the result of the pool is negative, then each constituent sample is reported as negative. The result report should include a comment that pooling was used during testing. Refer to the Warnings & Precautions section for additional information regarding decreased sensitivity of pool testing.
- Patients with symptoms inconsistent with negative results should be considered for individual testing.

- Positive or invalid results should be treated as presumptive positive unless or until they receive a negative result when re-tested individually. Patients in the positive pool should isolate until receiving a negative result.
- Positive or invalid results from media pooling should be deconvoluted and retested individually. Use the laboratory defined tracking system to ensure the correct individual samples are tested. If the result of the pool is invalid, each constituent sample should be re-tested as a separate individual sample to avoid potential delay in reporting valid patient results.
- If the invalid result is due to entire run failure or other instrument malfunctions, the pool may be re-tested if sufficient volume is available.
- For positive or invalid results from dry swab pooling samples new specimens should be collected from patients and tested individually.
- In both pooling types individual test results supersede the pool result.

# Interpreting Results

The recommended cycle cut-off is 40 cycles. Any amplification after cycle 40 should be considered negative. As this is not a quantitative assay, positivity must not be solely based on the Cq cutoff of a single target gene but should be an amalgam of Cq cutoff, visual analysis of amplification curve, and comparison of all targets. The user should repeat testing on any sample with questionable interpretation, as suggested in the results interpretation table.

## Table 3: QC Material Pass/Fail Criteria

| Control Type | Control Name | Used to Monitor | SARS-CoV-2 Orf1ab | SARS-CoV-2 S | RPC | Expected Cq values |
|---|---|---|---|---|---|---|
| Negative | NTC | Reagent and/or environmental contamination | - | - | - | None detected |
| Positive | PC | Substantial reagent failure including primer and probe integrity, failure in extraction procedure | + | + | + | < 40 |
| Extraction | RPC | Failure in lysis and extraction procedure, potential contamination during extraction, RT-PCR failure | - | - | + | < 40 |

## Table 4: Patient Specimen Results Interpretation

| SARS-CoV-2 Orf1ab target | SARS-CoV-2 S target | RPC | Result | Actions |
|---|---|---|---|---|
| + (Cq ≤ 40) | + (Cq ≤ 40) | ± | Positive | Report results to the sender and appropriate public health authorities. |
| - (Cq > 40) | + (Cq ≤ 40) | ± | Positive | Report results to sender and appropriate public health authorities. |
| + (Cq ≤ 40) | - (Cq > 40) | ± | Presumptive Positive | Re-extract the sample and run the rRT-PCR again. If the second run returns presumptive positive report presumptive positive results to sender and appropriate public health authorities.<br><br>For samples with a repeated presumptive positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and other SARS-like viruses for epidemiological purposes or clinical management. |
| - (Cq > 40) | - (Cq> 40) | - | Invalid | Re-extract the sample and run the rRT-PCR again. If the same result is obtained as the first run, report as Invalid. |
| - (Cq> 40) | - (Cq > 40) | + | Negative | Report results to sender. |

Look at your Go-Strips after your run has completed to check for any abnormalities such as bubbles or loss of sample. If this happens, we recommend re-running your sample.

***Note***: *Remember that after your run has completed, be careful when removing your Go-Strips and void filling caps. DO NOT remove only the void filling cap to avoid liquid splatter and PCR amplicon contamination.*

- When all SARS-CoV-2 targets are negative but the RPC is positive, the result should be considered as valid and negative.
- When all SARS-CoV-2 targets are positive but the RPC is positive or negative, the results should be considered as valid and positive.

- When all SARS-CoV-2 targets and the RPC is negative, the result is invalid. Re-extract the sample and run the rRT-PCR again. If the same result is obtained as the first run, a new specimen should be obtained.
- If only the SARS-CoV-2 S target is positive, and the RPC target is positive or negative, the result for SARS-CoV-2 is positive.
- If only the SARS-CoV-2 Orf1ab target is positive, and the RPC target is positive or negative, the result for SARS-CoV-2 is presumptive positive. A negative SARS-CoV-2 S (Target 1) result and a positive SARS-CoV-2 Orf1ab (Target 2) result is suggestive of:

  1) a sample at concentrations near or below the limit of detection of the test,
  2) a mutation in the Target 1 target region in the oligo binding sites,
  3) infection with some other Sarbecovirus (e.g., SARS-CoV or some other Sarbecovirus previously unknown to infect humans), or
  4) other factors.

  The sample should be retested. For samples with a repeated presumptive positive result, additional confirmatory testing may be conducted if it is necessary to differentiate between SARS-CoV-2 and other SARS-like viruses for epidemiological purposes or clinical management.
- If an NTC is run and the result is positive, then contamination may have occurred. Re-extract all samples within the extraction batch and re-test.

## Table 5: Pooled Patient Specimen Results Interpretation

| SARS-CoV-2 Orf1ab target | SARS-CoV-2 S target | RPC | Result Interpretation | Actions[c] |
|---|---|---|---|---|
| + (Cq ≤ 40) | + (Cq ≤ 40) | ± | Presumptive Positive[a,b] | Report pooled result to program administrator with notification that subjects in the positive pool should isolate until receiving a negative result when re-tested individually. Individual should not be cohorted with other individuals who have received a positive or presumptive positive result.<br><br>Individual samples from all subjects in the pool should be re-tested individually.<br><br>If only the Orf1ab target is detected, re-extract the sample and run the rRT-PCR again. If the second run returns presumptive positive report presumptive positive results to sender and appropriate public health authorities. |
| - (Cq > 40) | + (Cq ≤ 40) | ± | | |
| + (Cq ≤ 40) | - (Cq > 40) | ± | | |
| - (Cq > 40) | - (Cq> 40) | - | Invalid[b] | Repeat test on pooled sample one time.<br><br>If the result is Negative, report pooled result to program administrator and appropriate public health authorities.<br><br>If the result is Positive or Invalid, report the result as Presumed Positive to program administrator. A new sample from all subjects in the pool should be run and re-tested individually. |
| - (Cq> 40) | - (Cq > 40) | + | Negative[a,d] | Report pooled result to program administrator and appropriate public health authorities |

[a]Presumptive Positive" and "Negative" are the specific result interpretations for samples tested as part of the 10-swab or 10 media pooling procedure.

[b]Individuals included in a pool that returns a positive or invalid result should be treated as a presumptive positive unless or until they receive a negative result when re-tested individually. However, as most individuals in a positive pool will likely receive a negative result when re-tested individually, they should isolate until receiving a negative result when re-tested individually and should not be cohorted with other individuals who have received a positive or presumptive positive result. For serial testing programs, additional confirmatory testing for positive results may also be necessary, if there is a low likelihood of COVID-19, such as in individuals without known exposure to COVID-19 or residing in communities with low prevalence of infection.

[c]Results of follow-up testing are reported to the program administrator and appropriate public health authorities.

[d]Specimens with low viral loads may not be detected with pooled testing due to decreased sensitivity or increased interference from pooled testing. For serial testing programs, additional confirmatory testing for negative results may be necessary, if there is a high likelihood of COVID-19, such as an individual with a close contact with COVID-19 or with suspected exposure to COVID-19 or in communities with high prevalence of infection.

## Examples

The first screenshot below guides you through key components of the qualitative result screen followed by examples of the possible Biomeme SARS-CoV-2 Real-Time Rt-PCR test results as outlined in the Interpretation Table above. For use of the SARS-COV-2 test with alternative thermocyclers, please refer to Appendix 1 and Appendix 2.

### Qualitative Result Screen Components

1. **Export Your Results**
   Share your results via email or download to a shared drive (e.g. Google Drive).

2. **Fluorescent Channels**
   See which fluorescent channels were detected during your run (e.g. Green, Amber, Red).

3. **Well Selection**
   Toggle tabs to see your results per Go-Strip, per channel (e.g. Wells 1 - 3, 4 - 6, 7 - 9).

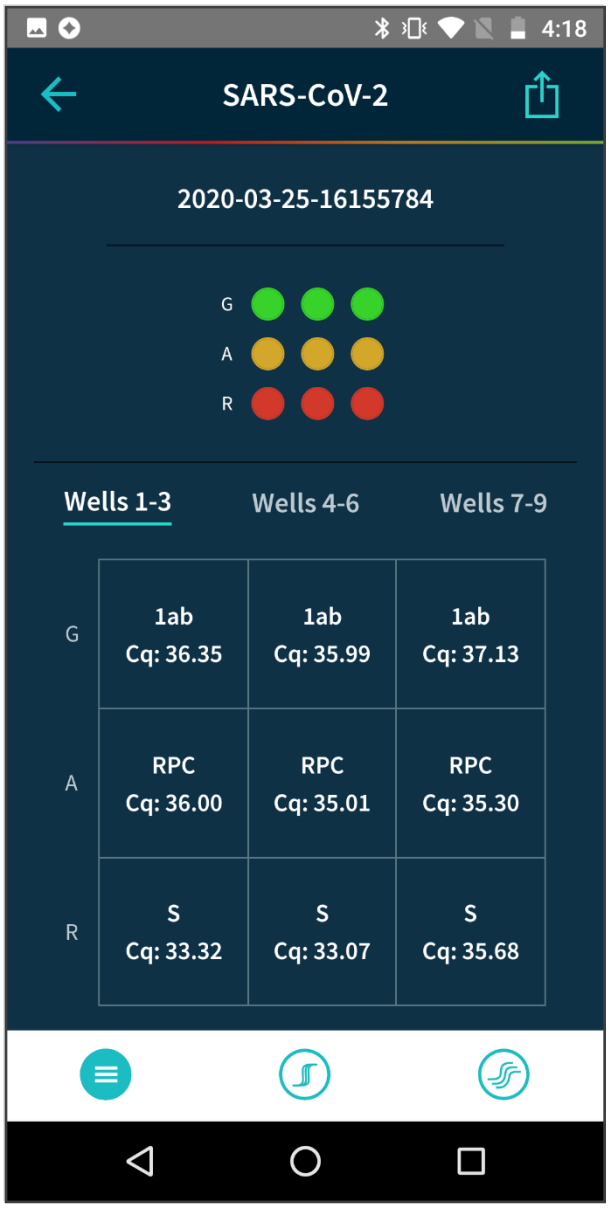
4. **Cq Values per Target/Sample**
   View Cq values for each of your targets per sample.

5. **Baselined Data**
   View amplification plots for your baselined data.

6. **Raw Data**
   View amplification plots for your raw data.

## Positive Results

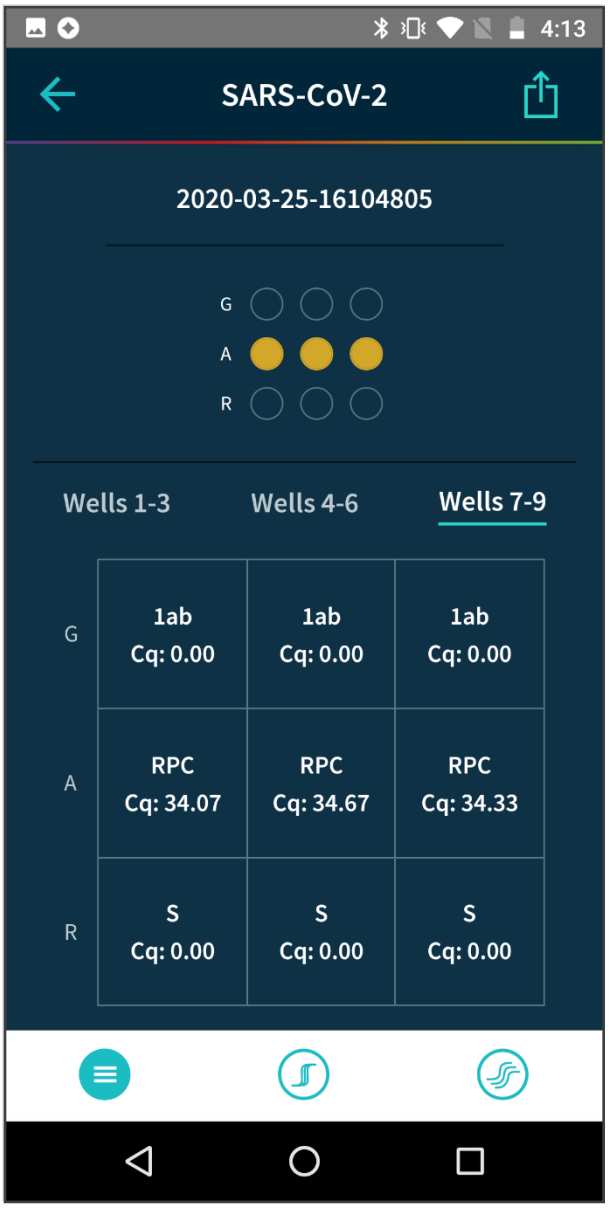
All targets detected




Report results to the sender and appropriate public health authorities.

## Negative Results

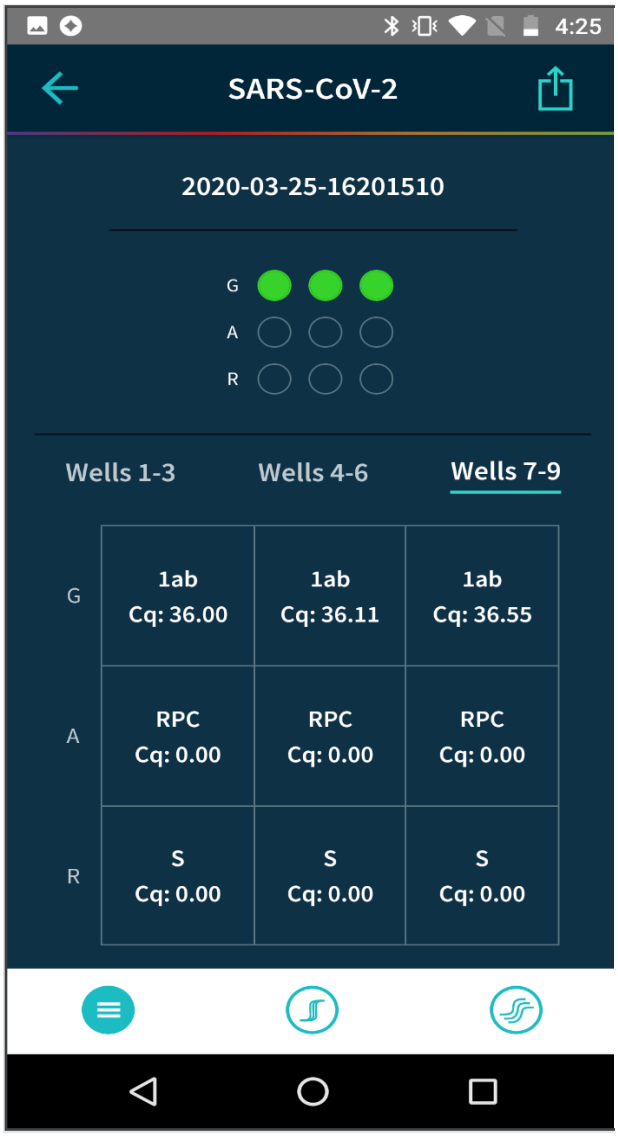
Only RPC detected




Report results to sender.

## Presumptive Positive Results

Only Orf1ab target detected

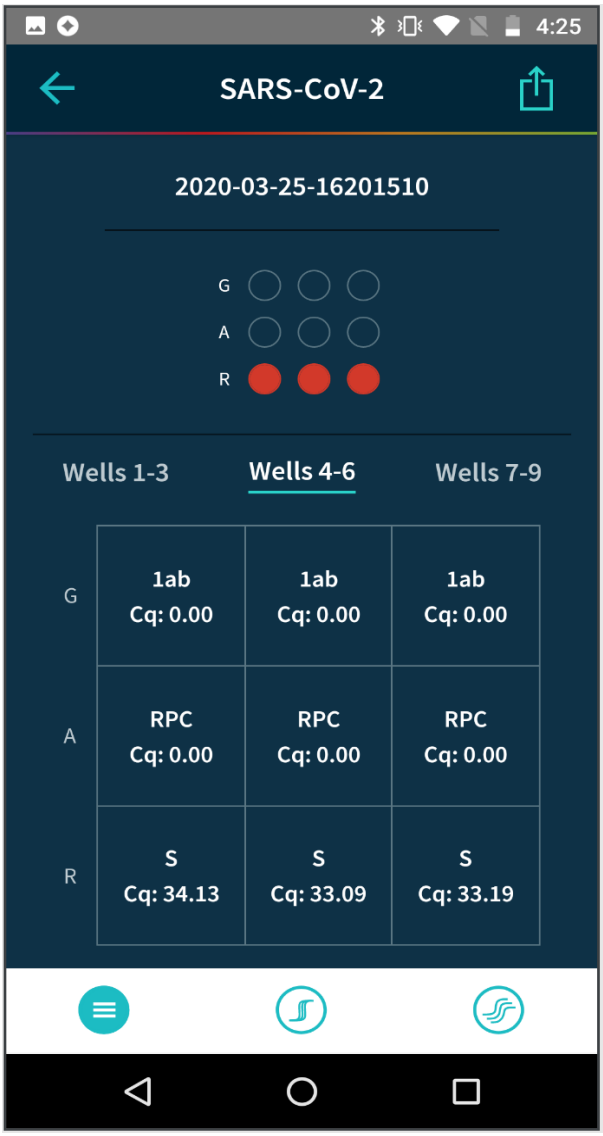



Re-extract the sample and run the rRT-PCR again. If the second run returns presumptive positive report presumptive positive results to sender and appropriate public health authorities.

For samples with a repeated presumptive positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and other SARS-like viruses for epidemiological purposes or clinical management.

## Positive Results

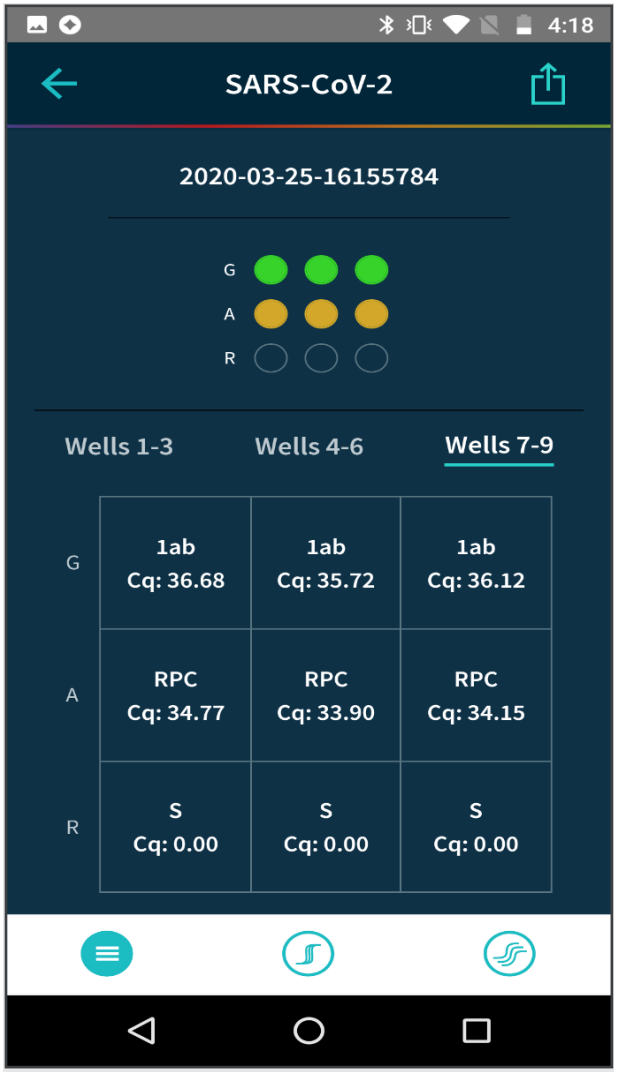
Only S target detected




Report results to the sender and appropriate public health authorities.

## Presumptive Positive Results

Orf1ab target & RPC detected
S target not detected

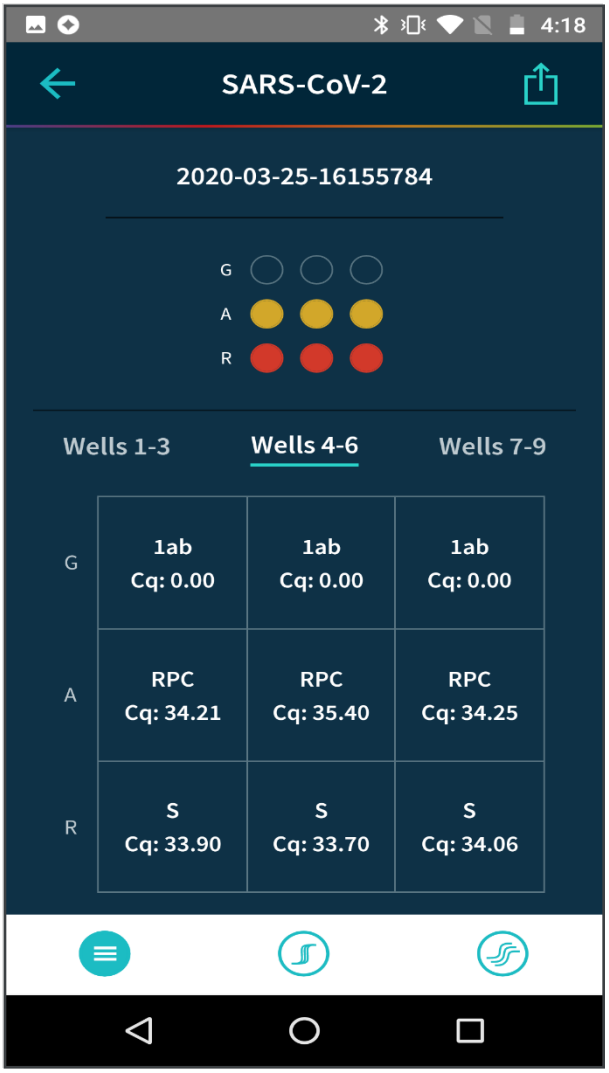



Re-extract the sample and run the rRT-PCR again. If the second run returns presumptive positive report presumptive positive results to sender and appropriate public health authorities.

For samples with a repeated presumptive positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and other SARS-like viruses for epidemiological purposes or clinical management.

## Positive Results

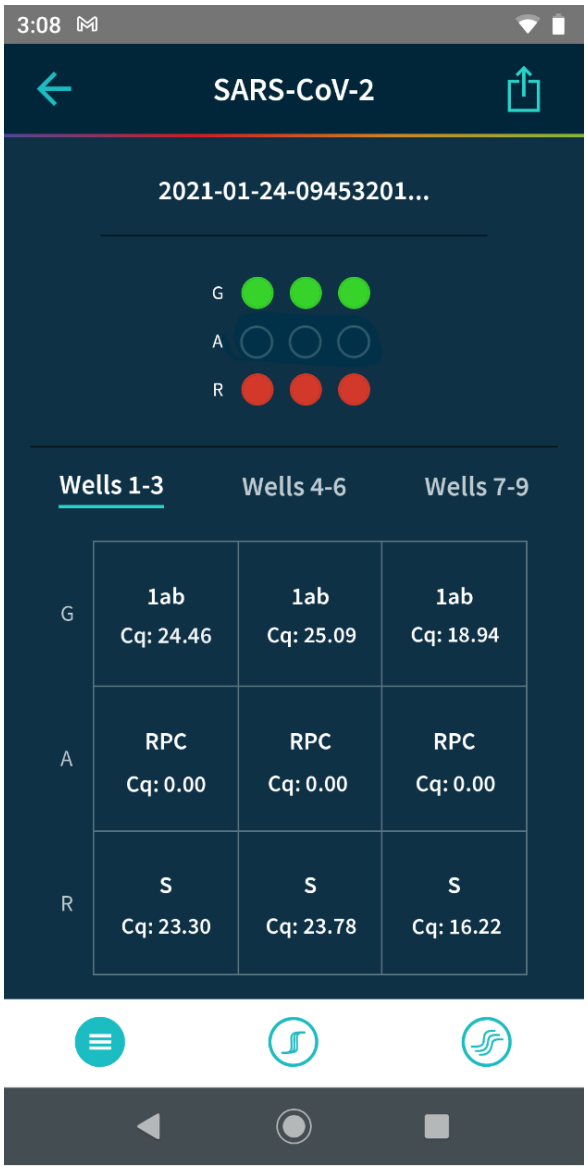
S target & RPC detected
Orf1ab target not detected




Report results to the sender and appropriate public health authorities.

## Positive Results

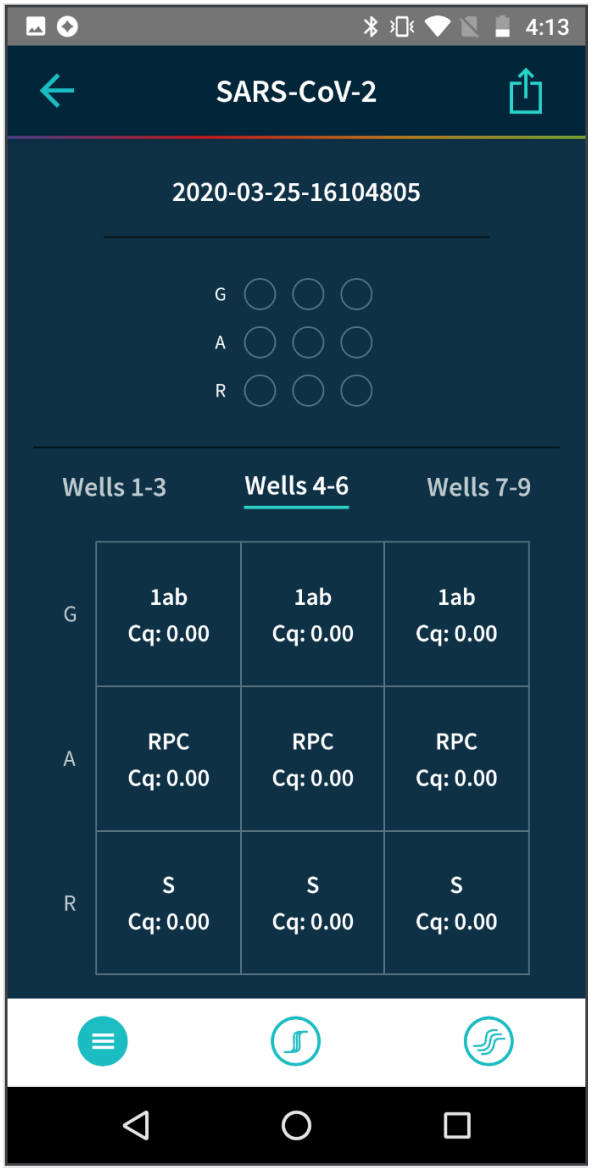
S target & Orf1ab detected
RPC not detected




Report results to the sender and appropriate public health authorities.

## Invalid Results

Nothing detected




Re-extract the sample and run the rRT-PCR again. If the same result is obtained as the first run, report as invalid.

# Assay Limitations

- The Biomeme SARS-CoV-2 Real-Time RT-PCR Test is for in vitro diagnostic use under FDA Emergency Use Authorization only. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
- Biomeme SARS-CoV-2 Real-Time RT-PCR Test performance was established using nasopharyngeal swab.

  *Note:* Nasal and oropharyngeal swab specimens, as well as nasopharyngeal wash/aspirate or nasal aspirate specimens are considered acceptable specimen types for use with Biomeme SARS-CoV-2 Real Time RT-PCR test.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.
- Extraction and amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.
- False-negative results may arise from:
  - Improper sample collection
  - Degradation of the viral RNA during shipping/storage
  - Specimen collection after nucleic acid can no longer be found in the specimen matrix
  - Using unauthorized extraction or assay reagents
  - The presence of RT-PCR inhibitors
  - Mutation in the SARS-CoV-2 virus
  - Failure to follow instructions for use
- False-positive results may arise from:
  - Cross contamination during specimen handling or preparation
  - Cross contamination between patient samples
  - Specimen mix-up
  - RNA contamination during product handling

- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated. The Biomeme SARS-CoV-2 Real-Time RT-PCR Test cannot rule out diseases caused by other bacterial or viral pathogens.
- Negative results do not preclude infection with SARS-CoV-2 virus and should not be the sole basis of a patient management decision. Negative results must be combined with clinical observations, patient history, and epidemiological information.
- Based on the *in silico* analysis, SARS-CoV and other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV-2 may cross-react with the Biomeme Orf1ab target. SARS-CoV is not known to be currently circulating in the human population, and therefore is highly unlikely to be present in patient specimens.
- Results from this test should be used in conjunction with clinical correlation with patient history and other diagnostic information available to the physician.
- Laboratories are required to report all positive results to the appropriate public health authorities.
- 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.

# Conditions of Authorization for Labs

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

To assist clinical laboratories using the Biomeme SARS-CoV-2 Real-Time RT-PCR Test, the relevant Conditions of Authorization are listed below, and are required to be met by laboratories performing the EUA test.

- Authorized laboratories[1] using the Biomeme SARS-CoV-2 Real-Time RT-PCR Test will include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using the Biomeme SARS-CoV-2 Real-Time RT-PCR Test will use the Biomeme SARS-CoV-2 Real-Time RT-PCR Test 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 the Biomeme SARS-CoV-2 Real-Time RT-PCR Test are not permitted.
- Authorized laboratories that receive the Biomeme SARS-CoV-2 Real-Time RT-PCR Test must notify relevant public health authorities of their intent to run the test prior to initiating testing.
- Authorized laboratories using the Biomeme SARS-CoV-2 Real-Time RT-PCR Test will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories will collect information on the performance of the test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: **CDRH-EUA-Reporting@fda.hhs.gov**) and Biomeme (**support@biomeme.com**, 267-930-7707) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.
- All laboratory personnel using the test must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use the test in accordance with the authorized labeling.
- Biomeme, its authorized distributor(s) and authorized laboratories using the Biomeme SARS-CoV-2 Real-Time RT-PCR Test will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1] 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.”

## Conditions related to pooling testing

- Authorized laboratories that previously used the Biomeme SARS-CoV-2 Real-Time RT-PCR Test must notify the relevant public health authorities of their intent to run the test with pooled samples prior to initiating testing.
- Authorized laboratories testing pooled specimens with your test must include with test result reports for specific individuals whose specimen(s) were the subject of pooling, a notice that pooling was used during testing and that "Individual specimens with low viral loads may not be detected due to the decreased sensitivity or increased interference when tested with pooled testing."
- Authorized laboratories must follow the Biomeme SARS-CoV-2 Real-Time RT-PCR Test pooling protocol that includes instructions for follow up for positive and invalid pools, including follow-up instructions to be provided to the organizer of the testing program. For media pooling, the instructions for follow up for positive and invalid pools must include deconvoluting to retest individual samples. For dry swab pooling, the instructions for follow up for positive and invalid pools must include reporting as "presumed positive" unless or until the individual is re-tested individually and must include instructions to collect a new specimen to be tested individually. However, as most individuals in a positive pool will likely receive a negative result when re-tested individually, the instructions must indicate that such individuals should isolate until receiving a negative result when re-tested individually and should not be cohorted with other individuals who have received a positive or presumptive positive result.
- Authorized laboratories testing pooled specimens with the Biomeme SARS-CoV-2 Real-Time RT-PCR Test must include with test result reports for specific individuals whose specimen(s) were the subject of pooling, a notice that their test result is "presumed positive" unless or until they are re-tested individually if the pool in which they were included returns a positive or invalid result. However, as most individuals in a positive pool will likely receive a negative result when re-tested individually, the report must include instructions to collect a new specimen to be tested individually and must indicate that such individuals should isolate until receiving a negative result when re-tested individually and should not be cohorted with other individuals who have received a positive or presumptive positive result.

- Authorized laboratories testing specimens using pooled testing with the Biomeme SARS-CoV-2 Real-Time RT-PCR Test must monitor the positivity rate of the specimens tested using pooled testing by calculating the percent positive results using a moving average (such as a rolling average updated daily using data from the previous 7-10 days).
- Authorized laboratories must keep records of specimen pooling test result data, daily testing totals including number of pooled test results, number of individuals tested and daily running average of percent positive results. For the first 12 months from the date of their creation, such records must be made available to FDA upon request within 48 business hours for inspection. After 12 months from the date of their creation, upon FDA request, such records must be made available for inspection within a reasonable time.

# Performance Characteristics

## Analytical Performance

### Analytical Sensitivity (Limit of Detection)

LoD studies determine the lowest detectable concentration of viral genomic RNA for both Orf1ab and S targets that consistently yields a 95% positivity. The LoD study was done by spiking in known concentrations of Genomic RNA from SARS-Related Coronavirus 2, Isolate USA-WSA1/2020 (BEI NR-52285, Lot: 70033320, Mftg Date: 11FEB2020) into individual clinical negative matrix (NP swab). The clinical negative matrix was mixed with BLB in the red section of Biomeme M1 sample prep cartridge for RNA 2.0 prior to addition of viral genomic RNA. The RNA control MS2 bacteriophage pellet was resuspended by provided resuspension buffer from Biomeme M1 sample prep cartridge for RNA 2.0 kit and added into the mix. Samples were extracted via Biomeme M1 sample prep cartridge for RNA 2.0 (REF 3000536). Real-Time RT-PCR assays were performed using Biomeme's SARS-CoV-2 Go Strips, (REF 3000555) on Biomeme Franklin three9 Real-Time PCR Thermocycler (REF 000003) and Android Smartphone W/Biomeme Go Mobile App (REF 1000013).

A preliminary LoD was determined by extracting and testing three 3-fold serial dilutions of the viral genomic RNA in negative NP swab matrix. A confirmation of LoD was determined by extracting and testing 20 replicates of two 3-fold serial dilutions of viral genomic RNA in negative NP swab matrix.

The preliminary LoD was then confirmed by testing 40 individual nasopharyngeal swab specimens spiked with 1.8 genomic copies/µL of SARS CoV-2 RNA (BEI Resources). The observed positivity rate among the 40 samples was 95% and 97.5% for the Orf1ab and S targets, respectively.

## Table 6: Preliminary LoD Study Data with Biomeme Franklin Thermocycler

| | % Reactivity | | |
|---|---|---|---|
| **Sample Concentration** | **Orf1ab Target** | **S Target** | **RPC Target** |
| 0.9 GE/µL | 80% (4/5) | 100% (5/5) | 100% (5/5) |
| 1.8 GE/µL | 100% (5/5) | 100% (5/5) | 100% (5/5) |
| 2.7 GE/µL | 100% (5/5) | 100% (5/5) | 100% (5/5) |
| 3.6 GE/µL | 100% (5/5) | 100% (5/5) | 100% (5/5) |

## Table 7: LoD Confirmation Data with Biomeme Franklin Thermocycler

| | % Reactivity | | |
|---|---|---|---|
| **Sample Concentration** | **Orf1ab Target** | **S Target** | **RPC Target** |
| 1.8 GE/µL | 95% (38/40) | 97.5% (39/40) | 100% (20/20) |
| 0.6 GE/µL | 90% (18/20) | 95% (19/20) | 100% (20/20) |

## Table 8: Final Limit of Detection

| Virus | Material | Limit of Detection (genome equivalent per uL) |
|---|---|---|
| Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) | Genomic RNA | 1.8 |

**FDA SARS-CoV-2 Reference Panel Testing**

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The extraction method and instrument used were Biomeme M1 Sample Prep Cartridge Kit for RNA 2.0 and Bio-rad CFX-96 with Bio-Rad CFX Maestro 1.1 version 4.1.2433.1219. The results are summarized in Table 9.

## Table 9: Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

| Reference Materials Provided by FDA | Specimen Type | Product LoD | Cross-Reactivity |
|---|---|---|---|
| SARS-CoV-2 | Nasopharyngeal Swabs | $1.8 \times 10^4$ NDU/mL | N/A |
| MERS-CoV | | N/A | ND |

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

An adaptive LoD Study was performed to establish equivalent assay performance to the Biomeme Franklin™ on the Bio-Rad CFX and the Applied Biosystems QuantStudio 5 thermocycler. 5 replicates each at 0.5x LoD, 1x LoD, 1.5x LoD, and 2x LoD were run on the alternative thermocyclers. Dilutions were made by spiking SARS-Related Coronavirus 2, Isolate USA-WSA1/2020 (BEI NR-52285, Lot: 70033320, Mftg Date: 11FEB2020) into negative clinical NP matrix. 4/5 replicates were positive at 0.5xLoD, while 5/5 replicates were positive at 1x LoD, for each alternative instrument being tested (see Table 9 and Table 10).

A confirmatory LoD study was performed at 1x LoD by spiking SARS-Related Coronavirus 2, Isolate USA-WSA1/2020 (BEI NR-52285, Lot: 70033320, Mftg Date: 11FEB2020) into 20 individual clinical negative NP matrices. All 20 replicates, or 100%, were positive across all instruments at this concentration (1.8 GE/uL) (see Table 12 and Table 13).

## Table 10: Bio-Rad CFX96 LoD

| | % Reactivity | | |
|---|---|---|---|
| **Sample Dilution** | **Orf1ab Target** | **S Target** | **MS2 Target** |
| 0.5x LoD | 100% (5/5) | 80% (4/5) | 100% (5/5) |
| 1x LoD | 100% (5/5) | 100% (5/5) | 100% (5/5) |
| 1.5x LoD | 100% (5/5) | 100% (5/5) | 100% (5/5) |
| 2x LoD | 100% (5/5) | 100% (5/5) | 100% (5/5) |

## Table 11: Applied Biosystems QuantStudio 5 LoD

| | % Reactivity | | |
|---|---|---|---|
| **Sample Dilution** | **Orf1ab Target** | **S Target** | **MS2 Target** |
| 0.5x LoD | 80% (4/5) | 100% (5/5) | 100% (5/5) |
| 1x LoD | 100% (5/5) | 100% (5/5) | 100% (5/5) |
| 1.5x LoD | 100% (5/5) | 100% (5/5) | 100% (5/5) |
| 2x LoD | 100% (5/5) | 100% (5/5) | 100% (5/5) |

## Table 12: Bio-Rad CFX96 LoD Confirmation

| | % Reactivity | | |
|---|---|---|---|
| **Sample Dilution** | **Orf1ab Target** | **S Target** | **MS2 Target** |
| 1.8 GE/µL | 100% (20/20) | 100% (20/20) | 100% (20/20) |

## Table 13: Applied Biosystems QuantStudio 5 LoD Confirmation

| | % Reactivity | | |
|---|---|---|---|
| | **Orf1ab Target** | **S Target** | **MS2 Target** |
| 1.8 GE/µL | 100% (20/20) | 100% (20/20) | 100% (20/20) |

The performance equivalence between Biomeme M1 sample prep cartridge for RNA 2.0 (carrier RNA) and RNA 2.0 NC (no carrier RNA) was tested by using 3 different concentrations at 1X established LoD and 3X above and below LoD by spiking SARS-Related Coronavirus 2, Isolate USA-WSA1/2020 (BEI NR-52285, Lot: 70033320, Mftg Date: 11FEB2020) into clinical negative nasopharyngeal swab matrix (Biomeme Preservation Buffer). Five extractions were performed for each concentration for both Biomeme M1 sample prep with carrier RNA and no carrier RNA. Each extraction was run in single PCR replicate (Table 14).

## Table 14: Equivalence Between Carrier/No Carrier Sample Prep

| | | % Reactivity | | |
|---|---|---|---|---|
| Sample Dilution | | Orf1ab target | S target | MS2 target |
| 0.6 GE/µL | Carrier RNA | 100% (5/5) | 100% (5/5) | 100% (5/5) |
| | No Carrier RNA | 100% (5/5) | 100% (5/5) | 100% (5/5) |
| 1.8 GE/µL | Carrier RNA | 100% (5/5) | 100% (5/5) | 100% (5/5) |
| | No Carrier RNA | 100% (5/5) | 100% (5/5) | 100% (5/5) |
| 5.4 GE/µL | Carrier RNA | 100% (5/5) | 100% (5/5) | 100% (5/5) |
| | No Carrier RNA | 100% (5/5) | 100% (5/5) | 100% (5/5) |

The equivalence performance between BD VTM and Biomeme Preservation Buffer was tested at 1X established LoD by spiking SARS-Related Coronavirus 2, Isolate USA-WSA1/2020 (BEI NR-52285, Lot: 70033320, Mftg Date: 11FEB2020) into clinical negative nasopharyngeal swab matrix (collected in either BD VTM or Biomeme Preservation Buffer). Five extractions were performed for both collection media. Each extraction was run in single PCR replicate (Table 15).

## Table 15: Equivalence Between BD VTM and Biomeme Preservation Buffer

| | | % Reactivity | | |
|---|---|---|---|---|
| | | Orf1ab target | S target | MS2 target |
| 1x LoD | Biomeme Preservation Buffer | 100% (5/5) | 100% (5/5) | 100% (5/5) |
| | BD VTM | 100% (5/5) | 100% (5/5) | 100% (5/5) |

The limit of detection for the KingFisher Flex Purification system with MagMax Viral/Pathogen Nucleic Acid isolation kit was determined by performing a tentative LoD using a 10-fold dilution series at 1.8 GE/uL, 0.18 GE/uL and 0.018 GE/uL by spiking SARS-Related Coronavirus 2, Isolate USA-WSA1/2020 (BEI NR-52285, Lot: 70033320, Mftg Date: 11FEB2020) into clinical negative matrix using Biomeme preservation buffer. 5 extraction preps were performed per concentration using the KingFisher system and run single PCR replicates using Biomeme's SARS-CoV-2 Go-Strips (REF3000555) on Bio-Rad CFX-96 thermocycler. The lowest concentration that gave 5/5 of positive results (1.8 GE/uL) was tested for a confirmatory LoD using 20 replicates. 20/20 replicates in the confirmatory LoD were detected therefore we performed another tentative LoD.

Concentrations picked for the second tentative LoD were 0.9 GE/uL, 0.54 GE/uL and 0.36 GE/uL. 5 extraction preps per concentration and single PCR replicates were performed. The lowest concentration that gave 5/5 of positive results (0.9 GE/uL) was tested for a confirmatory LoD

using 20 replicates. 19/20 replicates were detected. Therefore, the new established LoD for KingFisher instrument is 0.9 GE/uL.

## Table 16: KingFisher system with MagMax Viral/Pathogen kit LoD confirmation on Bio-Rad CFX-96

| | % Reactivity | | |
|---|---|---|---|
| | Orf1ab Target | S Target | MS2 Target |
| 0.9 GE/µL | 100% (19/20) | 100% (20/20) | 100% (20/20) |

## Analytical Reactivity (Inclusivity)

Orf1ab and S - BLASTn analysis queries alignments were performed with the oligonucleotide primer and probe sequences of the Biomeme SARS-CoV-2 Real-Time RT-PCR Test with publicly available nucleic acid sequences for SARS-CoV-2 in the betacoronavirus nucleotide sequence database as of July 1, 2020 to demonstrate the predicted inclusivity of the Biomeme SARS-CoV-2 Real-Time RT-PCR Test. The Orf1ab primers and probe show 100% homology to greater than or equal to 99.96% of significant sequences returned. The S primers and probe show 100% homology to greater than or equal to 99.87% of significant sequences returned. A small number of significant sequence alignments showed a single mismatch in the primer or probe regions. No sequence was found to contain more than a single mismatch in each primer and probe in the assay. No sequence was found to contain more than a single mismatch in more than one of the primers and probe in either the Orf1ab or S assays.

Biomeme continuously monitors and analyzes genetic sequences of emerging variants of concern. For the most up-to-date variant inclusivity analysis visit: info.biomeme.com/covid-19

**Variants of Concern Inclusivity Analysis**

Several variants of concern which contain multiple mutations and or deletions in the Orf1ab and S genes have emerged in the fall of 2020. BLASTn analysis queries alignments were performed with the oligonucleotide primer and probe sequences of the Biomeme SARS-CoV-2 Real-Time RT-PCR Test with publicly available nucleic acid sequences for SARS-CoV-2 from GISAID.

- The search parameters automatically adjust for short input sequences, the word size is 16 and expected threshold is 10.
- The Hitlist size is for Inclusive is 250.

- The match and mismatch scores are 1 and -2, respectively; The penalty to create and extend a gap in an alignment is 0 and 2.5 respectively.

**United Kingdom New Variant VUI 202012/01, 20I/501Y.V1**

The United Kingdom has reported a new variant, termed VUI 202012/01 (Variant Under Investigation, year 2020, month 12, variant 01), which is defined by multiple spike protein mutations. This variant is classified in clade GR, lineage B.1.1.7. Genome sequences (n=250) of SARS-CoV-2 (filtered for clade GR, lineage B.1.1.7 containing mutation Spike_N501Y) were downloaded from GISAID with collection dates from the 2nd of November 2020 to the 17th of December 2020. BLASTn analysis alignments were performed with the oligonucleotide primer and probe sequences of the Biomeme SARS-CoV-2 multiplex assay against all 250 genomes.

- Oligonucleotide primer and probe sequences of the Biomeme SARS-CoV-2 multiplex assay (Orf1ab and S gene targets) have 100% homology to the 250 genomes analyzed of SARS-CoV-2 variant VUI 202012/01.

**South Africa New Variant 20H/501Y.V2 Inclusivity Analysis**

South Africa has reported a novel mutation combination in the Spike receptor binding site, which is defined by multiple spike protein mutations. This variant is classified in clade GH, lineage B.1.351. Genome sequences (n=267) of SARS-CoV-2 (filtered for clade GH, lineage B.1.351 containing mutation Spike_N501Y) were downloaded from GISAID with collection dates from the 2nd of November 2020 to the 10th of December 2020. BLASTn analysis alignments were performed with the oligonucleotide primer and probe sequences of the Biomeme SARS-CoV-2 multiplex assay against all 267 genomes.

- Oligonucleotide primer and probe sequences of the Biomeme SARS-CoV-2 multiplex assay S gene target has 100% homology to the 267 genomes analyzed of SARS-CoV-2 South Africa Clade GH, lineage B.1.351.

- Oligonucleotide primer sequences of the Biomeme SARS-CoV-2 multiplex assay Orf1ab gene target has 100% homology to 99% of the genomes analyzed (n=264 of 267) of SARS-CoV-2 South Africa Clade GH, lineage B.1.351.

- Three sequences (n=3) were found to have 95% homology containing single mismatch in Orf1ab F or R primers 5' ends.

**Brazil Variant 20J/501Y.V3 Inclusivity Analysis**

Brazil has reported a strain with multiple spike gene mutations as well as a deletion in Orf1ab gene. This variant is classified as 20J/501Y.V3. Genome sequences (n=178) of SARS-CoV-2 (filtered for clade GR variant 484K.V2) were downloaded from GISAID with collection dates from the 9th of October 2020 to the 10th of January 2021. BLASTn analysis alignments were performed with the oligonucleotide primer and probe sequences of the Biomeme SARS-CoV-2 multiplex assay against all 178 genomes.

- Oligonucleotide primer and probe sequences of the Biomeme SARS-CoV-2 multiplex assay (Orf1ab and S gene targets) have 100% homology to the 178 genomes analyzed of SARS-CoV-2 variant 20J/501Y.V3.

**California Variant GH/452R.V1, B.1.429, CAL20.C**

California has reported a novel variant with a mutation L452R in the Spike gene. This variant is classified in clade GH, lineage B.1.429. Genome sequences (n=273) of SARS-CoV-2 (filtered for clade GH, lineage B.1.429 containing mutation Spike_L452R) were downloaded from GISAID with collection dates from the 28th of September 2020 to the 18th of January 2021. BLASTn analysis alignments were performed with the oligonucleotide primer and probe sequences of the Biomeme SARS-CoV-2 multiplex assay against all 273 genomes.

- Oligonucleotide primer and probe sequences of the Biomeme SARS-CoV-2 multiplex assay S gene target has 100% homology to 99.6% of the (n=272 of 273) genomes analyzed of SARS-CoV-2 Clade GH, lineage B.1.429.
  - One sequence was found to have 96% homology containing single mismatch in the S Probe.
- Oligonucleotide primer sequences of the Biomeme SARS-CoV-2 multiplex assay Orf1ab gene target has 100% homology the 273 genomes analyzed of SARS-CoV-2 Clade GH, lineage B.1.429.

## Analytical Specificity (Exclusivity and Cross-reactivity)

- Cross-reactivity of the Biomeme SARS-CoV-2 Real-Time RT-PCR Test was evaluated in an in-silico analysis against normal and pathogenic organisms associated with the respiratory tract.
- Biomeme Orf1ab Assay - The forward primer and probe sequences showed high sequence homology to Bat SARS-like coronaviruses. The reverse primer sequence showed high sequence homology to Bat SARS-like and human SARS coronavirus as well as *Candida albicans*. Combining primers and probe, no significant homologies with the human genome, other coronaviruses, common respiratory flora, human microflora and other viral pathogens that would predict potential false positive rRT-PCR results.
- Biomeme S Assay - Only the forward primer sequence showed high sequence homology to Bat SARS-like coronavirus. No other high sequence homology was observed for the forward primer sequence. The reverse primer and probe sequence showed no significant homologies with the human genome, other coronaviruses, common respiratory flora, human microflora and other viral pathogens that would predict potential false positives rRT-PCR results.
- In summary, the Biomeme SARS-CoV-2 Real-Time RT-PCR Test, a multiplex assay (Orf1ab and S), designed for the specific detection of SARS-CoV-2, showed no significant combined homologies with human genome or human microflora that would predict potential false positive RT-PCR results. Only the Orf1ab assay demonstrated significant homology among all assay components for bat SARS-like coronaviruses. However, the risk of false positive results due to potential reactivity with bat SARS coronaviruses not known to currently infect humans is low.

## Endogenous and Exogenous Interference Studies

The following substances were tested for inhibitory effects on RT-PCR using respiratory specimens positive for RNA viruses processed with the Biomeme M1 Sample Prep for RNA 2.0. No interference with any of the substances was observed.

## Table 17: List of Endogenous/Exogenous Substances Tested

| Interfering Substance | Active Ingredient | Concentration Tested |
|---|---|---|
| Blood | Human blood | 2% (v/v) |
| Throat lozenges | Benzocaine, menthol | 0.15 mg/mL |
| Saline Nasal Spray | Sodium chloride | 0.026 mg/mL |
| No Drip Nasal Mist | Oxymetazoline hydrochloride (0.05%) | 10% (v/v) |
| Extra Strength Nose Drops | Phenylephrine hydrochloride (1%) | 10% (v/v) |
| Saline Nasal Spray with Aloe | Sodium chloride (0.65%) | 10% (v/v) |
| Zicam, Nasal Swab Gel | Luffa operculata, Galphimia glauca, histaminum hydrochloricum, sulfur | 10% (w/v) |
| Flonase/Nasal corticosteroid | Fluticasone propionate (50mcg/ spray) | 10% (v/v) |

A separate study was conducted to test the effect of mucin on the SARS-CoV-2 Real-Time RT-PCR Test. In this study, three replicates of VTM containing SARS-CoV-2 RNA at 3X the LoD with or without 2% mucin were extracted using the Biomeme M1 Sample Prep Cartridge for RNA 2.0. All replicates were positive for SARS-CoV-2 with the Biomeme SARS-CoV-2 Real-Time RT-PCR Test.

## Clinical Evaluation

A total of 98 positive and 57 negative nasopharyngeal swab samples previously tested with an FDA EUA RT-PCR assay were tested with the Biomeme SARS-CoV-2 Real-Time RT-PCR Test. The results are presented in Table 18 below.

### Table 18: Overall Performance Estimate Based On Single Positive Target Algorithm

| | | EUA Authorized Comparator Method | |
|---|---|---|---|
| | | Positive | Negative |
| Biomeme | Positive | 95 | 1† |
| | Negative | 3* | 56 |

†Sample was retested by another comparator with results negative
*One sample was retested by another comparator with results positive

PPA: 96.9% (95% CI: 91.3% to 99.4%)
NPA: 98.3% (95% CI: 90.6% to 100%)

### Contrived Clinical Samples

The performance of the Biomeme SARS-CoV-2 Real-Time RT-PCR Test was evaluated using contrived clinical samples. A total of 60 contrived positive samples were prepared for testing by spiking individual negative clinical nasopharyngeal swab (NP) specimen matrix with known concentrations of genomic RNA from SARS-Related Coronavirus 2, Isolate USA-WSA1/2020 (BEI NR-52285, Lot: 70033320, Mftg Date: 11FEB2020). Prior to the addition of SARS-CoV-2 RNA, sample matrix was pre-mixed with Biomeme Lysis Buffer (BLB) from the Biomeme M1 Sample Prep Cartridge for RNA 2.0 nucleic acid extraction kit and the RPC (see Table ).

Of the 60 contrived positive samples, 40 contained SARS-CoV-2 RNA at 1x the LoD (same samples as those in the LoD confirmation study), 10 contained SARS-CoV-2 RNA at 2x the LoD, and the remaining 10 contained SARS-CoV-2 RNA between 3x and 5x the LoD. An additional 30 individual negative nasopharyngeal swab specimens were also included in the study. RNA from each sample was manually extracted using the Biomeme M1 Sample Prep Cartridge for RNA 2.0 with a total elution volume of 850µL. The results of the Biomeme SARS-CoV-2 Real-Time RT-PCR Test are shown in Table  below.

## Table 19: Clinical Evaluation with Contrived Nasopharyngeal Swab Specimens

| RNA Concentration (relative to LoD) | RNA Concentration (GE/µl) | Number of Positives | Mean Ct | |
|---|---|---|---|---|
| | | | Orf1ab | S |
| 1x | 1.8 | 39/40[a] | 34.75 | 33.51 |
| 2x | 3.6 | 10/10 | 33.19 | 31.14 |
| 3x | 5.4 | 5/5 | 33.46 | 32.36 |
| 4x | 7.2 | 3/3 | 30.96 | 29.71 |
| 5x | 9 | 2/2 | 31.24 | 30.05 |
| Negative | 0 | 0/30 | NA | NA |

[a] Samples prepared at 1X LoD are the same as those tested in the LoD confirmation study.

PPA at 1-2x LoD = 98.0% (95% CI: 89.4% to 100%)
PPA at 3-5x LoD = 100% (95% CI: 69.2 to 100%)
NPA= 100% (95% CI: 88.4 to 100%)

## Appendix 1: Loading SARS-CoV-2 Go-Plates for Bio-Rad CFX Maestro 96-well plate instruments

1. Open the contents of a Biomeme SARS-CoV-2 Go-Plate (REF# **3000562**).
    - If not loading the entire 96-well Go-Plate, cut the desired number of wells away from the Go-Plate using sterile scissors.
    - Insert the unused portion of the Go-Plate back into the foil pouch.
    - Remove any excess air from the pouch and reseal the ziplock.
    - Ensure the ziplock is fully sealed to maximize shelf-life of the unused reactions.
2. Place the wells you into to use into a 96-well plate tray for setup of your PCR run.
3. Working on one row at a time, carefully peel away the foil from the wells.
4. Attach a pipette tip to a 20µL fixed volume pipette (REF# **3000011**) or use your own 20uL pipette.
5. Unscrew the cap of your first purified sample and transfer 20µL of the extracted RNA into the first reaction well.

    *Note: The strip connections between the tubes of your Go-Plate will face the back of the thermocycler once inserted. When transferring your extracted RNA into the different reaction wells, replicate this orientation to ensure accurate result interpretation (e.g. transfer sample 1 into the far-left reaction well of your first Go-Plate Well, moving from a left to right orientation.*

6. Pipette up and down 3-5 times to mix your PCR reaction. When mixing your samples try to avoid introducing bubbles.

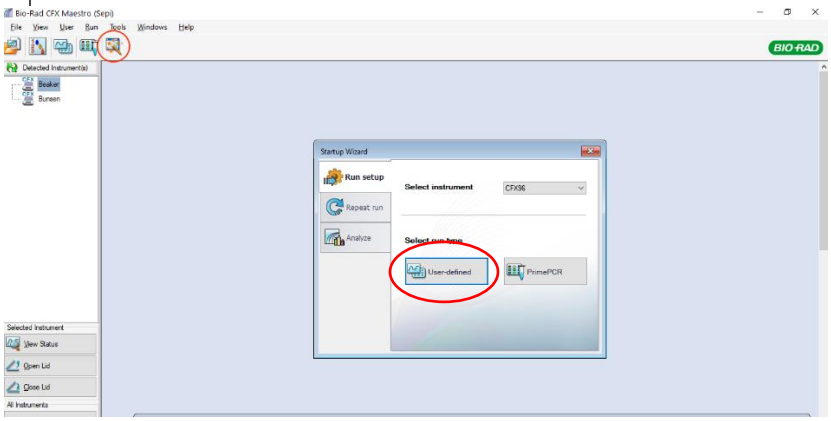
    *Note: If bubbles have been introduced, remove them from the lower portion of the PCR tubes by gently tapping them against your work surface before sealing. Bubbles may remain at the top of the tube, but bubbles at the bottom are not acceptable.*
7. Discard your pipette tip and repeat this process for the remaining reaction wells. Once all reaction wells are filled, apply an optical adhesive sealer. Firmly press down with a plastic sealer while moving around the outer edges of the top of the to ensure a good seal. Cut away any excess adhesive when less than 96 wells are used.

    *Note: You can use a plate spinner to make sure your PCR reactions are at the bottom of the plate.*

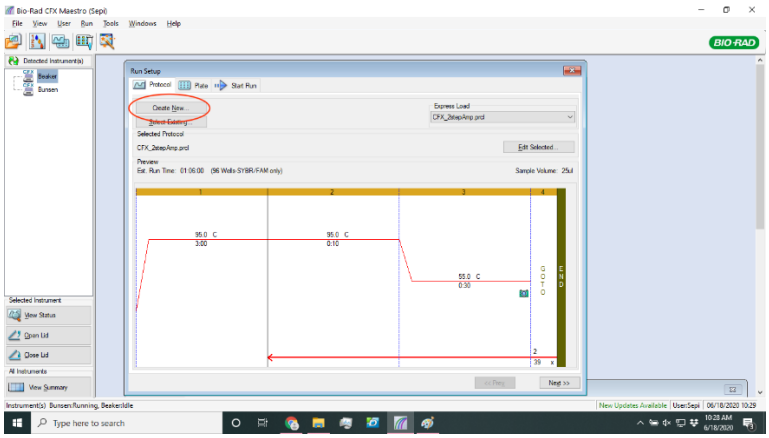
### Loading the Bio-Rad CFX-96:

1. Turn on Bio-Rad CFX-96 instrument
2. Open the Bio-Rad CFX Maestro 1.1 version 4.1.2433.1219 (REF 1855195)

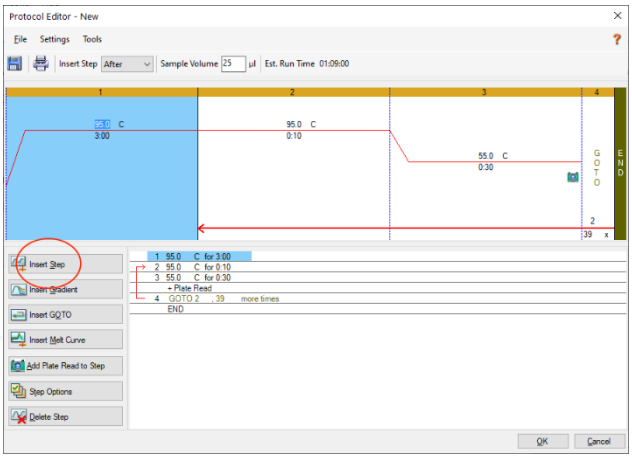
3. Select “Start Up Wizard” shown below:




4. Select “User-defined” tab. This will take you to Run set up page. Then click on “Create New” as shown below:



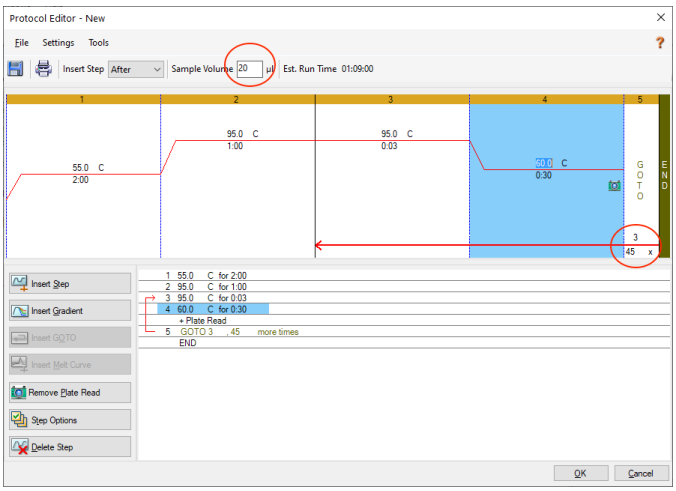

5. On the protocol editor page, click on “Insert Step” to add the RT step to the protocol



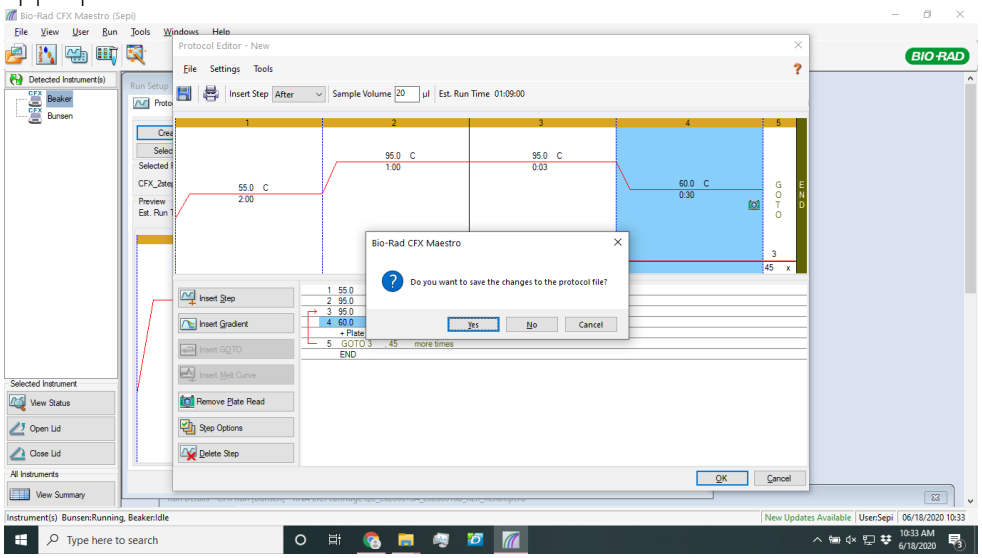

6. Set up your PCR thermocycling parameters as follows:
   a. Step 1 (RT): 55 C for 2 minutes

b. Step 2 (Initial Denature): 95˚C for 1 minute
c. Step 3 (Denature): 95˚C for 3 seconds
d. Step 4 (Anneal): 60˚C for 30 seconds

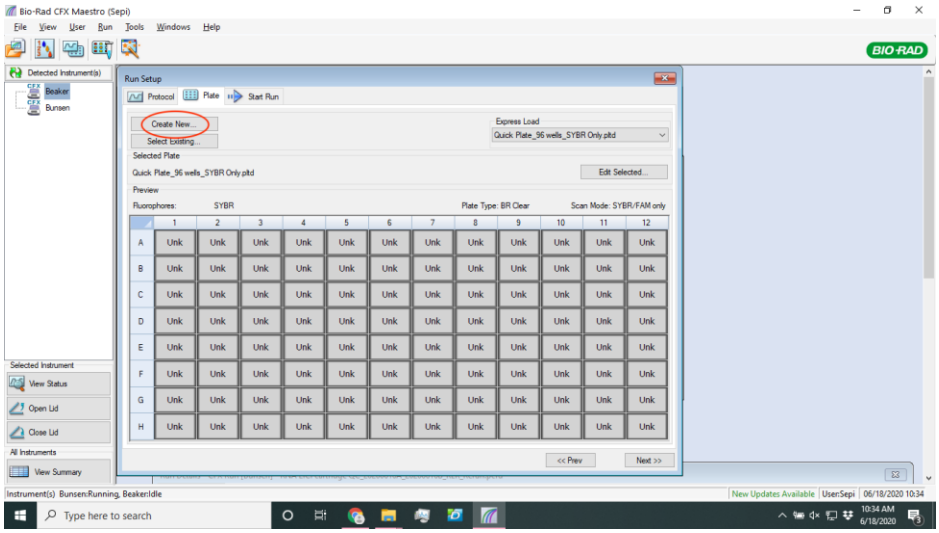
7. Change the cycles from a default of 39X to 45X.
8. Change the reaction volume shown below from default 25 uL to 20 uL.



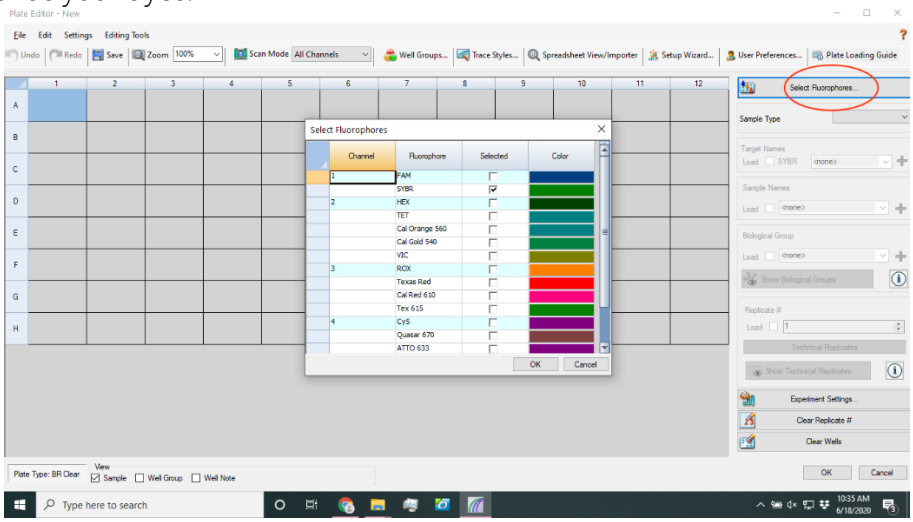

9. Press “Ok” once you are finished setting up the thermocycling parameters. The program will now ask whether you want to save your protocol file. Click on “Yes” and save your file where appropriate.



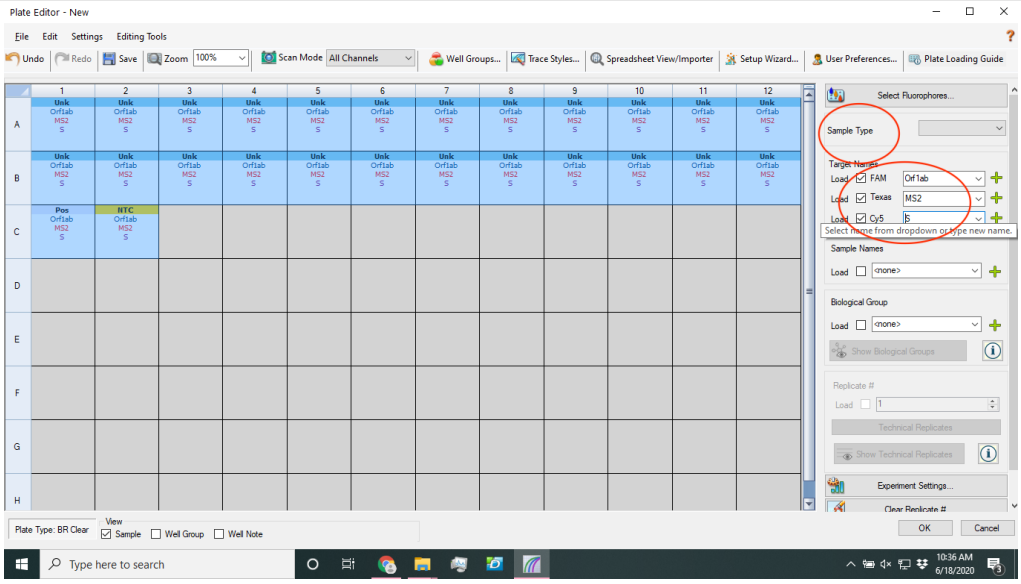

10. Once finished press “Next”. This will take you plate set up screen. Click on “Create New”. Now you will set up your plate layout.



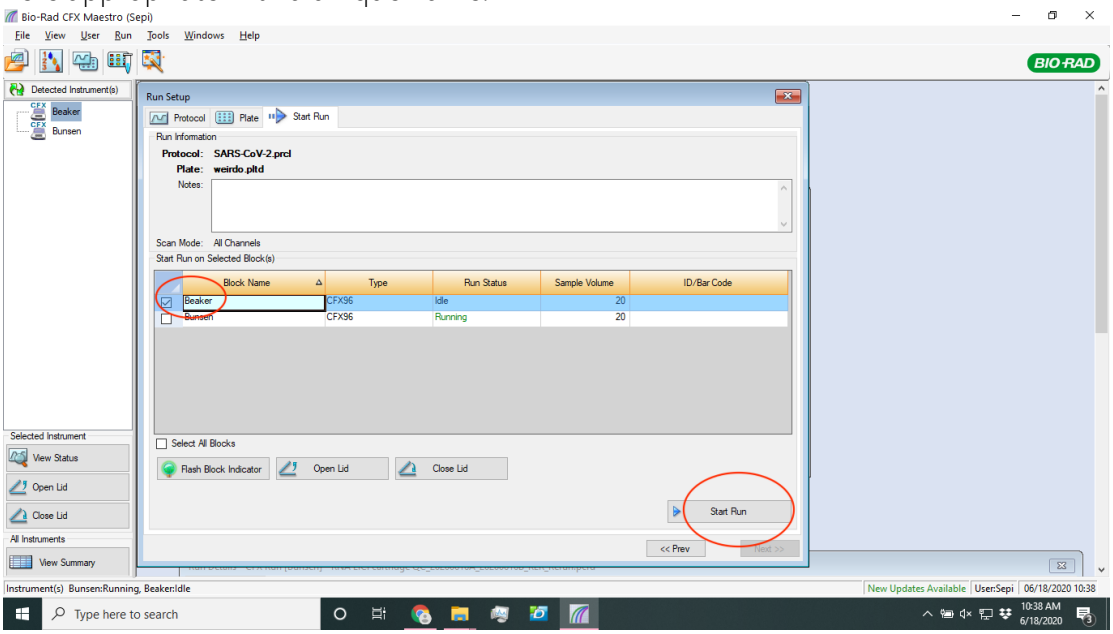

11. To start click on “Select Fluorophores”. From the list you want to select “FAM, Texas Red, and Cy5” as your dyes.



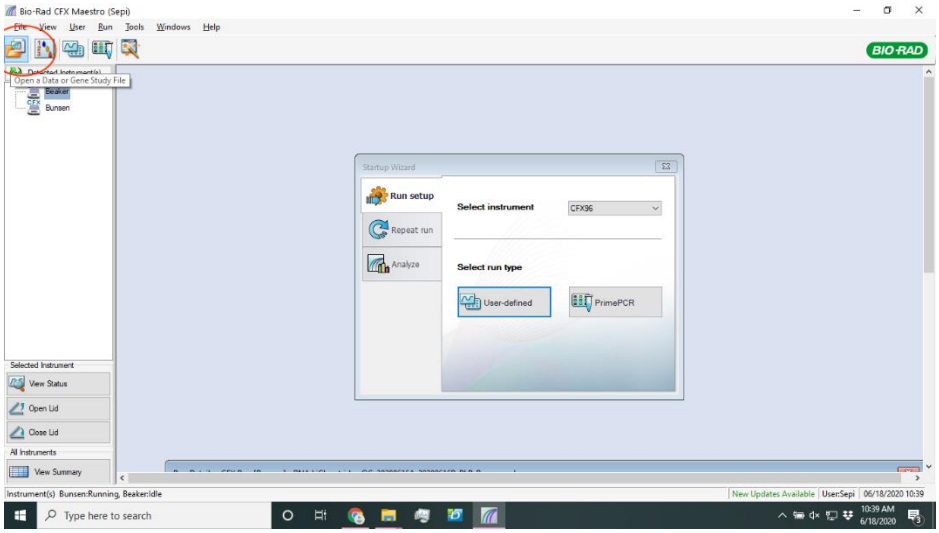

12. Depending on how you have loaded your plate, you can select “Sample Type” and give either “Unknown”, “Positive control”, or “NTC” tags to each well. For example, in the image below, 24 unknown samples were selected with one positive control and one NTC.
13. Name each target by typing the target name into each of the appropriate dyes:
    a. FAM: “Orf1ab”
    b. Texas Red: “MS2” or “RPC”
    c. Cy5: “S”



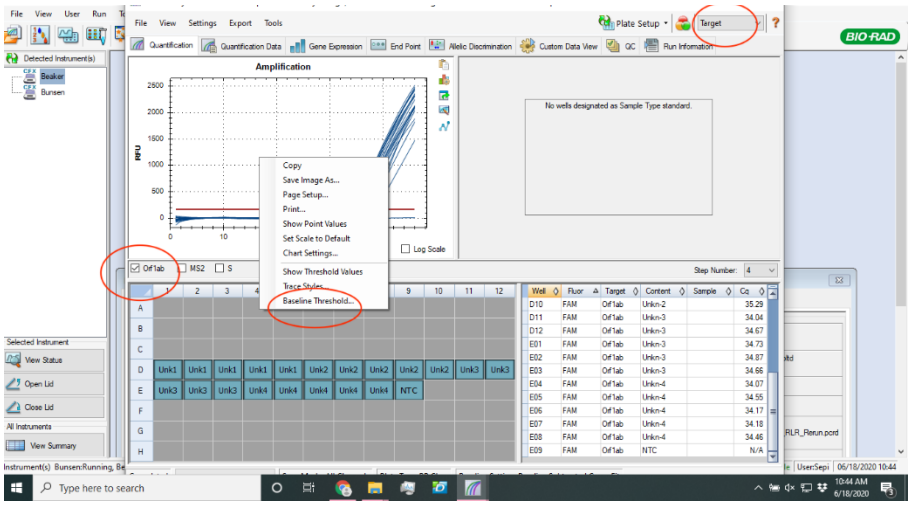

14. The software will ask you whether you want to save the plate setup. Click on “Yes” and save your plate file where appropriate.
15. Press on next to go to the “Start Run” page. If multiple Bio-Rad CFX-96 instruments are connected to the computer, select the appropriate one. Click on “Start Run”. The software will ask you again to save your data file. Click on “Yes” and save your data file where appropriate with a unique name.



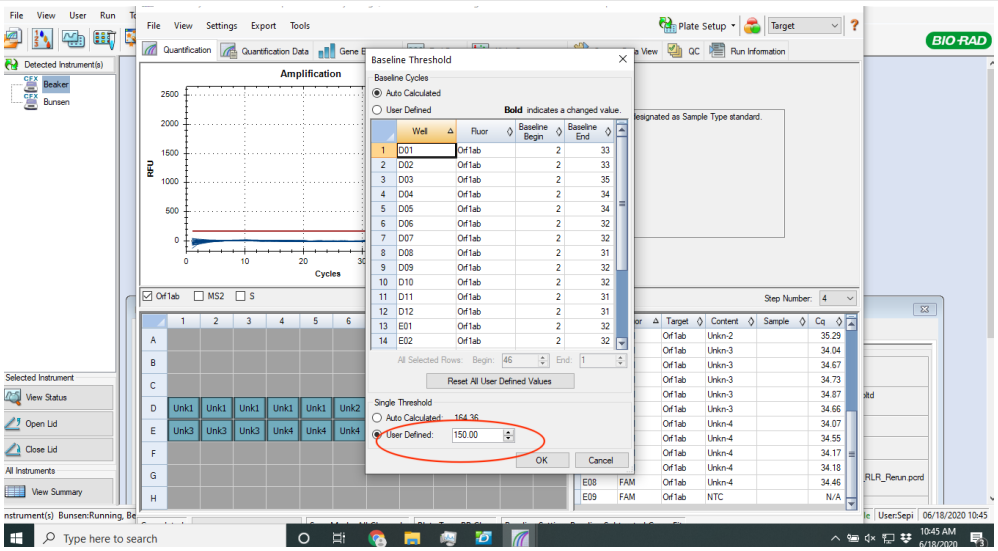

16. Once the run is finished, the result will pop up automatically on the screen. If it does not, then you can click on the “Open a Data or Gene Study File” icon shown below and open up your data file with the unique ID to view the results.



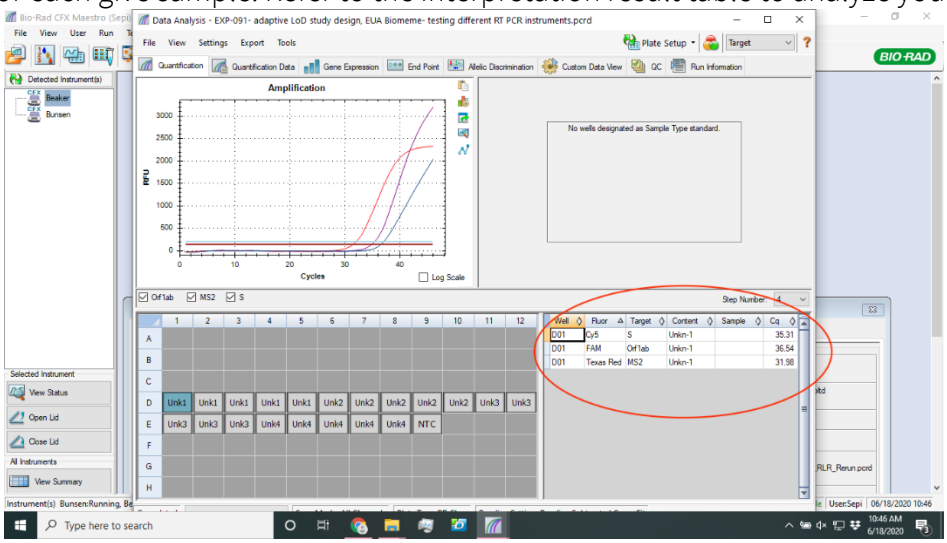

17. From the results screen, pick “Target” from the tab on the right-hand corner of the screen shown below. Select only one target, for example Orf1ab as shown. Right click on the screen and select “Baseline Threshold”.




18. On the baseline threshold page, click on “User Defined” and change the value to a specific number for each target:
    a. Orf1ab = 150
    b. S= 200
    c. RPC or MS2 = 200




19. Once the thresholds are set for each target, you can begin analyzing your sample by clicking on each well. On the right side of the page (shown below), you can see the Cq values for each give sample. Refer to the interpretation result table to analyze your data.



## Appendix 2: Loading SARS-CoV-2 Go-Plates for Applied Biosystems Quant Studio 5 96-well plate instruments

1. Open the contents of a Biomeme SARS-CoV-2 Go-Plate (REF#3000562).
    - If not loading the entire 96-well Go-Plate, cut the desired number of wells away from the Go-Plate using sterile scissors.
    - Insert the unused portion of the Go-Plate back into the foil pouch.
    - Remove any excess air from the pouch and reseal the ziplock.
    - Ensure the ziplock is fully sealed to maximize shelf-life of the unused reactions.
2. Place the wells you into to use into a 96-well plate tray for setup of your PCR run.
3. Working on one row at a time, carefully peel away the foil from the wells.
4. Attach a pipette tip to a 20µL fixed volume pipette (REF# 3000011) or use your own 20 uL pipette
5. Unscrew the cap of your first purified sample and transfer 20µL of the extracted RNA into the first reaction well.

    *Note: The strip connections between the tubes of your Go-Plate will face the back of the thermocycler once inserted. When transferring your extracted RNA into the different reaction wells, replicate this orientation to ensure accurate result interpretation (e.g. transfer sample 1 into the far-left reaction well of your first Go-Plate Well, moving from a left to right orientation.*

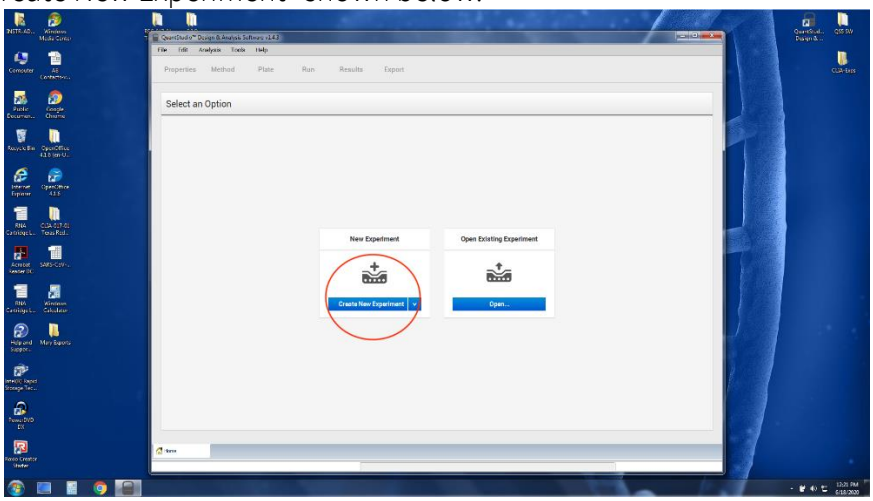
6. Pipette up and down 3-5 times to mix your PCR reaction. When mixing your samples try to avoid introducing bubbles.
    *Note: If bubbles have been introduced, remove them from the lower portion of the PCR tubes by gently tapping them against your work surface before sealing. Bubbles may remain at the top of the tube, but bubbles at the bottom are not acceptable.*

7. Discard your pipette tip and repeat this process for the remaining reaction wells. Once all reaction wells are filled, apply an optical adhesive sealer. Firmly press down with a plastic sealer while moving around the outer edges of the top of the to ensure a good seal. Cut away any excess adhesive when less than 96 wells are used.
8. Turn on the QuantStudio5. Once the instrument is booted up, press the “open drawer” button on the touch screen of the instrument
9. Carefully load your reaction wells into the blue plate adapter provided with the QuantStudio5. Close the adapter.
10. Load the adapter containing your reaction wells into the 96 well block of the QuantStudio5.
11. Close the drawer by pressing on the “open drawer” button.

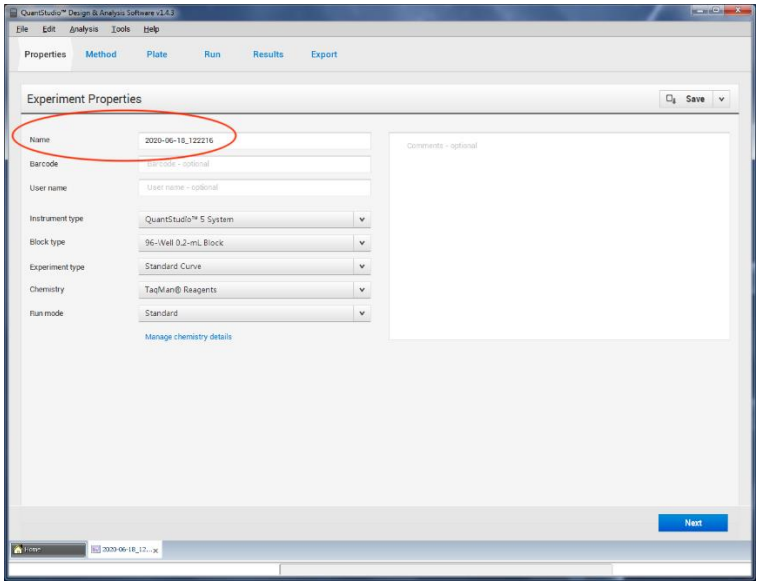
*Note: You can use a plate spinner to make sure your PCR reactions are at the bottom of the plate.*

## Loading the QuantStudio5:

1. Open the QuantStudio™ Design and Analysis Software
2. Select "Create New Experiment" shown below:

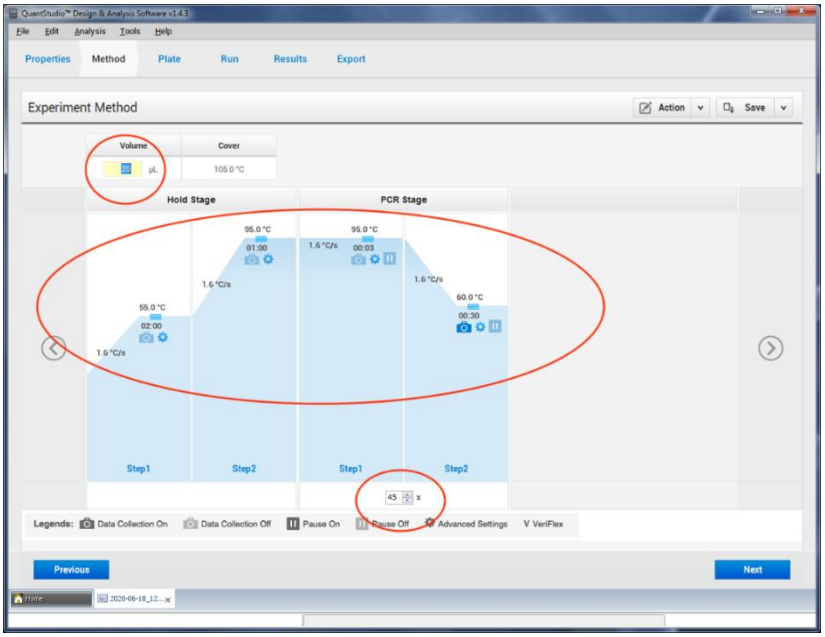



3. Change the run name if needed as shown in the image below. Once you have entered a unique name, click on "Next".

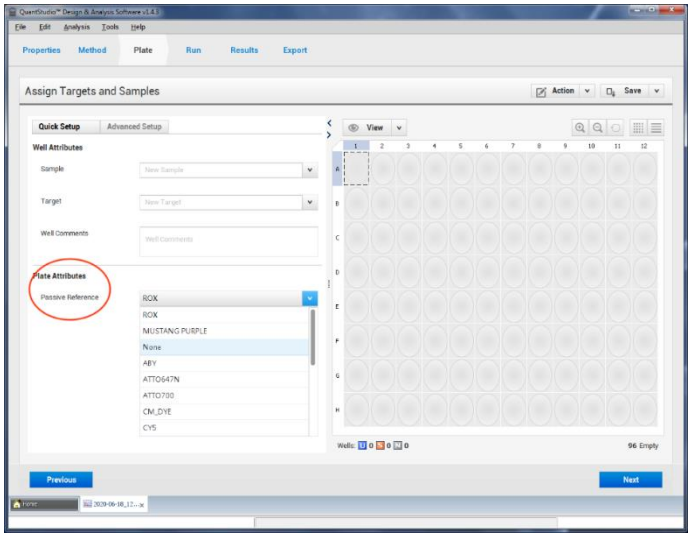



4. On the "method" tab make sure that PCR thermocycling parameters are set as follows:
   a. Step 1 (RT): 55 C for 2 minutes
   b. Step 2 (Initial Denature): 95 C for 1 minute

   c. Step 3 (Denature): 95 C for 3 seconds
   d. Step 4 (Anneal): 60 C for 30 seconds
5. Change the cycles to 45X cycles
6. Change the reaction volume shown below from default 50 uL to 20 uL.



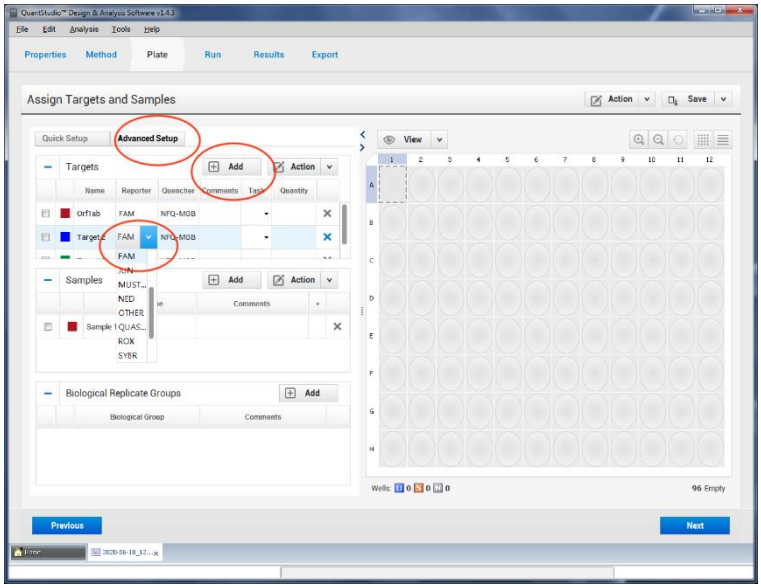

7. Press next once you are finished setting up the thermocycling parameters. In the “Plate” tab, click on the drop down on “Passive reference” and change the default from “ROX” to “None”.



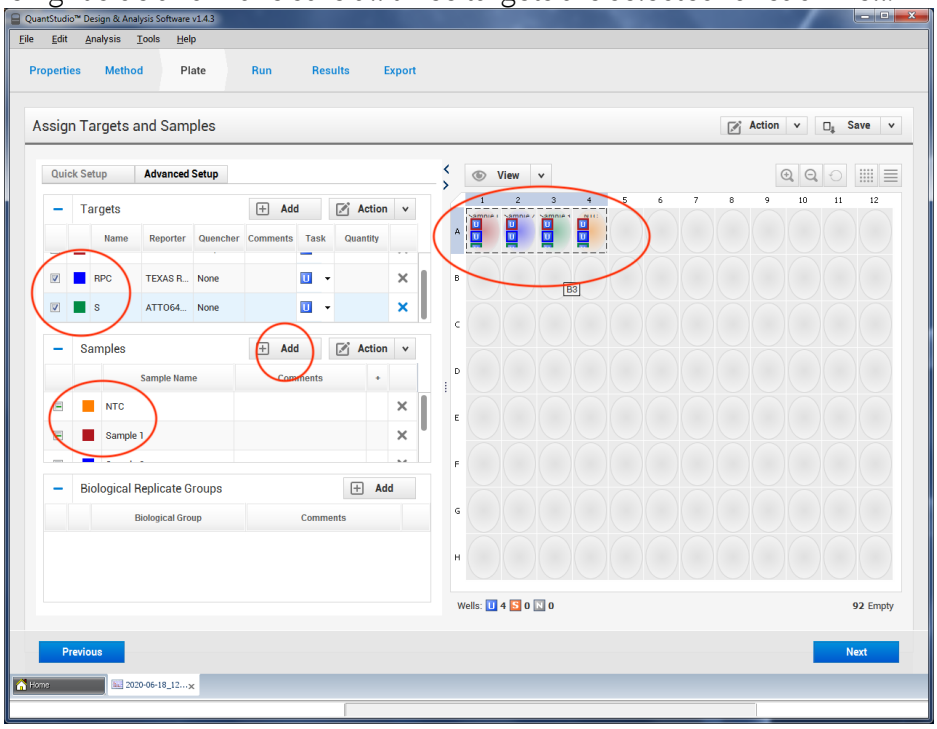

8. In the plate tab, click on “Advanced setup”. In that page, click on the “Add” button as shown below and add 2 other targets (total of 3 targets). Change the name of the target and pick appropriate reporter dye for each target:
   a. FAM : Orf1ab
   b. TexasRedX: RPC

c. ATTO647N: S

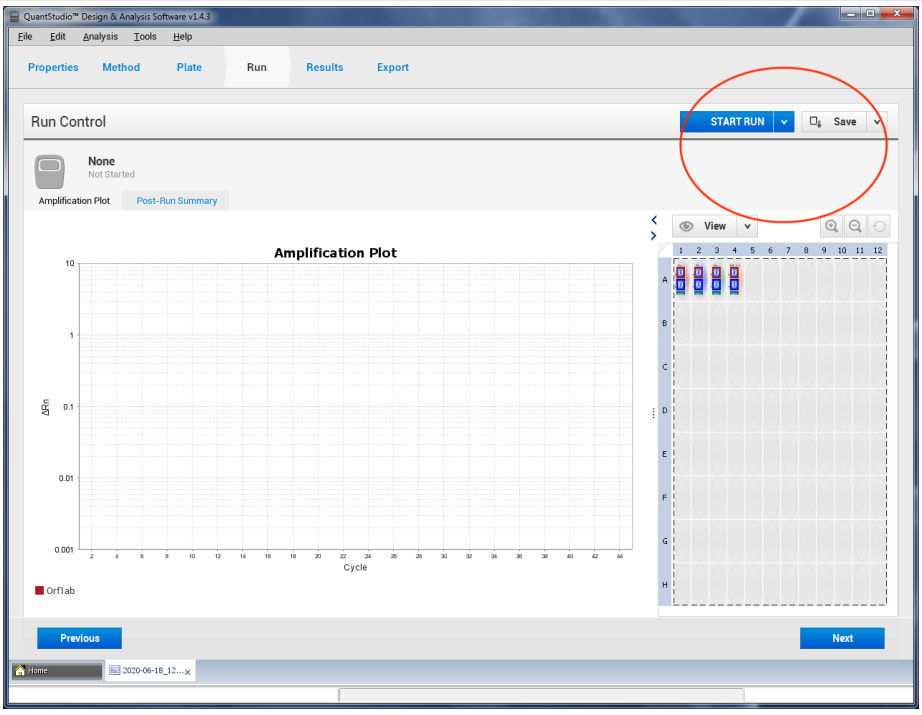
***Note**: Make sure that your instrument is calibrated with specified dyes (FAM, ATTO647N and TexasRedX) before use.*



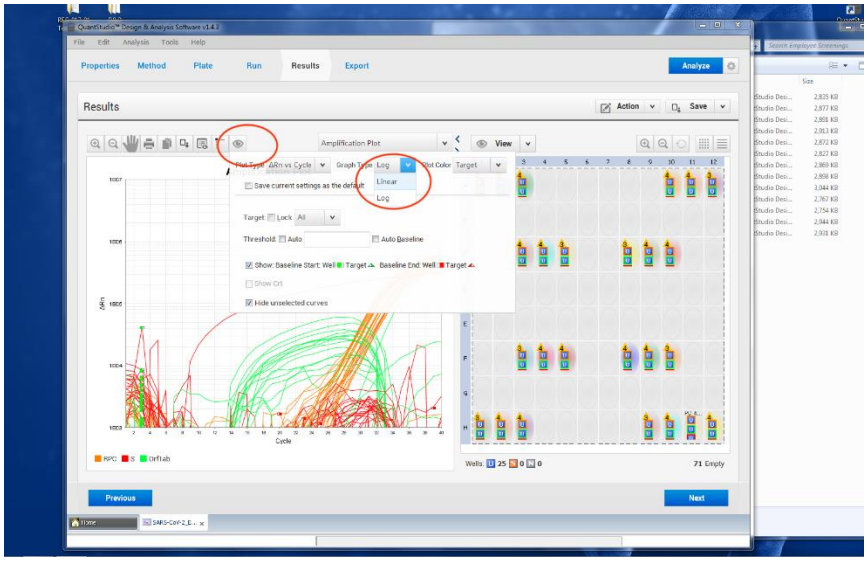

9. Click on the "Add button" shown below to add number of samples you are running based on your plate layout. You can change the sample name as preferred. Click on each well on the right side and make sure all three targets are selected for each well.



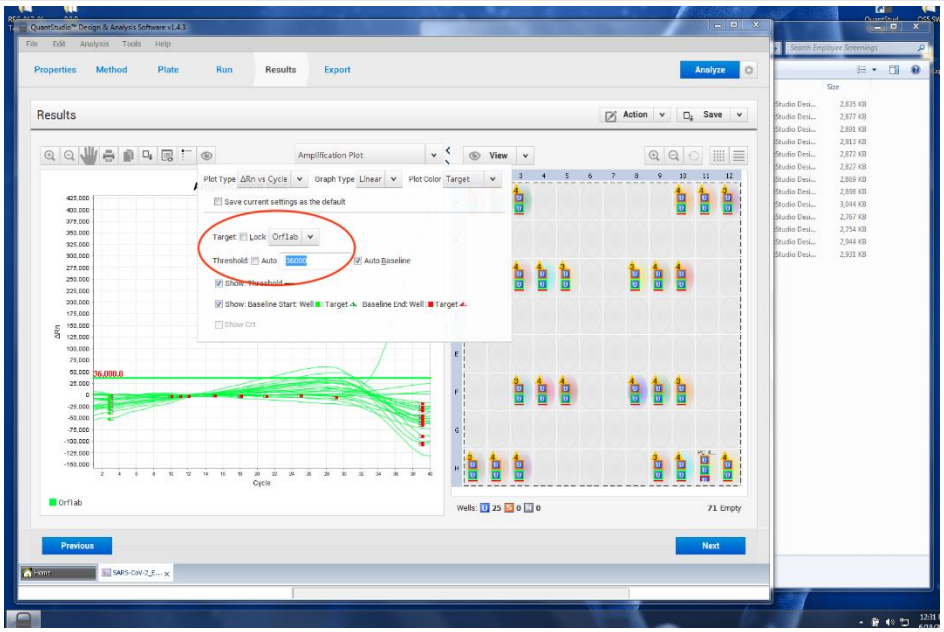

10. Click next, now you want to save your run where you prefer and then click on "Start Run".



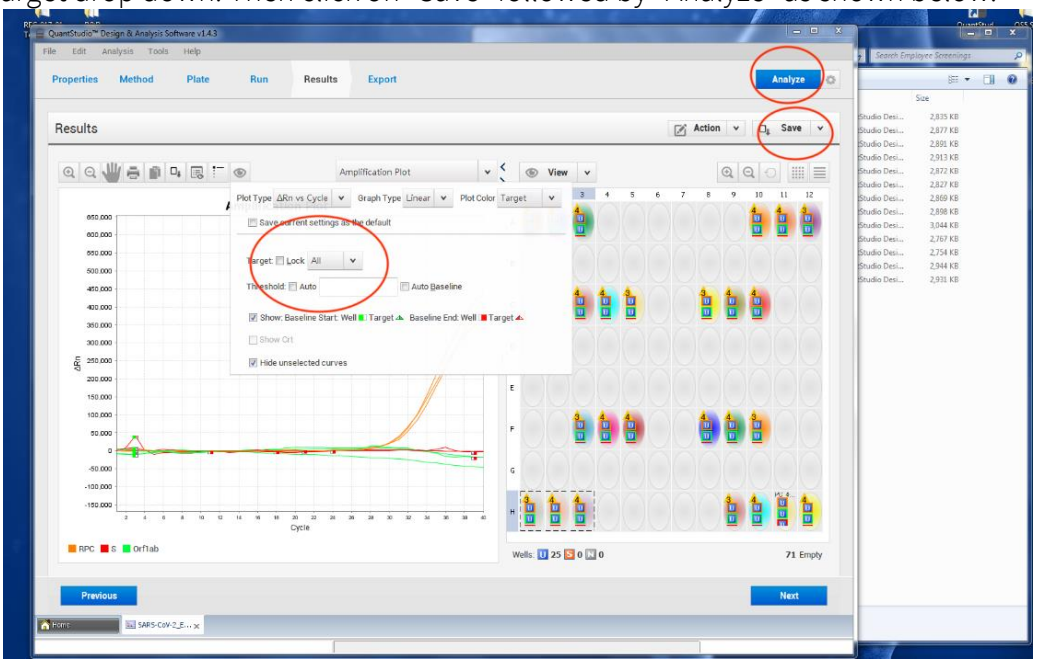

11. Once your run is finished and on “Results” tab, click on the “eye” icon and from the drop-down show in the image, change the graph type from “Log” to “Linear”.



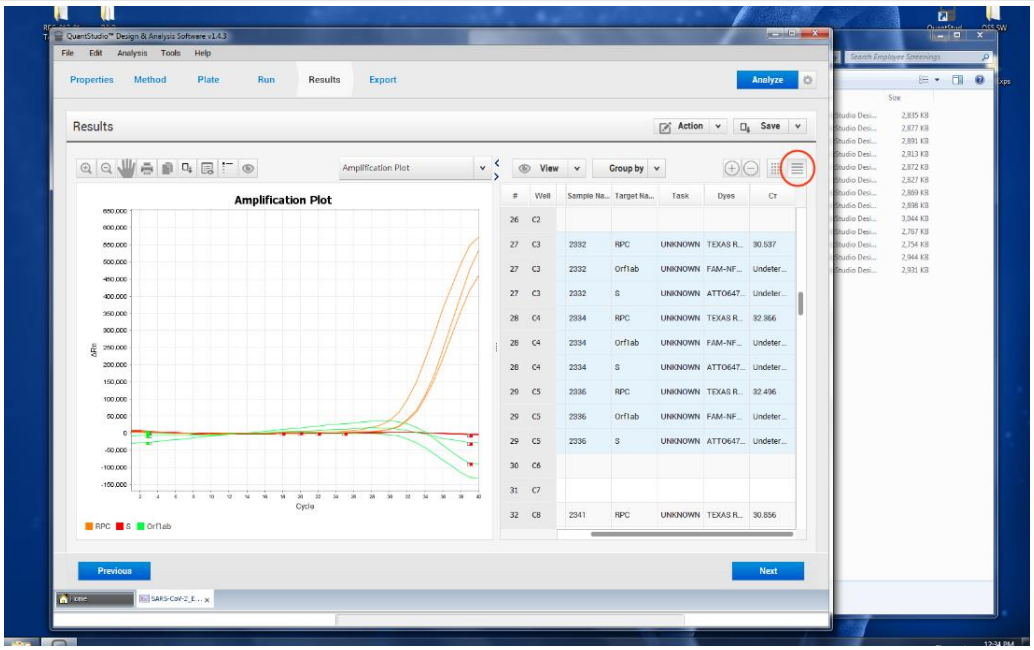

12. Next, from the “Target” drop down, pick each target one by one and change the threshold from auto to the following:
    a. Orf1ab: 36000
    b. S: 38000
    c. RPC: 56000




13. Once you have set the threshold for all the targets individually, click on “All” from the target drop down. Then click on “Save” followed by “Analyze” as shown below.




14. As shown in the image below, click on the tab and you can see the CT values given for each sample and target. Refer to interpretation result table to analyze your data.


QuantStudio™ Design & Analysis Software v1.4.3
File
Edit
Analysis
Tools
Help
Properties
Method
Plate
Run
Results
Export
Analyze
Results
Action
Save
Amplification Plot
View
Group by
Amplification Plot
ΔRn
Cycle
RPC
S
Orf1ab
# Well Sample Na... Target Na... Task Dyes CT
26 C2
27 C3 2332 RPC UNKNOWN TEXAS R... 30.537
27 C3 2332 Orf1ab UNKNOWN FAM-NF... Undeter...
27 C3 2332 S UNKNOWN ATTO647... Undeter...
28 C4 2334 RPC UNKNOWN TEXAS R... 32.866
28 C4 2334 Orf1ab UNKNOWN FAM-NF... Undeter...
28 C4 2334 S UNKNOWN ATTO647... Undeter...
29 C5 2336 RPC UNKNOWN TEXAS R... 32.496
29 C5 2336 Orf1ab UNKNOWN FAM-NF... Undeter...
29 C5 2336 S UNKNOWN ATTO647... Undeter...
30 C6
31 C7
32 C8 2341 RPC UNKNOWN TEXAS R... 30.856
Previous
Next

# Appendix 3: Extraction using the Kingfisher Flex Purification System

Before you begin

Determine the number of required reactions based on the number of patient samples to be processed, plus one Negative Control per plate.

- Prepare fresh 80% Ethanol using Ethanol, Absolute, Molecular Biology Grade and Nuclease free Water (not DEPC-Treated) for the required number of reactions, sufficient for 1 mL per reaction, plus 10% overage.

- Label the short side of each KingFisher™ Deepwell 96 Plate (4):

| Label | Number of plates |
|---|---|
| Sample plate | 1 |
| Wash 1 | 1 |
| Wash 2 | 1 |
| Elution plate | 1 |

- Label the short side of the KingFisher™ 96 KF microplate (1):

| Label | Number of plates |
|---|---|
| Tip comb | 1 |

Note: The following items can be used to hold the tip comb instead of the KingFisher™ 96 KF microplate:
  - Tip Comb Presenting Plate for KF 96
  - Nunc™ MicroWell™ 96-Well Microplate, Flat Bottom
  - Nunc™ MicroWell™ 96-Well Microplate, barcoded
  - ABgene™ 96–Well Polypropylene Storage Microplate
  - ABgene™ 96–Well 1.2–mL Polypropylene Deepwell Storage Plate
  - Nunc™ F96 MicroWell™ Black Polystyrene Plate
  - Nunc™ F96 MicroWell™ White Polystyrene Plate
  - KingFisher™ Deepwell 96 Plate
- Mark the Negative Control well on the plate.

## Extract RNA—Automated method (200-µL sample input volume)

The following procedure uses components from the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit or the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation

### Set up the instrument (200-µL sample input volume)

1. Ensure that the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head is set up with the KingFisher™ Flex 96 Deep-Well Heating Block.

*IMPORTANT! Failure to use the proper magnetic head and heat block results in lower yields and potential harm to the instrument.*

2. Ensure that the MVP_2Wash_200_Flex program has been downloaded from the product page and loaded onto the instrument.

### Prepare sample plate (200-µL sample input volume)

Prepare the processing plates according to the following table. Cover the plates with a temporary seal (such as MicroAmp™ Clear Adhesive Film), then store at room temperature for up to 1 hour while you set up the sample plate.

| Plate ID | Plate position | Plate type | Reagent | Volume per well |
|---|---|---|---|---|
| Wash 1 Plate | 2 | KingFisher™ Deepwell 96 Plate | Wash Buffer | 500 µL |
| Wash 2 Plate | 3 | | 80% Ethanol | 1000 µL |
| Elution Plate | 4 | | Elution Solution | 50 µL |
| Tip Comb Plate | 5 | Place a KingFisher™ 96 tip comb for DW magnets in a KingFisher™ 96 KF microplate | | |

Note: The following items can be used to hold the tip comb instead of the KingFisher™ 96 KF microplate:

- Tip Comb Presenting Plate for KF 96
- Nunc™ MicroWell™ 96-Well Microplate, Flat Bottom
- Nunc™ MicroWell™ 96-Well Microplate, barcoded
- ABgene™ 96–Well Polypropylene Storage Microplate
- ABgene™ 96–Well 1.2–mL Polypropylene Deepwell Storage Plate
- Nunc™ F96 MicroWell™ Black Polystyrene Plate

- Nunc™ F96 MicroWell™ White Polystyrene Plate
- KingFisher™ Deepwell 96 Plate

### Prepare Binding Bead Mix (200-µL sample input volume)

Prepare the required amount of Binding Bead Mix on each day of use.

1. Vortex the Total Nucleic Acid Magnetic Beads to ensure that the bead mixture is homogeneous.
2. For the number of required reactions, prepare the Binding Bead Mix according to the following table:

| Component | Volume per well [1] |
|---|---|
| Binding Solution | 265 µL |
| Total Nucleic Acid Magnetic Beads | 10 µL |
| Total volume per well | 275 µL |

[1] Include 10% overage when making the Binding Bead Mix for use with multiple reactions.

3. Mix well by inversion, then store at room temperature.

### Prepare sample plate (200-µL sample input volume)

1. Add 5 µL of Proteinase K to each well in the KingFisher™ Deepwell 96 Plate labeled "Sample Plate".
2. Add 200 µL of sample to each sample well.
3. Add 200 µL of Nuclease-free Water (not DEPC-Treated) to the Negative Control well.
4. Invert the Binding Bead Mix 5 times gently to mix, then add 275 µL to each sample well and the Negative Control well in the Sample Plate.

   *Note: Remix Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples or wells. Binding Bead Mix is viscous, so pipet slowly to ensure that the correct amount is added. DO NOT reuse pipette tips to add Binding Bead Mix to the samples, as the high viscosity will cause variations in the volumes added.*

5. Add 5 µL of MS2 Phage Control to each sample well and to the Negative Control well.

Process the samples (200-µL sample input volume)

1. Select the MVP_2Wash_200_Flex on the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head.
2. Start the run, then load the prepared plates into position when prompted by the instrument.
3. After the run is complete (~22 minutes after start), immediately remove the Elution Plate from the instrument, then cover the plate with MicroAmp™ Clear Adhesive Film.
   IMPORTANT! To prevent evaporation, seal the plate containing the eluate immediately.

The samples are eluted in 50 µL of Elution Solution (see "Prepare the processing plates (200-µL sample input volume)").

*Note: Significant bead carry over may adversely impact RT-PCR performance.*

Place the Elution Plate on ice for immediate use in real-time RT-PCR.

# Technical Support

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Philadelphia, PA 19108

**Phone**: 267-930-7707
**FAX:** 855-940-0157
**Email**: support@biomeme.com

The customer is responsible for compliance with regulatory requirements that pertain to their procedures and uses of the instrument. The information in this guide is subject to change without notice.

**DISCLAIMER**: TO THE EXTENT ALLOWED BY LAW, BIOMEME INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

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| Revision | Date | Description |
|---|---|---|
| 1.0 | April 7, 2020 | New document |
| 1.1 | May 15, 2020 | Updated Intended Use.<br>Updated Tables 1, 2, 3, 4, 6.<br>Added Tables 7, 8, 10.<br>Updated Interpreting Results section.<br>Updated NTC and PC instructions.<br>Added Biomeme Go OS compatibility statement.<br>Changed 'General Guidelines' section to be more specific.<br>Added note to 'Add RNA Process Control (RPC)' section for incubating cartridges in bulk.<br>Updated 'RNA Extraction Using M1 Sample Prep Cartridge' section.<br>Added statement regarding FDA's independent review of validation.<br>Added storage recommendation to 'Prepare RNA Process Control' section.<br>Updated 'Positive Control Material' section.<br>Added Additional Clinical Performance Data.<br>In 'Placing Go-Strips into Franklin™ Thermocycler' section, split tables into 2 for running PCR w/ and w/o External Controls.<br>Added additional screenshots to 'Patient Sample Pass/Fail Criteria' section.<br>Grammatical/clarity updates.<br>Updated Contents tables. |
| 1.2 | June 10, 2020 | Updated reference to BEI genomic material.<br>Updated LoD section<br>Removed limitations from Intended U se<br>Removed Bulk Vials alternative form factor<br>Summarized Table 7<br>Included Comparator Method information<br>Updated Table 4, Table 1, Table 2 |
| 1.3 | June 19, 2020 | Removed Table 7 as it was a duplicate of Table 5<br>Removed Sample Collection Materials and added note<br>Added note about use of alternative thermocyclers<br>Clarified Clinical and LoD Studies tables<br>Added Appendix 1 and Appendix 2<br>Cleaned up text for clarity |
| 1.4 | June 19, 2020 | Updated Sample Storage information<br>Removed requirement for procedures to be performed in BSL2 laboratory<br>Reorganized placement of Table 6, Table 12, Table 13, and Table 14<br>Added description of Adaptive LoD Study of alternative thermocyclers<br>Corrected positivity rate of LoD study |

| | | |
|---|---|---|
| 1.5 | July 20, 2020 | Removed Swab Collection section<br>Removed shaking VTM step prior to adding sample as it is only required for NP swab sample types<br>Removed limitations statement mentioning use in CLIA labs as it is a duplicate<br>Reorganized Clinical Evaluation section to lead with Clinical Data and follow with Contrived Specimen data<br>Combined Clinical Evaluation data into one table (Table 5)<br>Added detail about use of NP swabs for clinical samples<br>Updated LoD procedure for clarity<br>Added summary of equivalence between carrier/no carrier and BD/Biomeme VTM |
| 1.6 | July 21, 2020 | Updated Table 5 |
| 1.7 | Aug 20, 2020 | Added white thermocycler to Table 2 and page 19 |
| 1.8 | Dec 14, 2020 | Added reference panel testing information.<br>Updated table numbers.<br>Added test descriptions for tables 13 & 14 |
| 1.9 | Feb 03, 2021 | Added variant inclusivity information<br>Fixed a typo in long term storage temperature<br>Added Appendix 3 - Kingfisher extraction instructions<br>Added Kingfisher performance information<br>Updated the table numbers |
| 2.0 | May 26, 2021 | Added sample pooling information<br>Reformatted |
| 2.1 | June 21, 2021 | Clarified the authorized indications |
| 2.2 | Oct 27, 2021 | Revised resuspended RPC storage time<br>Document formatting |
| 3 | Mar 30, 2022 | Corrected version to whole integer to algin with document control number scheme<br>Addition of 10 media pooling to the intended use<br>Added a limitation about variant performance<br>Added an additional positive test result screenshot<br>Clarified the procedure for presumptive positive test results<br>Added Table 5: Pooled Patient Specimen Results Interpretation<br>Updated Biomeme address to 401 N Broad Street, Suite 222, Philadelphia, PA 19108<br>Added “Go Dx” as a mobile app option |