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Evaluation of the Biomeme Franklin Human Performance Factors: Final Report

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The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

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U.S. Army Combat Capabilities Development Command Chemical Biological Center (CCDC CBC) scientists completed an evaluation of the human performance factors related to the execution of COVID-19 testing using the SARS-CoV-2 Test Kit including (1) ease of use, (2) workflow development and assessment, and (3) time to results and sample throughput. The assay was found to require a fairly complex laboratory in order to safely process the samples due to the need for a biosafety cabinet to contain any SARS-CoV-2 containing aerosols. The workflow required nearly 3.5 h to complete 7 extractions, set up the seven RT-PCR reactions plus the two control RT-PCR reactions, run the RT-PCR on the Biomeme Franklin thermocycler, and interpret results. Therefore, throughput was estimated at 42 samples per 24 period on a single Franklin thermocycler.

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PREFACE

The work described in this report was authorized under project no. R.0035825.211.1. The work was started and completed in April 2020.

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

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EXECUTIVE SUMMARY

Combat Capabilities Development Command (CCDC) Chemical Biological Center (CBC) completed an evaluation of the human performance factors related to the execution of COVID 19 testing using the SARS-CoV-2 Test Kit. Testing began on April 13, 2020 and was completed on April 16, 2020.

The SARS-CoV-2 Test Kit pairs Biomeme's M1 Sample Prep Cartridge Kit for RNA 2.0 extraction kit with their room-temperature stable SARS-CoV-2 Go-Strips assay. The Go-Strips are amplified by RT PCR using their portable Franklin™ Real-Time qPCR Thermocycler. The Franklin™ thermocycler companion mobile app, Biomeme Go, is operated on a ruggedized android-based cell phone device that is used to scan test kits, control the FranklinTM thermocycler in either online or offline mode, and display easily interpretable test results. In on-line mode, Biomeme Go conveniently syncs data to the Biomeme Cloud for further evaluation and archiving.

Chemical Biological Center (CBC) scientists evaluated the following human factor and/or test kit performance metrics including 1) Ease of Use, 2) Overall Work flow, and 3) Time to Results. The Biomeme SARS-CoV-2 Instructions for Use (IFU) and Performance Characteristics v1.2 document that Biomeme submitted to the FDA for Emergency Use Authorization (EUA) was used as the base method. The workflow described in the IFU documentation was supplemented with distance learning products produced by Biomeme including videos and instruction manuals available on their website. Previous CBC scientist experiences with RT-PCR as well as COVID-19 diagnostics related training materials produced by the U. S. Army Medical Center of Excellence (MedCoE) and available online were also used to inform clinical sample handling, personal protective equipment selection, and biosafety cabinet hygiene recommendations.

CBC scientists found the assay required a fairly complex laboratory in order to safely process the samples due to the need for a biosafety cabinet to contain any SARS-CoV-2 containing aerosols. Using the CLIA complexity matrix taken from FDA policies, CCDC CBC scientists scored the test complexity an 11 out of possible 21. The lack of manufacturer supplied controls and the requirement to store positive control samples at -80 °C severely limit the locations at which the test could be performed accurately. The manufacturer developed video tutorials were beneficial for interpreting subjective language in the IFU such as performing

CCDC CBC scientists developed a protocol for the full analysis workflow which was executed over approximately 60 sample extractions to determine the time required to complete each portion of the method. They concluded that nearly 3.5 hours are required to complete 7 extractions, set-up the seven RT-PCR reactions plus the two control RT-PCR reactions, run the RT-PCR on the Biomeme Franklin™ thermocycler, and interpret results. Therefore, approximately 6 iterations of 7 samples (42 total samples) could be assayed in each 24 period on a single Franklin™ thermocycler.

The evaluators acknowledge that the time for sample to answer using the consecutive extraction procedure they employed is much longer than the time required to complete sequential extraction where the extraction of all 7 samples is initiated simultaneously. According to the manufacturer, this method had been their intent when developing the IFU and they will modify the IFU document to clarify their recommended workflow. Using this methodology, they report they can extract 7 patient samples, set-up the seven RT-PCR reactions and two control RT-PCR reactions, run the RT-PCR on the Franklin™ thermocycler, and interpret results in approximately 1.5 hours. This would yield a total 24 hour throughput of about 16 cycles of 7 (112 total samples). This is over 2.5 times more samples than the CCDC CBC developed method. CCDC CBC scientists caution that there is a large risk of cross-contaminating samples during sequential processing. The possibility of increasing the false positive rate should give any clinician pause and CBC scientists suggest a thorough risk evaluation be conducted before adopting the time-saving technique.

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EVALUATION OF THE BIOMEME FRANKLIN HUMAN PERFORMANCE FACTORS: FINAL REPORT

1. INTRODUCTION

Combat Capabilities Development Command (CCDC) Chemical Biological Center (CBC) was tasked by the Joint Program Office for Chemical and Biological Defense (JPEO-CBD) with providing a Performance Evaluation of the Biomeme, Inc. SARS-CoV-2 Test Kit in support of its efforts to rapidly field the kit as a screening tool used during soldier in-processing. Evaluation of the assay performance had already been completed by NorthWell Health as part of the Food and Drug Administration (FDA) *in vitro* diagnostic filing for use under Emergency Use Authorization (EUA). Therefore, this evaluation focused on human performance factors rather than assay performance metrics.

The SARS-CoV-2 Test Kit included the following Biomeme components and products: the M1 Sample Prep Cartridge Kit for RNA 2.0 extraction kit; room-temperature stable SARS-CoV-2 Go-Strips assays for amplification of two SARS-CoV-2 gene targets, *Orf1ab* and *S*, as well as an internal control gene target from bacteriophage MS2 also known as the RNA Process Control (RPC); and the Franklin™ Real-Time qPCR Thermocycler. A lyophilized form of the MS2 bacteriophage and a rehydration buffer were provided as part of the SARS-CoV-2 Go-Strips Kit. A companion mobile application (app), Biomeme Go, run on a ruggedized android-based cellphone device, was used to scan test kits for reagent tracking, control the Franklin™ Real-Time qPCR Thermocycler, and quickly interpret test results. In on-line mode, Biomeme Go conveniently synced data to the Biomeme Cloud for further evaluation and data archiving.

Biomeme has solicited the FDA for approval of the kit in "Other Authorized Labs—Patient Care Settings" thus there is an expectation that the kit can be safely and effectively used outside the confines of a clinical laboratory where engineering controls such as a biosafety cabinet (BSC) may not be present. All tests were performed with water blanks. Due to current policy of handling SARS-CoV-2 with Biosafety Level-2 (BSL-2) precautions, the negative "samples" were handled as if they are actual patient samples and opened only inside a BSC. The samples were packaged as if they were collected at a patient bedside and transported to the processing location. Nucleic acid extraction and RT-PCR was performed according to the Biomeme SARS-CoV-2 Real-Time RT-PCR Test Instructions for Use (IFU), v1.2 (Appendix B). Handling of clinical samples, decontamination of the BSC, and avoidance of cross-contamination was supplemented by user laboratory experience and online training materials produced by the U. S. Army Medical Center of Excellence (MEDCoE) available for the BioFire FilmArray and Cepheid GeneXpert COVID-19 diagnostic platforms available at https://www.milsuite.mil/book/groups/ngds-covid19-training.

Chemical Biological Center (CBC) scientists evaluated the following human factor and/or test kit performance metrics. Methods for the evaluation of each follow in separate sections.

Ease of Use

Work flow

Time to Results

2. METHODS

2.1 Assessment of Ease of Use

The Biomeme SARS-CoV-2 Test Kit is intended for use in Other Authorized Testing Locations—Patient Care Settings; therefore, there is an expectation that use of the kit is straightforward and simple. Tests that can be performed in this setting are also referred to as CLIA Waived. As noted by the Centers for Disease Control "although CLIA requires that waived tests must be simple and have a low risk for erroneous results, this does not mean that waived tests are completely error-proof. Errors can occur anywhere in the testing process, particularly when the manufacturer's instructions are not followed and when testing personnel are not familiar with all aspects of the test system[1]. " The FDA complexity scoring criteria was used as a guide for assessing ease of use, Table 1. Additionally, all errors made during processing of each sample were tallied and assigned an impact as Minor, Major, or Catastrophic. Minor errors were defined as those with no expected impact on sample result. Major errors were considered those that could potentially alter results or create the need to retest a sample. A catastrophic error were those that inhibit the performance of the test or result in loss or potential adulteration of the sample. Operator opinions following the evaluation were also compiled as subjective metrics.

[1]https://www.cdc.gov/labquality/waived-tests.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fclia%2Fwaived-tests.html (last accessed April 10, 2020)

Table 1. Complexity Assessment Taken from FDA Policies

Parameter	Score = 1	Score = 3
Knowledge	(A) Minimal scientific and technical knowledge is required to perform the test; and (B) Knowledge required to perform the test may be obtained through on-the-job instruction.	Specialized scientific and technical knowledge is essential to perform pre-analytic, analytic or post-analytic phases of the testing.
Training and Experience	Minimal training is required for pre-analytic, analytic and post-analytic phases of the testing process; and (B) Limited experience is required to perform the test.	Specialized training is essential to perform the pre-analytic, analytic or post-analytic testing process; or Substantial experience may be necessary for analytic test performance.
Reagents and materials preparation	(A) Reagents and materials are generally stable and reliable; and (B) Reagents and materials are prepackaged, or premeasured, or require no special handling, precautions or storage conditions.	(A) Specialized training is essential to perform the pre-analytic, analytic or post-analytic testing process; or (B) Substantial experience may be necessary for analytic test performance.
Characteristics of operational steps	Operational steps are either automatically executed (such as pipetting, temperature monitoring, or timing of steps), or are easily controlled.	Operational steps in the testing process require close monitoring or control, and may require special specimen preparation, precise temperature control or timing of procedural steps, accurate pipetting, or extensive calculations.
Calibration, quality control, and proficiency testing materials	(A) Calibration materials are stable and readily available; (B) Quality control materials are stable and readily available; and (C) External proficiency testing materials, when available, are stable.	(A) Calibration materials, if available, may be labile; (B) Quality control materials may be labile, or not available; or (C) External proficiency testing materials, if available, may be labile.
Test system troubleshooting and equipment maintenance	(A) Test system troubleshooting is automatic or self-correcting, or clearly described or requires minimal judgment; and (B) Equipment maintenance is provided by the manufacturer, is seldom needed, or can easily be performed.	(A) Troubleshooting is not automatic and requires decision-making and direct intervention to resolve most problems; or (B) Maintenance requires special knowledge, skills, and abilities.
Interpretation and judgment	(A) Minimal interpretation and judgment are required to perform pre-analytic, analytic and post-analytic processes; and (B) Resolution of problems requires limited independent interpretation and judgment.	(A) Extensive independent interpretation and judgment are required to perform the pre-analytic, analytic or post-analytic processes; and (B) Resolution of problems requires extensive interpretation and judgment.

Note: A score of 2 will be assigned to a criteria heading when the characteristics for a particular test are intermediate between the descriptions listed for scores of 1 and 3.

2.2 Assessment of Work Flow

CBC scientists evaluated the overall workflow described in the Biomeme IFU and produced several recommendation on the development of a sample processing workflow. There was little information in the instructions detailing decontamination procedures between samples nor warnings regarding the ubiquity of RNase and DNase enzymes that could destroy the sample during or after the extraction steps. The U. S. Army Medical Center of Excellence (MEDCoE) is tasked with developing training and doctrine for the evaluation of diagnostic samples. Many of the precautions, decontamination methods, and personal protective equipment (PPE) requirements were taken from training materials they have produced for evaluating COVID-19 samples on the BioFire Defense FilmArray and Cepheid GeneXpert systems as included at https://www.milsuite.mil/book/groups/ngds-covid19-training/pages/biofire-filmarray and https://www.milsuite.mil/book/groups/ngds-covid19-training/pages/cepheid-genexpert, respectively, as accessed on Monday April 13, 2020.

2.3 Assessment of Time to Result

CBC evaluated the time required to go from a collected sample to final results and determine the difference between a novice user and an experienced user. All users were trained using distance learning products produced by Biomeme. These included evaluation of video products produced by Biomeme, instruction manuals for the M1 Sample Prep Cartridge Kit for RNA 2.0 and Franklin™ thermocycler, and the SARS-CoV-2 Real-Time RT-PCR Test IFU, v1.2. Three operators performed the evaluation. Operators 1 and 2 had experience with RT-PCR methodology and aseptic technique. Operator 3 had less experience with RT-PCR methodology and little experience with aseptic technique. The average time required to perform the first two tests were used as the metric for the "novice user," the average time required to perform tests 3-10 were recorded as "proficient user", and the average time required to perform all additional tests were recorded as "expert user." The third user was evaluated as an "on-the-job" trainee using a see one, do one model after at least one of the initial users had completed enough tests to progress to "expert user." The time required for the user to complete their first extraction was recorded as "on-the-job trainee user" as a further metric. The improvement with subsequent extractions was also recorded.

3. RESULTS

3.1 Ease of Use

3.1.1 FDA Complexity Matrix

The FDA complexity scoring criteria was used as a guide for assessing ease of use, Table 1. CBC scientists recorded observations and opinions when performing the human factors evaluation then applied their finding to each of the parameters in the table. The lower the score, the less complex a method is considered to be. CLIA Waived tested are expected to obtain a score of 7 (minimum score).

3.1.1.1 FDA Parameter: Knowledge (Score = 2)

The knowledge parameter was score as a 2. There is minimum scientific knowledge required to perform the sample extraction using the M1 Sample Prep Cartridge Kit for RNA 2.0 because the user does not need to understand the science behind each step of the process to yield a suitable RNA extraction. Likewise, no scientific knowledge is required to accurately add the RNA samples into the Go-Strips containing pre-aliquoted, lyophilized reagents. The Biomeme Go App on the smartphone device walks the user through the process of starting a run. There is, however, some knowledge required to perform the result interpretation. The system displays the results in tabular form with the quantification cycle (Cq) value for each assay within the tube. Although the instructions simply recommend that positives are considered any sample with a Cq values $\leq$40, the manufacturer states that "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." This implies the user is trained in and knowledgeable of all aspects of interpreting amplification plots. The system allows the user to toggle to views of the amplification curves with and without background subtraction. Interpretation of the curves could easily confuse a user with little or no knowledge of typical RT-PCR results. Additionally, there is little information in the IFU regarding methods of minimizing cross-contamination, aseptic technique, and/or best practices for maintaining laboratorian safety.

3.1.1.2 FDA Parameter: Training and Experience (Score = 1)

The training and experience parameter was scored as a 1. The extraction and RT-PCR set-up, Franklin™ thermocycler interaction, and result screen views are quite easy to learn. A single see one, do one training activity was sufficient to train a new user with no RT-PCR experience and this user obtained acceptable results throughout their first 5 test samples. Additionally, Biomeme has videos available on their website (https://help.biomeme.com/how-to-videos) showing each step of the analysis workflow. These were easy to access and especially were vital in learning the recommended speed with which to pump the syringe plunger during each step of the sample extraction and learning the recommended process for removing the syringe tip from the cassette. The instructions simply stated to pump the syringe plunger slowly (most steps) or rapidly (drying step). The instructions made to reference to removing the tip after each step. All steps were easily transferred to a naive technician via on-the-job training.

3.1.1.3 FDA Parameter: Reagents and materials preparation (Score = 3)

The reagents and materials preparation parameter was scored the highest metric at a 3. The Go-Strips contain all reagents required to perform the RT-PCR of the extracted samples. The user simply adds 20 µL of extracted RNA to the appropriate tube. The extraction kit contains everything required to complete the extraction except the RNA Process Control (RPC) that is added into the lysis buffer after adding the sample. Biomeme has a lyophilized pellet that is rehydrated in a provided buffer in order to prepare the RPC. The buffer is 5mL and only 20 µL is used per extraction. Therefore, there is enough material for 250 extractions per RPC kit. There are no instructions detailing short term or long term storage requirements for the rehydrated RPC. We suggest the manufacturer include instructions on storage of the control material and

that laboratories/test sites make aliquots of the rehydrated RPC to reduce the chance of introducing contamination into a large sample, especially in laboratories performing lower throughput operations. Additionally, the manufacturer states that positive and negative control reactions should be performed with each set of 7 patient samples, but these reagents are to be provided by the laboratory. A laboratory must have substantial experience to determine the LOD of the system as installed in their laboratory and prepare a suitable positive control for each set, and validate the performance of the material. The IFU instructs the end-user to store the positive control material at -80 °C. Most patient care settings are not likely to have this capability.

3.1.1.4 FDA Parameter: Characteristics of operational steps (Score = 2)

The characteristics of operational steps parameter was scored as a 2 as it is intermediate between the requirements for a score of 1 and 3. All extraction and RT-PCR set-up steps are manual, though they don't require overly close monitoring because of their simplicity. The drying step is supposed to be performed for 20 pumps or until no liquid sprays out of the tip when plunging. Since the tip is inserted into the cassette, it is impossible to tell whether liquid is still spraying out of the tip. Further, the IFU states samples should be incubated with the lysis buffer for 10 minutes. There are no options listed for shorter or longer incubations nor is there a description of the effect of incubations for incorrect time periods. Liquid handling via micropipettes is a manual step. Biomeme provides a 20 µL fixed volume pipette and a 200 µL transfer pipet as optional catalog items if the laboratory performing the assay does not have them. Knowledge of how to use various pipettes is needed. The ability to decontaminate the extraction rack between samples is also of paramount importance in order to reduce the likelihood of sample cross-contamination. The current rack is 3D printed and rough textured. Operators found that it may be difficult to thoroughly decontaminate. Biomeme will soon be producing the rack by injection molding and should test the design to ensure it can be thoroughly decontaminated prior to making it available to end users.

3.1.1.5 FDA Parameter: Calibration, Quality Control, and Proficiency Testing Materials (Score = N/a)

CBC scientists did not assess a score for the calibration, quality control, and proficiency testing materials because (1) no calibration materials are described in the literature accompanying the SARS-CoV-2 Test Kits nor on the Biomeme website, (2) a source for the positive control material was described in the IFU but no determination of its availability was made during this evaluation, and (3) there are no proficiency samples available to score their stability.

3.1.1.6 FDA Parameter: Test system troubleshooting and equipment maintenance (Score = 1)

CBC scientists scored the test system troubleshooting and equipment maintenance parameter as a 1. Detailed descriptions of the most likely encountered issues, specifically communication loss between the controller android phone and the Franklin™ thermocycler, are included in the IFU and are available in the support pages of the Biomeme website referenced prior. No user supplied maintenance is described for the Franklin™ thermocycler.

3.1.1.7 FDA Parameter: Interpretation and Judgement (Score = 2)

CBC scientists scored the interpretation and judgement parameter as a 2 due solely to the fact the system reports results of the qualitative tests as Cq values that must be converted by the user to "negative," "positive," and "invalid" rather than simply showing the qualitative result on the screen.

3.1.2 Errors Encountered During the Assessment

The most common error that occurred during sample processing was performing the incorrect number of pumps in one or more sections of the cartridge. This error commonly occurred during the single pump portions of the workflow where the technician would do two pumps instead one for the salt wash or drying wash steps. This error occurred less than 5 times during the processing of the 60 (< 8.3 %) samples. The IFU does not specify what actions to take, if any, if this error occurs and there is no way to know its impact on the results; therefore, we evaluated this error as **MAJOR**.

Another error that commonly occurred was caused by the size of the M1 kit pouch. The size and packaging of the components of the kit caused occasional mishandling of the extraction syringe and/or extraction column. Most often, the column was situated in the bottom of the pouch, but, sporadically, it was present to the side or on top of the cartridge and would fall out of the pouch when the technician removed the cartridge. Additionally, the syringe barrel flange would sometimes get caught on the zipper closure portion of the pouch. To aseptically remove the syringe from each pouch, technicians typically employed a method where they tore open the pouch and worked the syringe up by squeezing from the bottom of the pouch. Twice, the flange caught on the zipper and developed enough potential energy that when the syringe did move past the zipper, it exited the pouch with additional velocity and landed on the BSC surface. All errors caused by mishandling of the pouch components were evaluated as **MINOR** because a new M1 kit pouch could easily be obtained.

During two extractions (3.33 %), the Luer-Lok fitting between the syringe and extraction column released when trying to remove the column from one of the cartridge sections. This is likely due to the technician slightly twisting the syringe while employing the forward and backward rocking motion required to remove the tip from each locations on the sample preparation cassette. Since the tip was inside the cartridge section and the syringe did not contact any other items in the workspace, the error was evaluated as **MINOR.**

RECOMMENDATION: The pouch containing components of the M1 Sample Prep Cartridge Kit for RNA 2.0 should be approximately 25% larger and the purification tip containing the silica resin should be pre-installed on the syringe. Re-positioning the zipper closure to the long axis of the pouch would also be beneficial for aseptic handling of the

CBC scientists found the void-filling caps difficult to handle without touching the portion that goes into the RT-PCR tubes. Removing the caps from the bag and orienting them

properly took significant care. Mishandling the caps could introduce contamination into the RT-PCR reactions.

Technicians also found that the placement of the small Go-Strips behind the 2mL tubes containing the RNA extracts made the tube hard to see unless the rack was moved far forward in the biosafety cabinet. Although not experienced in this evaluation, one technician noted that users should ensure that pipet tips do not contact the 2mL tubes when transferring sample into the reaction tubes. The difference in height could cause inadvertent touching of the pipet tip when reaching over the taller tubes. Another technician noted that the Go-Strips sat loosely in the rack and were easily knocked out of the rack when picking up the 2mL RNA extraction containing tubes. This mistake was categorized as **MINOR** because the Go-Strip was replaced when this occurred.

One *potentially* **CATASTROPHIC** error occurred (1.67 %). First, when performing the final elution, the technician noticed a bubble of sample form on the top of the foil when pressing the syringe plunger down. This could easily lead to cross-contamination of the sample or introduction of RNase into the extract—especially if more than 1 sample were processed in the BSC simultaneously. The liquid remained contained on the top of the cassette and did not run over the sides where it would have significantly contaminated the cassette rack. This issue is exacerbated by the finding by all three technicians that there was often notable spatter when the tip was removed from the foil covering. The forward and backward rocking motion recommended by the manufacturer in their videos was beneficial in enlarging the holes produced when the foil was pierced, but the lip on the column tip tends to grab onto the edges of the holes when removing the syringe tip.

RECOMMENDATION: The manufacturer should include the recommendation to rock the syringe forward and backward to enlarge the holes in the foil cover prior to removing the tip in the M1 Sample Prep Cartridge Kit for RNA 2.0 instruction manual and/or the Biomeme SARS-CoV-2 Instructions for Use. It is possible that not all users will view the videos prior to beginning extractions and this method is instrumental in reducing the amount of liquid spatter and potential contamination.

RECOMMENDATION: The manufacturer should remove the hose barb on the end of the silica resin-containing tip to facilitate removal of the tip from each hole in the foil cover and reduce the chance of spatter.

The Biomeme Go application loaded onto the ruggedized cell phone was very intuitive and easy to use. The software guides the user through each step of connecting the phone to the Franklin™ thermocycler by either a USB or Bluetooth connection and details the steps of placing the reaction tubes into the thermocycler in the correct orientation. The software automatically syncs data to the Biomeme cloud environment when a data connection (Wi-Fi) is available. The Biomeme Go application requires that operators input a username and password when it is run the first time. Users must have their username and password handy along with an

internet connection. Afterward, the application will run in off-line mode and automatically sync whenever a data connection is available.

3.1.3 **Overall Conclusion: Ease of Use**

Overall, CBC scientists found the M1 Sample Prep Cartridge Kit for RNA 2.0 and SARS-CoV-2 Go-Strip assays easy to use. Only 3 manual pipetting steps were required—200 µL sample added to cassette, 20 µL RPC added to cassette, and 20 µL RNA extract added to Go-Strip—paired with a syringe based extraction procedure. Most mistakes that were made during the 60 samples evaluated were categorized as MINOR and were rectified by simply replacing a component. We are unsure how the system could be used in a patient care setting, however, due to the requirement to use a biosafety cabinet and the number of steps. This system would likely fit better in a moderate or high complexity laboratory and this is re-enforced by our scoring of the kit as **11 out of a maximum 21 points** using the FDA complexity scoring matrix. Reagents and Materials Preparation was given the highest individual component score of 3 due to the need to add the RPC to each extraction, and the need to have positive control material available at a specified quantity. The positive control requirement is compounded by its need for storage at -80 °C. Most patient care settings are not likely to have this capability.

3.2 Evaluation of Workflow

To simulate the workflow that would be used with actual patient or soldier samples, all samples were extracted inside a BSC Type II which was decontaminated with 10 % (v/v) bleach solution followed by 70 % (v/v) isopropanol. Additionally, as per the Biomeme IFU, the BSC was decontaminated between sample extractions.

NOTE: The Biomeme Instructions for Use, v1.2 and accompanying instruction manuals for the M1 Sample Prep Cartridge Kit for RNA 2.0 (v1.0) and Franklin Real-Time PCR Thermocycler & Biomeme Go App (v1.0) were utilized. These documents indicate 7 patient samples plus both positive and negative control samples should be analyzed simultaneously.

The Biomeme IFU document states to clean the work space between samples, care should be taken to avoid cross-contamination between samples, and the sample should be incubated in the lysis buffer for 10 minutes. CCDC CBC scientists interpreted this guidance such that only 1 sample should be extracted at a time. Subsequent discussions with Biomeme indicated that the company intended that all 7 RNA extractions be started at one time and the samples be sequentially extracted starting after the first sample had completed its 10 minute incubation. Overlapping of the extractions, while not affecting the workflow steps, would significantly shorten the time-to-results.

Due to the occurrence of spattering when removing the syringe tip from the extraction cassette and potential contamination of the cartridge rack, we **DO NOT RECOMMEND** overlapping the extractions. Laboratories should perform their own workflow and risk assessment.

A protocol detailing the steps performed by CBC scientists is included in Appendix A and includes all steps from setting up the biosafety cabinet to putting the reaction tubes onto the Franklin™ thermocycler. CBC scientists wore two layers of gloves when handling the sample, performing the extraction, and loading the extracted RNA into the reaction tubes. This was done so that the gloves could be easy changed without removing the scientists' hands from the BSC thus increasing operator safety. CBC scientists had a full complement of liquid handling micropipettes and did not evaluate the 20 µL fixed volume pipette available from Biomeme. Additionally, they used a 20-200 µL micropipette to transfer the sample into the extraction cartridge and did not evaluate the transfer pipets also available from Biomeme.

3.2.1 RNA Extraction

Unless the biosafety cabinet is situated immediately adjacent to the point of sample collection, all samples will require packaging for transport to the laboratory. To simulate this, all samples were packaged in Zip Lock style bags labeled with sample information. The packaging was decontaminated with bleach solution prior to entry into the BSC. Using a double set of gloves, the tubes were removed from the packaging, wiped with bleach prior to and

immediately following opening them to remove the 200 µL necessary for the extraction, then placed on a bleach soaked wiper. The operator would then doff the outer set, start the 10 minute count-down timer, and return the reclosed tube to the sample packaging by inverting the bag then picking up the tube. The RPC was added to the sample-containing lysis buffer during the 10 minute sample incubation.

RECOMMENDATION: The extraction cassette can be labeled with the sample ID using a Sharpie® or similar style marker. This would be imperative if multiple samples were being extracted simultaneously. Use of indelible/alcohol resistant ink is also recommended depending on the decontamination procedures used to wipe the rack.

CBC scientists noticed that the technician performing the extractions in the Biomeme training videos held the syringe near the Luer-Lok which places the hand just above the foil covering of the cassette. With this placement, the user could easily contaminate their gloves with any sample material that is present on the foil cover, especially at or near the lysis buffer well. As an alternative, we recommend users hold the syringe near the barrel flange. This would keep their hands free of the cassette surface and allows them to see when the entire contents of each well have been drawn into the syringe. However, the cassette is not locked into the cartridge rack during extraction. The user must support the cartridge both when rocking the syringe forward and backward and while removing the tip from each well of the cassette. There are many times during the RNA extraction process that an operator's gloves could become contaminated with sample or extraction reagents.

The IFU states to pump a designated number of times slowly except during the drying step. The video training material was used to determine that "slowly" meant approximately 2-3 seconds per plunger cycle. The syringe was allowed to fill completely with each buffer and there was often a slight lag between reaching maximum plunger pull and filling of the syringe due to the viscosity of the buffer. The IFU notes that liquid from one well should not be transferred to the subsequent well; however, CBC scientists often noted there appeared to be residual liquid in the column tip after fulling depressing the syringe plunger. Additionally, the protocol states that the drying step should be done for 20 pumps or until no additional liquid sprays from the column tip. It is difficult to ascertain whether enough pumps have been completed while the tip is inside the well. For this evaluation, 20-25 pumps were employed.

The extracted RNA was transferred to a 1.5mL conical bottom microcentrifuge tube because CBC scientists did not have immediate access to an appropriate 2mL screw cap tube. The microcentrifuge tubes substituted by CBC easily fell over when placed in the rack. It would have been preferable that the recommended tube was included as part of the kit rather than a separate catalog number. There is no recommendation in the IFU for storage of RNA extracts after setting up the RT-PCR. We recommend storage $\leq$ -20 °C. There was plenty of volume remaining in the event a retest were required.

RECOMMENDATION: Biomeme could include the 2mL tube with the extraction kit to ensure proper fit in the extraction rack.

3.2.2 Go-Strip Set-Up

The SARS-CoV-2 Go-Strip Assays are packaged in a pouch containing a plastic 96-well tray holding the Go-Strips. Each set of 3 Go-Strip tubes were covered by a foil cover containing a small tag. The Go-Strips should be placed into the corresponding wells of the extraction tray with the tag positioned to the left to orient the strips correctly based on the IFU.

CBC scientists preferred to perform a glove change after handling each extracted RNA to reduce the chance of cross-contamination, but, in the interest of time and materials, chose to simply wipe their gloved hands with a bleach soaked wiper between samples. A set of Go-Strips were set up and capped with the void-filling caps prior to moving to the next set of reactions. Two pair of gloves were worn when adding sample into the reaction tubes and the outer set was doffed prior to handling the void-filling caps. A fresh outer layer was donned prior to starting the next set of reactions.

Of note, it is common practice to have separate, designated areas for nucleic acid extraction and RT-PCR set-up, but there was no discussion of this in the Biomeme IFU. Also, CBC scientists noted it was odd that a positive control reaction would be set up prior to setting up sample reactions. A recommended change to the IFU would be to move the positive control reaction from being the second reaction set up to being last.

RECOMMENDATION: Make void-filling caps easier to handle aseptically.

RECOMMENDATION: Move the positive control reaction so that it is the last reaction set up to ensure no samples are cross-contaminated with the positive control.

3.2.3 Franklin™ Thermocycler and Biomeme Go Application

The reactions were transferred to the Franklin™ thermocycler once all of the void-filling caps were in place. For this evaluation, the Franklin™ thermocycler was positioned on a benchtop near the BSC used for RNA extraction and RT-PCR set-up. The Franklin™ thermocycler is controlled by a companion application, Biomeme Go, which is loaded onto a ruggedized android-based cellphone. The application is intuitive, but the process of entering 9 sample IDs into the interface is cumbersome due to the size of the input boxes. The font is small,

the input boxes are hard to select, and the keyboard is cramped. The application contains the capability to scan in the sample IDs if they are barcoded. This feature was not assessed in this evaluation. Because the Franklin™ thermocycler doesn't require a physical connection to the android-based cellphone unit, there is no mandate that the android unit be a cellphone—**a ruggedized tablet may be a much better option and allow easier input of sample ID information**.

The Biomeme Go application instructs the user to fill the Go-Strips with sample as part of the set-up. Since RT-PCR is the last step, the Go-Strips were filled prior to taking them to the thermocycler. These steps are easily skipped in the application, but serve as a good reminder to users so that no required set-up steps are missed.

For user auditory and visual feedback that the instrument is powering on, the Franklin™ thermocycler makes a sound as servo motors rotate inside the unit and the LEDs on the front of the device light up. Pictograms are located under the lid to remind the user of the orientation requirement when loading Go-Strips. Additionally, when the Bluetooth button is depressed correctly such that Bluetooth communications activates, a small LED lights and blinks until a connection is established with the Biomeme Go App. It appears the Go App only transfers data from the Franklin™ thermocycler when the screen is active. If the screen is dark (default setting is to darken after 1 min of inactivity), the Biomeme Go App doesn't automatically transfer the data. Perhaps a beep should sound when a run is complete to prompt the user to reactivate the android device and ensure data is transferred immediately.

3.2.4 Run Results

Of the 60 samples that were evaluated with the workflow developed, only 1 sample resulted in an unexpected result—the RPC did not amplify. The sample was re-extracted as per the IFU then the original sample was run alongside the re-extracted sample and another sample that had performed as expected. The RPC amplified in all 3 samples; therefore, the possibility that the technician did not add the RPC during the first extraction was discounted. No further analysis was performed to determine the root cause of the single RT-PCR failure obtained with the workflow.

3.2.5 Overall Conclusion: Workflow

The workflow for the SARS-CoV-2 Kit is straightforward and doesn't require many consumables other than those included by the manufacturer. CBC scientists did not use the 2mL tubes recommended by Biomeme due to short project time lines but, rather, substituted a 1.5mL conical bottom tube present in their stock. The value of using the 2mL tubes was realized and is discussed in Section 3.2.1. CBC scientists evaluated the workflow based on experience and supplemented their knowledge with doctrinal materials available from MEDCoE for the evaluation of COVID-19 samples on other nucleic acid amplification platforms. The use of double gloves is an example of workflow modifications that were not designated in the Biomeme IFU. The workflow was exercised over 4 days and a total of 60 samples divided between 3 technicians. Only 1 sample generated an unexpected result but the root cause of the unamplified RPC was not established.

3.3 Evaluation of Time to Result

NOTE: The Biomeme IFU document states the workspace should be cleaned between samples, care should be taken to avoid cross-contamination between samples, and the sample should be incubated in the lysis buffer for 10 minutes. CCDC CBC scientists interpreted this guidance such that only 1 sample should be extracted at a time.

Subsequent discussions with Biomeme indicated that the company had intended that all 7 RNA extractions be started at one time and the samples be sequentially extracted starting after the first sample had completed its 10 minute incubation. Overlapping of the extractions would **SIGNIFICANTLY SHORTEN** the time-to-results from those reported here. Biomeme has indicated that they would update the IFU to more clearly specify the option to overlap extractions.

With overlapping extractions, Biomeme states they can perform approximately 25 extractions per hour and the overall sample-to-answer time for 7 samples plus controls is 88 minutes (less than 1.5 hours). This timeline was not evaluated by CBC.

Overlapping sample extractions **would significantly increase the possibility** of sample cross-contamination. The workflow utilized and potential steps where cross-contamination could occur are discussed in Section 3.2.

3.3.1 Time Required for Go-Strip Loading

The time required to set-up Go-Strip reaction tubes, place them on the Franklin™ thermocycler, and confirm the RT-PCR started successfully was dependent on the number of reactions set-up with 3 reactions taking significantly less time than 9 reactions. Since the customer was concerned with the time required to complete full runs, only the time to load all 9 reactions is reported. The overall average time (mm:ss) to load a full complement of reactions was **15:46**.

3.3.2 Time Required for RT-PCR

The time required (mm:ss) for the Franklin™ thermocycler to complete a full RT-PCR amplification cycle was **56:08**.

3.3.3 Time Required for RNA Extraction: Novice User

Both operators evaluated the protocols and online training videos available in the support section of the Biomeme website (https://help.biomeme.com). There were discussions about setting up a webinar style training with Biomeme staff, but the time line for this project didn't allow the users the opportunity to establish this training method. The times recorded for performing all extractions begin with a clean BSC and end after the BSC is again deconned. The amount of time included in the extraction timing for the quick decon of the BSC with a bleach wipe, followed by an isopropanol wipe, was between 2.5 to 3.5 minutes. The time (mm:ss) required for novice users was 22:17 and 22:35 for operators 1 and 2, respectively. This corresponds to minimum overall times (hh:mm) for consecutive sample to answer for 7 samples of 03:48 and 03:50, respectively.

3.3.4 Time Required for RNA Extraction: Proficient User

Proficient operators—those performing between 3 and 10 extractions--like novices, also included the time to go from a clean BSC through extraction and decon the BSC again. Time required for proficient users was **21:30** and **17:02** for operators 1 and 2, respectively. This corresponds to minimum overall times (hh:mm) for consecutive sample to answer for 7 samples of **03:42** and **03:11**, respectively. Note: operator 2 did not use the double gloving technique discussed in the workflow section, Section 3.2.

3.3.5 Time Required for RNA Extraction: Expert User

Despite the 4.5 minute difference in the time required for proficient users to complete the RNA extraction, the time required for expert users—those having performed more than 10 extractions—converged due to operator 2 following the workflow that had been worked out that included using two layers of gloves as discussed in Section 3.2. Time required for the operators to extract these samples was **18:52** and **19:12** for operator 1 and 2, respectively. This corresponds to minimum overall times (hh:mm) for consecutive sample to answer for 7 samples of **03:24** and **03:26**, respectively.

3.3.6 On-the-job Training Knowledge Transfer

Operator 2 and Operator 3 completed an on-the-job training using the see one, do one framework after Operator 2 had become an expert as per the designations used in this evaluation. While demonstrating the RNA extraction, Operator 2 took **22:16** (mm:ss) to demonstrate the technique. Operator 3 took **38:39** (mm:ss) to complete their first extraction. Of note, Operator 3 had less knowledge of RT-PCR and RNA extraction than both Operators 1 and 2 and expressed confusion of some of the aseptic techniques and decontamination protocols that were added to the workflow to minimize cross-contamination and increase operator safety. As Operator 3 completed 4 additional extractions independently, the time required (mm:ss) dropped to **27:53**. This corresponds to a minimum overall time (hh:mm) for consecutive sample to answer for 7 samples of **04:27**, more than an hour more than either Operator 1 or 2. When asked for commentary on the Biomeme M1 Sample Prep Cartridge Kit for RNA 2.0, Operator 3 replied that, overall, they found sample prep to be less challenging with each iteration and felt proficient

by the fourth sample. They concluded that the process could be learned quickly by novice operators.

3.3.7 Overall Conclusion: Time to Result

Operators already proficient in sample processing and RT-PCR procedures received training by reading through the provided Biomeme SARS-CoV-2 Real-Time RT-PCR Test IFU, v1.2 as well as reviewing demonstration videos on the Biomeme website. After this review, the operators felt confident they could perform the testing without additional training. When performing single sample processing using the sample prep kit as experienced users, they averaged about 19 minutes which included decontamination of the BSC following RNA extraction. Loading the Go-strips to run on the Franklin™ thermocycler took an average of 15 minutes and the Franklin™ thermocycler run time was just over 56 minutes. Therefore, at minimum, extraction of 7 consecutive patient samples would take 133 minutes (2.25 hours) and set-up of the Go-Strips and cycling on the Franklin™ thermocycler would take an additional 71 minutes for a total of 204 minutes (nearly 3.5 hours).

4. CONCLUSIONS

The Biomeme SARS-CoV-2 Real-Time RT-PCR Test, run on the FranklinTM thermocycler is a fairly straight-forward test using pre-aliquoted reagents contained in an extraction cartridge and Go-Strips that a user can use to evaluate a sample for the presence of SARS-CoV-2 RNA. Biomeme has submitted the test to the FDA for clearance for use in patient care settings otherwise known as CLIA Waived diagnostic labs. CCDC CBC evaluated the ease of use and the time required to go from sample to result while following the Instructions for Use document, v1.2 that was submitted to the FDA. The assay was found to require a fairly complex laboratory in order to safely process the samples due to the need for a biosafety cabinet to contain any SARS-CoV-2 containing aerosols. Using a CLIA complexity matrix taken from FDA policies, CCDC CBC scientists scored the test complexity an 11 out of possible 21. The lack of manufacturer supplied controls and the requirement to store positive control samples at -80 °C severely limit the locations at which the test could be performed accurately.

CCDC CBC scientists developed a protocol that was an amalgam of experience, online training materials produced by MEDCoE available for other SARS-CoV-2 diagnostics, Biomeme Instructions for Use documentation and user manuals, and video training materials produced by Biomeme. This protocol was executed over approximately 60 sample extractions and the times required to complete each portion of the method were recorded. They concluded that nearly 3.5 hours are required to complete 7 extractions, set-up the seven RT-PCR reactions plus the two control RT-PCR reactions, run the RT-PCR on the Biomeme Franklin™ thermocycler, and interpret results. Therefore, approximately 6 cycles of 7 samples (42 total samples) could be assayed in each 24 period on a single Franklin™ thermocycler.

The evaluators acknowledge that the time for sample to answer using the consecutive extraction procedure they employed is much longer than the time required to complete sequential extraction where the extraction of all 7 samples is initiated simultaneously.

According to the manufacturer, the time required to complete 7 extractions, set-up the seven RT-PCR reactions and two control RT-PCR reactions, run the RT-PCR on the Franklin™ thermocycler, and interpret results is approximately 1.5 hours. This would yield a total 24 hour throughput of about 16 cycles of 7 (112 total samples). This is over 2.5 times more samples than the CCDC CBC developed method. CCDC CBC cautions that there is a large risk of cross-contaminating samples during sequential processing. The possibility of increasing the false positive rate for a disease mandating 14 day quarantine procedures and automated reporting of all positive results should give any clinician pause and suggests a thorough risk evaluation be conducted before adopting the time-saving technique.

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APPENDIX A
CCDC CBC recommended protocol for testing a sample using the Biomeme SARS-CoV-2 Real-Time RT-PCR Test

NOTE: This protocol is performed following the Biomeme SARS-CoV-2 Real-Time RT-PCR Test IFU, v1.2. The times reported in this evaluation began after the operator donned all PPE including lab coat, eye protection, and gloves, and prepared a clean BSC. Additionally, operators wore an N95 respirator as shown in the MEDCoE training materials and as a face covering during the COVID-19 pandemic as per OSD003652-20. The use of double gloves and decontamination (decon) of the biosafety cabinet (BSC) is not specified in the Biomeme SARS-CoV-2 Real-Time RT-PCR Test IFU, v1.2. Rather, these steps were performed based on previous operator experiences when handling potential infectious materials and performing RT-PCR activities.

1. Set-up

NOTE: Cleaned hood should contain P20 and P200 tips, micropipettes, and an empty biohazardous waste receptacle.

1.1 Obtain Biomeme M1 Sample Prep Cartridge Kit for RNA 2.0, RNA Process Control (RPC), extraction rack, and 1.5mL snap-cap transfer vial. Spray items with IPA and place into BSC (Note: Do not spray transfer vial packaging if there is a paper backing).
1.2 Prepare wipe soaked with diluted bleach and place in BSC.
1.3 Obtain sample in Zip Lock style sample bag. Wipe sample container with diluted bleach and place into the hood on the bleach soaked wiper.
1.4 Setup kit:
- 1.4.1 Tear open M1 kit and push up from the bottom of the packaging to get the syringe and cartridge out. Take care not to push the column out.
- 1.4.2 Take the syringe out and hold up from surfaces or place to the side. Do not allow the end of the syringe to contact any surfaces.
- 1.4.3 Push the cartridge out of the packaging and place it in the tray.
- 1.4.4 Carefully arrange the column within the packaging so the Luer-Lok is facing up. Secure the syringe to the column by rotating clockwise to lock the Luer-Lok adapter.
- 1.4.5 Puncture the cartridge in the red section twice.
- 1.4.6 Place the syringe with the attached column to the side or back into the original packaging. Be sure not to touch any other surfaces with the column tip.

2. Prepare sample for extraction

2.1 Don a second pair of examination gloves.
2.2 Open storage bag and retrieve the sample vial.
2.3 Wipe the sample tube with the bleach soaked wiper placed into the hood in Step 1.2.
2.4 Mix sample by vigorously inverting or by vortexing for 10 seconds.
2.5 Open the tube and pipet 200 µL of the sample into the red section of the cartridge.
2.6 Close the sample tube and wipe it with the bleach soaked wiper.
2.7 Doff outer gloves and start the countdown timer for the 10 minute incubation.
2.8 Touching only the exterior of the sample packaging, return the sample to the packaging, seal, and store appropriately.

3. RNA Process Control (RPC)

3.1 While the sample is incubating, add 20 µL of RPC to the red section of the cartridge containing the sample.
3.2 Wipe the RPC tube with bleach soaked wiper.
3.3 Remove RPC from BSC and return to storage.

NOTE: The Biomeme protocol does not state a storage condition for the rehydrated RPC. CBC scientists chose to store it at 4 °C based on previous experience with MS2 bacteriophage.

4. RNA Extraction

4.1 At the end of 10 minutes, don a second pair of gloves, and proceed with manufacturer protocol for processing the sample through the cartridge.
- 4.1.1. Lysis: 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
- 4.1.2. Use a forward then backward rocking motion to enlarge the hole, remove the syringe with the attached sample prep column from the red section, and puncture the red-orange section twice.
- 4.1.3. Protein Wash: Press column tip to the bottom of the red-orange well containing Biomeme Protein Wash (BPW) and draw the BPW 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.
- 4.1.4. Use a forward then backward rocking motion to enlarge the hole, remove the syringe with the attached sample prep column from the red-orange section and puncture the orange section twice.
- 4.1.5. Salt Wash: Press the column tip to the bottom of the orange section of the cartridge containing the Biomeme Wash Buffer (BWB) and 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.
- 4.1.6. Use a forward then backward rocking motion to enlarge the hole, remove the syringe with the attached sample prep column from the orange section and puncture the yellow section twice.

4.1.7. Drying Wash: Press the column tip to the bottom of the yellow well of the cartridge containing the Biomeme Drying Wash (BDW) and 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.

4.1.8. Use a forward then backward rocking motion to enlarge the hole, remove the syringe with the attached sample prep column from the yellow section and puncture the blue section **once**.

4.1.9. Air Dry: Press the column tip to the bottom of the blue well of the cartridge and 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.

4.1.10. Use a forward then backward rocking motion to enlarge the hole, remove the syringe with the attached sample prep column from the blue section and puncture the green section twice.

4.1.11. Extraction: Press the column tip to the bottom of the green well of the cartridge containing Biomeme Elution Buffer (BEB) and elute the bound RNA from the column by drawing the BEB fluid all the way up through the syringe and slowly pumping it back out for a total of 5 pumps.

4.1.12. Transfer: Draw the BEB back up into the syringe then use a forward then backward rocking motion to enlarge the hole, remove the syringe with the attached sample prep column from the green well and expel the eluted RNA into the transfer tube.

4.1.13. Once the sample is in the transfer tube the operator can discard the sample prep kit components. The syringe, column, and cartridge are placed back into the original packaging then placed into the biohazard waste.

4.1.14. Wipe the transfer tube with the bleach soaked wiper and place into the appropriate sample slot on the extraction tray.

4.1.15. Doff outer layer of gloves and decon immediate working area of BSC to include pipettes, tip boxes, sample tray, and sample vial.

4.1.16. Proceed to the next sample, and repeat until all 9 samples are complete.

4.1.17. Once all samples are complete, perform a full decon of the BSC prior to setting up the Biomeme SARS-CoV-2 Go-Strips.

5. Set-up Biomeme SARS-CoV-2 Go-Strip Assay Tubes

5.1 Obtain Biomeme SARS-CoV-2 Go-Strips and void filling caps from its packaging and place into the BSC.

5.2 Place a wiper soaked with diluted bleach into the BSC.

5.3 Ensure the pellets in the Go-Strips are at the bottom of the tube by gently but firmly tapping the vials on the surface.

5.4 Place Go-strips into the appropriate areas on the tray with the small tag on the foil covering oriented to the left.

5.5 Don a second set of gloves over the first pair.

5.6 Remove the foil covering from one Go-strip at a time.

5.7 Add 20 µL of controls/samples to one well at a time. Pipette up and down 3 – 5 times to mix the PCR reaction. Be sure to limit the generation of bubbles while mixing.

5.8 Wipe gloved hands with dilute bleach soaked wiper after handling each sample.

5.9 Repeat 5.6 and 5.7 until all three wells of a strip are filled.

5.10 Doff outer gloves and place a void filling cap into the Go-Strip assuring to align the Go-Strip and void filling cap so that the strip connections are visible through the cap cutouts. NOTE: The cap will be slightly loose.

5.11 Repeat steps 5.5 through 5.10 until all 3 Go-Strips are filled and capped.

Load Go-Strips into Biomeme Franklin™ thermocycler and perform RT-PCR using the Biomeme Go App

6.1 Confirm there are no bubbles at the bottom of the Go-Strip assays.

6.2 Open the lid of the thermocycler and insert the Go-Strips into the appropriate 3-well spots, making sure the strip connections are visible through the void filling cap cutouts and are facing the back of the thermocycler.

6.3 Open the Biomeme Go app on the phone and follow the prompts to start the run. See user manual for troubleshooting.

6.3.1. Scan the SARS-CoV-2 assay barcode using the phone's camera. Confirm information displayed is correct.

6.3.2. Choose the number of Go-Strips added and the layout of the samples

6.3.3. Enter Sample IDs

6.3.4. Confirm Go-Strips were loaded properly

6.3.5. Turn on Franklin™ thermocycler by pressing the power button.

NOTE: user will hear servo motors and see LED display on front of device.

6.3.6. Choose the method of connecting to the Franklin™ thermocycler. If Bluetooth, follow prompts to press the Bluetooth connection button on the Franklin then ensure the correct Franklin™ thermocycler is chosen from the list that populates.

6.3.7. Close lid and start run.

6.4 Use the SARS-CoV-2 Real-Time RT-PCR Test IFU to interpret results and confirm Cq values to a qualitative "positive/negative/invalid" call.

APPENDIX B
Biomeme SARS-CoV-2 Real-Time RT-PCR Test Instructions for Use, v1.2

Biomeme SARS-CoV-2 Real-Time RT-PCR Test Instructions For Use v1.2

Brief Overview

The Biomeme SARS-CoV-2 Real-Time RT-PCR Test is a qualitative multiplex assay for *in vitro* diagnostic (IVD) use on Biomeme's Franklin™ Real-Time PCR System. 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

Biomeme SARS-CoV-2 for use on the Biomeme's Franklin Real-Time PCR System is a real-time RT-PCR test intended for qualitative detection of RNA from SARS-CoV-2 in nasopharyngeal and oropharyngeal (throat) swab samples from patients who meet COVID-19 clinical and/or epidemiological criteria. Testing is limited to Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a certified laboratories with FDA Emergency Use Authorization FDA for performing SARS-CoV-2 testing. Other Authorized Testing Locations - Patient care settings. The Biomeme SARS-CoV-2 test is only for use under the Food and Drug Administration's Emergency Use Authorization.

Results are for the identification of SARS-CoV-2 RNA in nasopharyngeal and oropharyngeal (throat) swab samples during 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 target detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

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

The Biomeme SARS-CoV-2 Real-Time RT-PCR Test is intended for use by trained 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.

***Note**: Nasal swabs and mid-turbinate swabs are considered acceptable specimen types for use with Biomeme's SARS-CoV-2 Real Time RT-PCR test, but performance with these specimen types has not been established. Testing of nasal and mid-turbinate nasal swabs (self-collected under supervision of or collected by a healthcare provider) is limited to patients with symptoms of COVID-19. Please refer to FDA's FAQs on Diagnostic Testing for SARS-CoV-2 for additional information.*

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]

The Biomeme SARS-CoV-2 Real-Time RT-PCR Test is a molecular *in vitro* diagnostic test that aids in the detection and diagnosis of SARS-CoV-2 and 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 nasopharyngeal and oropharyngeal (throat) swab specimens.

The term "qualified laboratories" refers to laboratories in which all users, analysts, and any person reporting results from use of this device are proficient in performing real-time RT-PCR assays.

[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.

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 qCPR 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.

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. Please contact support@biomeme.com for further instruction on running Go-Strips on the Bio-Rad CFX96, ABI 7500, or QuantStudio5 using the "fast" block. The Biomeme SARS-CoV-2 assay is also available in a 96-well Go-Plate format for direct use onBio-Rad CFX96, ABI 7500, or QuantStudio5 using the "fast" block.

Contents

The materials provided 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

The components listed below contain sufficient reagents to process 30 samples or quality control samples.

Source: REF#	Component	Description
BD: 220531 or Thermo Scientific™: R12515	BD Viral Transport Media (VTM) or MicroTest™ M5™ Viral Transport Media (VTM-M5)	Collect and maintain samples during transport and before molecular analysis
Biomeme: 3000567	200µL Transfer Pipette Pack	Pack of disposable transfer pipettes to transfer VTM into Extraction Kit
Biomeme: 3000536	Biomeme M1 Sample Prep Cartridge Kit for RNA 2.0	RNA Extraction Kit containing cartridges, syringes, and binding column tips
Biomeme: 3000011	20µL Fixed Volume Pipette Kit	20µL pipette and 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*	Pre-aliquoted 3-well PCR strips. Each well contains a 20µL lyophilized triplex reaction. Also includes RNA Process Control (MS2).

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

Biomeme SARS-CoV-2 Real-Time RT-PCR Test Instructions For Use v1.2

Additional Form Factors

SARS-CoV-2 Assays also come in two additional form factors:

- 3000562: Biomeme SARS-CoV-2 Go-Plates (96 rxns at 20 uL)
- 3000564: Biomeme SARS-CoV-2 Bulk Vials (65 rxns at 20 uL)

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

The following equipment and software is required to run the test and analyze results.

Source: REF#	Component	Description
Biomeme: 1000003	Biomeme Franklin three9 Real-Time PCR Thermocycler	Real-Time PCR Thermocycler
Biomeme: 1000013 or Biomeme: 1000012	Android Smartphone w/ Biomeme Go Mobile App or Rugged Android Smartphone w/ Biomeme Go Mobile App	Controller for Biomeme Franklin Thermocycler
Biomeme: 2000006	Biomeme Cloud	PCR Data Management Software
Provided by User	No Template Control (NTC)	Monitors contamination and primer-dimer formation that could produce false positive results
Provided by User	Positive Control (PC)	Control that is not exposed to the experimental treatment and is known to produce a positive result.

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

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.

- The assay is for *in vitro* diagnostic use under the FDA Emergency Use Authorization Only.
- 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 the local, state, and federal regulations.
- Safety Data Sheets are available upon request.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- 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.

- Reagents must be stored and handled as specified in Tables 1 and 2.

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. See the Swab Collection section below for collection procedure. Nasopharyngeal specimens can be stored at room temperature (15–30 °C) for up to 8 hours and refrigerated (2–8 °C) up to seven days until sample extraction is performed using Biomeme's M1 Sample Prep Cartridges.

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

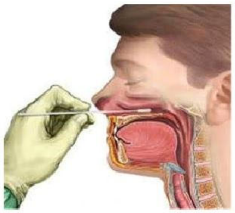
General Guidelines

- Do not use any of the materials supplied after their expiry dates.
- 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.
- All procedures should be performed in a BSL2 laboratory, and specimens handled within a Biological Safety Cabinet.
- Precautions must be taken to prevent cross contamination of samples.

Nasopharyngeal (NP) Swab Collection

Describe to the patient what they can expect during the NP collection and the importance of staying still to allow for the least discomfort and accurate collection. Additionally, encourage the patient to blow his or her noses to clear nasal passage.

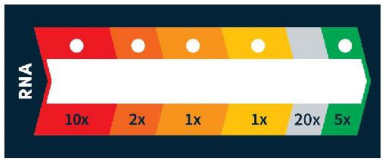
1. Put on a mask, eye protection, gloves and any other necessary PPE.
2. With the person's head in a neutral position, insert the swab into either nostril straight back (not upwards), along the floor of the nasal passage until you reach the posterior wall of the nasopharynx (generally half of the distance from the base of the nose to the front of the ear).



3. Rotate the swab gently then leave in place for a few seconds.
4. Carefully remove the swab without touching the sides of the nostril.
5. Open a viral transport tube (e.g. BD Viral Transport Media or MicroTest™ M5™ Viral Transport Media) and place the swab inside.
6. Break the swab at the indicated break line and cap the sample collection tube tightly. Shake the tube after capping.
7. Proceed to Sample Extraction.

***Note**: Nasal swabs and mid-turbinate swabs are considered acceptable specimen types for use with Biomeme's SARS-CoV-2 Real Time RT-PCR test, but performance with these specimen types has not been established. Testing of nasal and mid-turbinate nasal swabs (self-collected under supervision of or collected by a healthcare provider) is limited to patients with symptoms of COVID-19. Please refer to FDA's FAQs on Diagnostic Testing for SARS-CoV-2 for additional information.*

RNA Extraction Using M1 Sample Prep Cartridge



After collecting your sample, use Biomemes'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.
- **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.

Prepare RNA Process Control (MS2)

The Biomeme SARS-CoV-2 Real-Time RT-PCR Test includes RNA Process Control (RPC) and RPC Pellets (MS2). Extractions should be performed in batches of 9 total reactions, to include: one (1) negative control (NTC), one (1) positive control (PC), and seven (7) patient samples.

1. Remove the 2mL screw cap tube containing your RPC pellet to open the tube.
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 to the MS2 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.
6. Your RPC is now ready to add to your upcoming sample extractions (this will equal ~400 pfu per 20µL PCR reaction).

Add Your Sample

1. Vortex or shake your viral transport tube containing your swab sample for 10 seconds.
2. Open your M1 Sample Prep Cartridge pouch and remove the contents.
3. 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 luerlock syringe with column attached on a tube rack such that the tip of the column is not touching any surface.
4. Using a 200µL transfer pipette (REF# 3000567), or your own 200µL pipette, transfer 200µL of media from the transport tube containing your sample and add it into the red section of your sample prep cartridge.
5. 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 into the punctured red section of your sample prep cartridge.
2. After the sample has finished incubating for 10 minutes at room temperature (15–25°C) inside the red section of the cartridge, proceed to Lysis & Binding.

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, remove the tip of the sample prep column from the red section of the sample prep cartridge and gently pull back the plunger, wait a few seconds, and slowly push the plunger back down. You should notice some of the liquid 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 to remove excess buffer.
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.

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**.

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Transfer Extracted RNA to Storage Tube

1. After completing the 5th pump, draw up the entire fluid 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 a new set of materials for up to 7 total samples (+1 NTC and 1 PC) to optimize throughput of the Biomeme Franklin™ thermocycler.
2. Proceed to Loading Pure Sample into Go-Strips.

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.

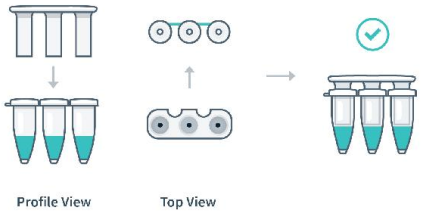
1. Open the contents of a Biomeme SARS-CoV-2 Go-Strips (REF# 3000555). Do not immediately discard the Go-Strips pouch as you'll need to scan the QR code in a later step.
2. Start with a single Go-Strip and remove the foil covering.
3. Attach a pipette tip to a 20µL fixed volume pipette (REF# 3000011) or prepare your own 20µL pipette.
4. Unscrew the cap of your no template control (NTC) and transfer 20µL to the **first** reaction well of your first Go-Strip. Pipette up and down 3-5 times to mix your PCR reaction. Discard your pipette tip. Recap your NTC tube.

   ***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).*

5. 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.*

6. Unscrew the cap of your positive control (PC) and transfer 20µL to the **second** reaction well of your first Go-Strip. Pipette up and down 3-5 times to mix your PCR reaction. Discard your pipette tip. Recap your PC tube.
7. Unscrew the cap of your first purified sample in the 2mL tube and transfer 20µL of the extracted RNA into the **third** reaction well of your Go-Strip. Pipette up and down 3-5 times to mix your PCR reaction.
8. Discard your pipette tip and repeat the process of transferring your samples only (no NTC or PC) for the remaining 6 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:



9. 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. If the cap touches liquid in the well bottom, it will wick up your reaction.

Placing Go-Strips into Franklin™ Thermocycler

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

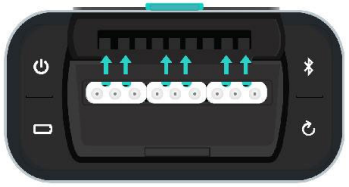
PCR Layout Example (for one full Franklin™ run)

Well	1	2	3	4	5	6	7	8	9
A	NTC	PC	S1	S2	S3	S4	S5	S6	S7

a. The Go-Strip containing samples NTC, PC, S1 should be inserted to the far left of the thermocycler.

b. The Go-Strip containing samples S2 - S4 should be inserted in the middle 3 wells of the thermocycler.

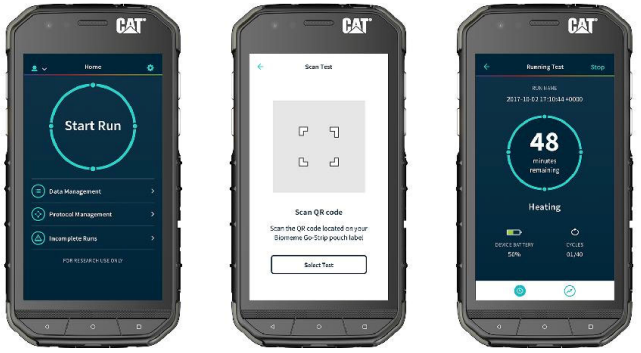
c. The Go-Strip containing samples S5 - S7 should be inserted to the far right of the thermocycler.



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 Go-Strip void filling cap to avoid liquid splatter and PCR amplicon contamination.*

Launch Biomeme Go App on Smartphone



Biomeme Go (REF# 1000013 or REF# 1000012) is an intuitive smartphone app that pairs wirelessly with it's real-time PCR thermocycler. 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 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 Go, tap **Start Run**.
3. Use the camera on your smartphone to scan the **QR code** printed on the Go-Strips pouch you opened earlier. If you experience trouble scanning, you can also choose to manually select your test.

   Note: *The first time you scan a QR code, you may be asked to give your QR scanner 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
Extension	No Extension

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'd 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 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 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 app and reconnected to the thermocycler. For more information on recovering and reattaching test data, please see help.biomeme.com.*

Positive Control Material

1. A new positive control (PC) is prepared by dilution of the target organisms (Genomic RNA from SARS-Related Coronavirus 2, Isolate USA-WSA1/2020 (BEI NR-522285, Lot: 70033320, Mftg Date: 11FEB2020) to 2x LoD, 9 replicates RNA extracted and eluate aliquoted into 22uL per tube. The PC RNA is stored in the -80 freezer and 1 PC is run with every patient batch run on the Franklin instrument by adding 20uL of the extracted PC RNA to the SARS-CoV-2 Go-strip.
    1. The Cq value for each run must be within +/- 3SD of the Cq values observed in the 2xLoD samples of the LoD Study from the assay's validation data.
    2. No more than 1 in 100 values may be omitted as an outlier.
2. PC Values are tracked over time and monitored in the monthly QA review.
    1. The observed PC Cq values will be observed over time and a graph will be plotted including each nucleic acid run to ensure that the data is continually reviewed for possible trends.
        1. The mean Cqs are within +/- 3SD across all samples tested, per target.
        2. No more than 1 in 100 values may be omitted as an outlier.
        3. Any trends outside of these parameters warrant further investigation.

Interpreting Results

Recommended cycle cut-off is 40 cycles. Any amplification after cycle 40 should be considered negative, however 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.

QC Material Pass/Fail Criteria

Control Type	Control Name	Used to Monitor	2019 nCoV ORF1ab	2019 nCoV S	MS2	Expected Cq values
Negative	NTC	Reagent and/or environmental contamination	-	-	-	None detected
Positive	PC	Substantial reagent failure including primer and probe integrity	+	+	+	< 40
Extraction	MS2	Failure in lysis and extraction procedure, potential contamination during extraction	-	-	+	< 40

- If the PC is negative for any of the assay targets, repeat the PCR. If the PC remains negative, re-extract the entire batch of samples. If the newly extracted PC is still negative, suspect system failure and discontinue testing until the source of failure is found and eliminated. Patient testing should not resume until the PC is performing as expected.
- If the NTC is positive for any of the assay targets, first repeat PCR. If the NTC remains positive, that batch of extracted samples are invalid and the user must repeat the extraction for the entire bath. If the newly extracted NTC is still positive, suspect contamination of the test system and discontinue testing until the source of contamination is found and eliminated. Patient testing should not resume until NTC is performing as expected.

Patient Sample Pass/Fail Criteria

nCoV orf1ab target	nCoV S target	RPC (MS2)	Result	Actions
+	+	±	Positive	N/A
If only one target is positive		±	Positive	N/A
-	-	-	Invalid	Re-extract the sample and run the rRT-PCR again. If the same result is obtained as the first run, report as Invalid.
-	-	+	Negative	N/A

This algorithm should be used in conjunction with recommended Cq cutoff value and visual analysis of amplification curves.

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 Go-Strip void filling cap to avoid liquid splatter and PCR amplicon contamination.*

Examples

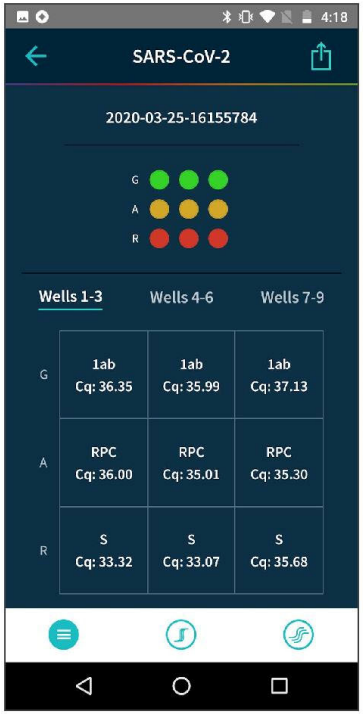
The first screenshot below guides you through key components of the qualitative result screen followed by examples of the possible Biomeme SARS-CoV-2 Real-Time RT-PCR Test results as outlined in the Interpretation Table above.

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 Result

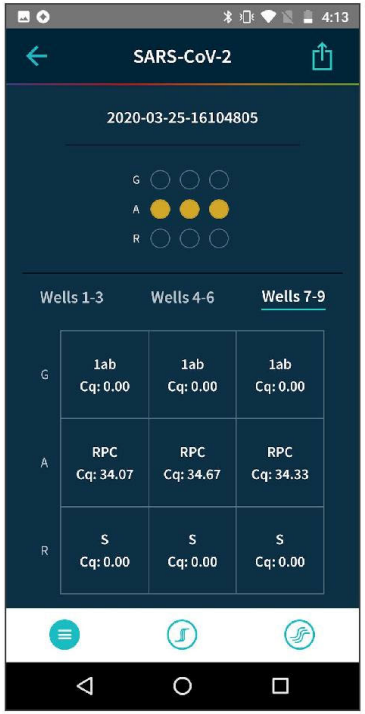
All targets detected



No additional action required other than reporting.

Negative Result

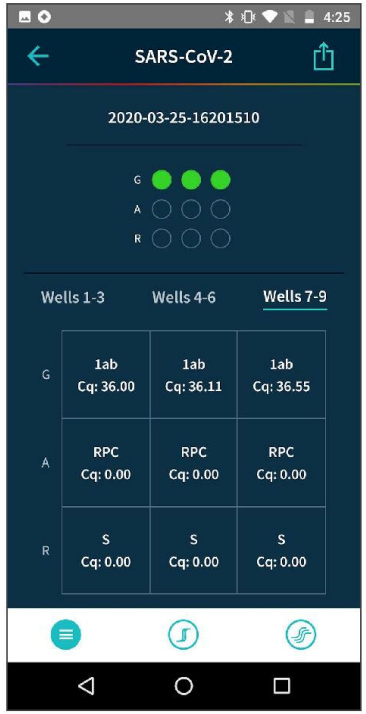
Only RPC detected



No additional action required other than reporting.

Positive Result

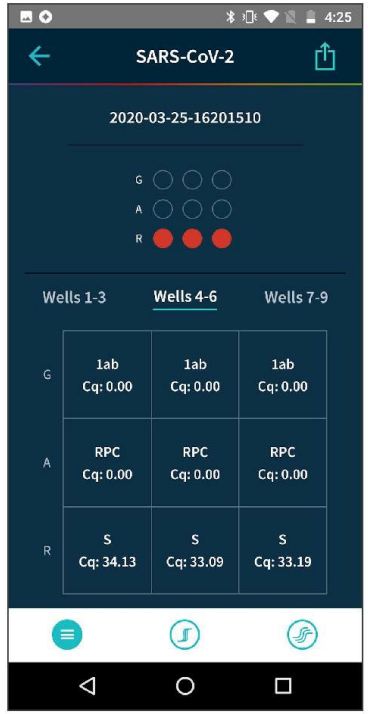
Only Orf1ab target detected



No additional action required other than reporting.

Positive Result

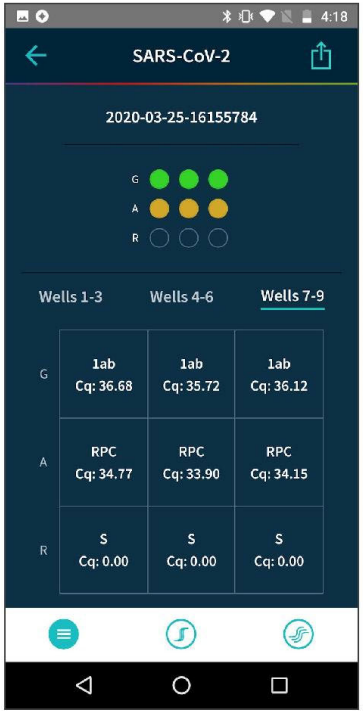
Only S target detected



No additional action required other than reporting.

Positive Result

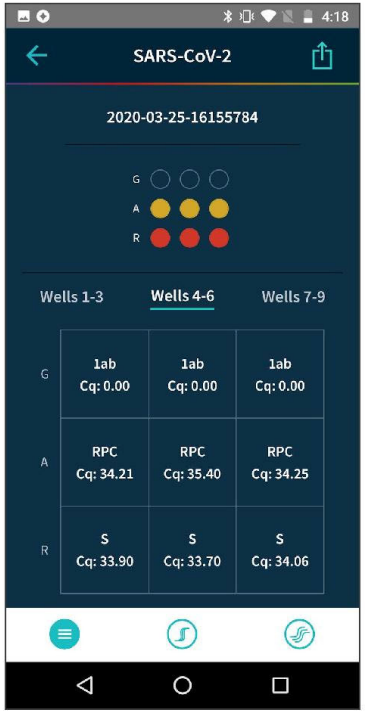
Orf1ab target & RPC detected
S target not detected



No additional action required
other than reporting.

Positive Result

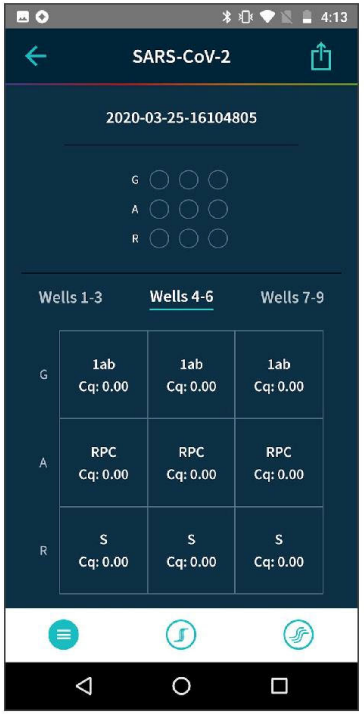
S target & RPC detected
Orf1ab target not detected



No additional action required
other than reporting.

Invalid

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 use of this assay as an *in vitro* diagnostic under the FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a. Other Authorized Testing Locations - Patient care settings. The Biomeme SARS-CoV-2 test is only for use under the Food and Drug Administration's Emergency Use Authorization.
- Biomeme SARS-CoV-2 Real-Time RT-PCR Test performance was established using nasopharyngeal swab. Other specimen types have not been evaluated and should not be tested with this assay.

  ***Note**: Nasal swabs and mid-turbinate swabs are considered acceptable specimen types for use with Biomeme's SARS-CoV-2 Real Time RT-PCR test, but performance with these specimen types has not been established. Testing of nasal and mid-turbinate nasal swabs (self-collected under supervision of or collected by a healthcare provider) is limited to patients with symptoms of COVID-19. Please refer to FDA's FAQs on Diagnostic Testing for SARS-CoV-2 for additional information.*

- 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.
- Laboratories are required to report all positive results to the appropriate public health authorities.

Conditions of Authorization for Labs

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

To assist clinical laboratories running the Biomeme SARS-CoV-2 Real-Time RT-PCR Test, the relevant Conditions of Authorization are listed verbatim 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 result reports of the Biomeme SARS-CoV-2 Real-Time RT-PCR Test, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

- Authorized laboratories using the Biomeme SARS-CoV-2 Real-Time RT-PCR Test will perform the Biomeme SARS-CoV-2 Real-Time RT-PCR Test as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to perform 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 (biomeme@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, it's 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] For ease of reference, this letter will refer to, "Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a certified laboratories with FDA Emergency Use Authorization FDA for performing SARS-CoV-2 testing. Other Authorized Testing Locations - Patient care settings" as "authorized laboratories."

Performance Characteristics

Clinical Evaluation

1. A master list of Sample IDs were generated, and labels made for synthetic specimens to be made for the Clinical Evaluation.
2. A technician not performing the Clinical Evaluation scrambled the cohort to eliminate bias.
3. A technician not performing the Clinical Evaluation:
    1. Created 30 "negative" specimens using 30 individual clinical negative specimens.
    2. Created 20 "positive specimens", 10 at a concentration @2x LoD and the remaining 10 @ 3x LoD (5 specimens), 4xLoD (3 specimens) and 5xLoD (2 specimens).

       ***Note**: 10 of the samples at 1xLoD in the LoD Study were counted as the 1xLoD "positives" towards the Clinical Evaluation.*
4. Acceptance Criteria:
    1. ≥ 95% agreement for the positive specimens @ 2x LoD.
    2. 100% agreement for all other specimens, including negative specimens.

As of March 24th, 2020, Biomeme has tested 30 "positive" and 30 "negative" specimens. Specimen type is NP swab. "Positive" specimens were created with varying concentrations, 10 @ 2xLoD, 10 @ 1XLoD and remaining 10 between 3x-5x LoD. "Negative" specimens were created using individual clinical negative matrix (NP swabs) specimens.

The Clinical Evaluation study was done by spiking in known concentration of Genomic RNA from SARS-Related Coronavirus 2, Isolate USA-WSA1/2020 (BEI NR-522285, Lot: 70033320, Mftg Date: 11FEB2020) into individual clinical negative matrix (NP swab), for "positive" samples. Individual negative matrix (NP swab) specimens were used as is for "negative" samples. The clinical negative matrix was mixed with BLB in red section of Biomeme M1 sample prep cartridge for RNA 2.0 prior to addition of viral genomic RNA and for 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. 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).

Table 4. Summary of Biomeme SARS-CoV-2 Real-Time RT-PCR Assay Generated By Testing Human Respiratory Specimens Collected From Individual NP Swabs

Specimen Type	Number of Specimens	Biomeme SARS-CoV-2 Assay Positive	Biomeme SARS-CoV-2 Assay Negative	Biomeme SARS-CoV-2 Assay Inconclusive
NP Swab	60	30	30	0

Positive percent agreement= 30/30= 100%
Negative Percent Agreement=30/30= 100%

Table 5. Detailed List of 50 samples run in the Clinical Evaluation

			Targets				
#	Description	Expected Result	orf1ab	MS2	S	Final Result	PASS/FAIL
1	NEG	NEG	NEG	POS	NEG	NEG	PASS
2	NEG	NEG	NEG	POS	NEG	NEG	PASS
3	3.6 genome equivalent per uL	POS	POS	POS	POS	POS	PASS
4	NEG	NEG	NEG	POS	NEG	NEG	PASS
5	5.4 genome equivalent per uL	POS	POS	POS	POS	POS	PASS

6	3.6 genome equivalent per uL	POS	POS	POS	POS	POS	PASS
7	7.2 genome equivalent per uL	POS	POS	POS	POS	POS	PASS
8	NEG	NEG	NEG	POS	NEG	NEG	PASS
9	NEG	NEG	NEG	POS	NEG	NEG	PASS
10	NEG	NEG	NEG	POS	NEG	NEG	PASS
11	NEG	NEG	NEG	POS	NEG	NEG	PASS
12	NEG	NEG	NEG	POS	NEG	NEG	PASS
13	5.4 genome equivalent per uL	POS	POS	POS	POS	POS	PASS
14	NEG	NEG	NEG	POS	NEG	NEG	PASS
15	7.2 genome equivalent per uL	POS	POS	POS	POS	POS	PASS
16	NEG	NEG	NEG	POS	NEG	NEG	PASS
17	NEG	NEG	NEG	POS	NEG	NEG	PASS
18	NEG	NEG	NEG	POS	NEG	NEG	PASS
19	5.4 genome equivalent per uL	POS	POS	POS	POS	POS	PASS
20	5.4 genome equivalent per uL	POS	POS	POS	POS	POS	PASS
21	5.4 genome equivalent per uL	POS	POS	POS	POS	POS	PASS
22	NEG	NEG	NEG	POS	NEG	NEG	PASS
23	NEG	NEG	NEG	POS	NEG	NEG	PASS
24	NEG	NEG	NEG	POS	NEG	NEG	PASS
25	NEG	NEG	NEG	POS	NEG	NEG	PASS
26	NEG	NEG	NEG	POS	NEG	NEG	PASS

27	NEG	NEG	NEG	POS	NEG	NEG	PASS
28	NEG	NEG	NEG	POS	NEG	NEG	PASS
29	9 genome equivalent per uL	POS	POS	POS	POS	POS	PASS
30	NEG	NEG	NEG	POS	NEG	NEG	PASS
31	NEG	NEG	NEG	POS	NEG	NEG	PASS
32	NEG	NEG	NEG	POS	NEG	NEG	PASS
33	NEG	NEG	NEG	POS	NEG	NEG	PASS
34	3.6 genome equivalent per uL	POS	POS	POS	POS	POS	PASS
35	3.6 genome equivalent per uL	POS	POS	POS	POS	POS	PASS
36	3.6 genome equivalent per uL	POS	POS	POS	POS	POS	PASS
37	NEG	NEG	NEG	POS	NEG	NEG	PASS
38	7.2 genome equivalent per uL	POS	POS	POS	POS	POS	PASS
39	3.6 genome equivalent per uL	POS	POS	POS	POS	POS	PASS
40	3.6 genome equivalent per uL	POS	POS	POS	POS	POS	PASS
41	3.6 genome equivalent per uL	POS	POS	POS	POS	POS	PASS
42	NEG	NEG	NEG	POS	NEG	NEG	PASS
43	NEG	NEG	NEG	POS	NEG	NEG	PASS
44	NEG	NEG	NEG	POS	NEG	NEG	PASS
45	3.6 genome equivalent per uL	POS	POS	POS	POS	POS	PASS
46	9 genome equivalent per uL	POS	POS	POS	POS	POS	PASS

47	3.6 genome equivalent per uL	POS	POS	POS	POS	POS	PASS
48	NEG	NEG	NEG	POS	NEG	NEG	PASS
49	NEG	NEG	NEG	POS	NEG	NEG	PASS
50	NEG	NEG	NEG	POS	NEG	NEG	PASS
NSC	No Sample Control (NSC)	NEG	NEG	NEG	NEG	NEG	PASS
NSC	No Sample Control (NSC)	NEG	NEG	NEG	NEG	NEG	PASS
NSC	No Sample Control (NSC)	NEG	NEG	NEG	NEG	NEG	PASS
NSC	No Sample Control (NSC)	NEG	NEG	NEG	NEG	NEG	PASS
	NTC	NEG	NEG	NEG	NEG	NEG	PASS
	NTC	NEG	NEG	NEG	NEG	NEG	PASS
	NTC	NEG	NEG	NEG	NEG	NEG	PASS
	NTC	NEG	NEG	NEG	NEG	NEG	PASS
	NTC	NEG	NEG	NEG	NEG	NEG	PASS
	NTC	NEG	NEG	NEG	NEG	NEG	PASS
	NTC	NEG	NEG	NEG	NEG	NEG	PASS
	NTC	NEG	NEG	NEG	NEG	NEG	PASS
	NTC	NEG	NEG	NEG	NEG	NEG	PASS
	NTC	NEG	NEG	NEG	NEG	NEG	PASS
	NTC	NEG	NEG	NEG	NEG	NEG	PASS

Analytical Performance

Analytical Sensitivity (Limit of Detection)

LoD studies determine the lowest detectable concentration of viral genomic RNA for both Orf1ab and S targets. The LoD study was done by spiking in known concentration of Genomic RNA from

APPENDIX B

	Rhinovirus
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Bordetella pertussis
	Mycoplasma pneumoniae
	Pneumocystis jiroveci (PJP)
	Candida albicans
	Pseudomonas aeruginosa
	Staphylococcus epidermis
	Staphylococcus salivarius

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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.

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Revision	Date	Description
1.0	April 3, 2020	New document
1.1	April 8, 2020	Updated Table 4 LOD, NTC and PC instructions
1.2	April 9, 2020	Updated NTC and PC instructions

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