revogene®

Revogene SARS-CoV-2 assay

For Emergency Use Authorization (EUA) Only

For use with the Revogene®

REF **410700** IVD **For *in vitro* Diagnostic Use** 8 C / 2 C **R_x Only**

INTENDED USE

The Revogene® SARS-CoV-2 assay is a real-time RT-PCR test intended for the qualitative detection of RNA from SARS-CoV-2 in nasopharyngeal, oropharyngeal, anterior nasal, and mid-turbinate nasal swab specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, that meet requirements to perform high or moderate complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in nasopharyngeal, oropharyngeal, anterior nasal, and mid-turbinate nasal swab specimens during the acute phase of infection. Positive results are indicative of the presence of SARS CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all test 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 Revogene SARS-CoV-2 assay is a single-use test intended for use by qualified laboratory personnel specifically instructed and who are proficient in performing testing using the Revogene instrument. The Revogene SARS-CoV-2 assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

SUMMARY AND EXPLANATION OF THE TEST

The World Health Organization (WHO) received a report of an outbreak of respiratory illness on December 31, 2019[1]. The source of this outbreak was Wuhan City, Hubei Province, China[1]. Authorities identified this outbreak as coronavirus disease 2019 (COVID-19). On March 11, 2020, the COVID-19 outbreak was characterized as a pandemic by the WHO[2]. The virus was subsequently named SARS-CoV-2 by the International Committee for Taxonomy of Viruses (ICTV)[3]. The coronaviruses are a group of enveloped, non-segmented positive-sense RNA viruses which may cause illness in animals or humans[4]. In humans, coronaviruses are known to cause a variety of clinical outcomes, including asymptomatic infection, mild upper respiratory infection, severe lower respiratory disease including pneumonia and respiratory failure, and in some cases, death. The availability of specific and sensitive assays for the detection of the virus is essential for accurate diagnosis of cases, assessment of the extent of the outbreak, monitoring of intervention strategies, and surveillance studies.

The Revogene SARS-CoV-2 assay is a rRT-PCR *in vitro* diagnostic test designed to identify nucleic acid from SARS-CoV-2 associated with respiratory tract infection, from a single swab specimen. The Revogene SARS-CoV-2 reagents of Revogene SARS-CoV-2 assay contain primers and probes targeting RNA from the nucleocapsid protein (N) gene of the SARS-CoV-2 coronavirus. Two (2) controls are also incorporated in the system, one (1) Microfluidic Control and one (1) Internal Control. The assay minimizes operator intervention from the time the single-use microfluidic cartridge (named PIE hereafter) containing the sample is placed in the Revogene carousel until results are available. Each patient specimen (nasopharyngeal swab, oropharyngeal swab. anterior nasal swab (nasal), or mid-turbinate nasal swab specimens) collected in authorized transport media (see the Storage and Stability section) is directly transferred into the Revogene SARS-CoV-2 PIE using a Disposable Transfer Tool (DTT). The PIE is designed to perform viral lysis and the homogenized sample is automatically delivered by centrifugation to sample wells containing dried master mix.

PRINCIPLE OF THE PROCEDURE

The Revogene automates sample homogenization, dilution, cell lysis, conversion of RNA templates into DNA using reverse transcription, nucleic acid amplification and detection of the amplified PCR products. User intervention is only required to transfer the sample into the PIE and insert the PIEs into the Revogene carousel.

Each PIE is a fully integrated closed device into which a sample is dispensed and processed through different microfluidic chambers and channels which allow for the sample processing (i.e., sample homogenization, sample dilution and cell lysis) and subsequent rRT-PCR steps (**Figure 1**). The liquid from a single sample is transferred by centrifugation from one chamber to the next in sequence. All reagents specific for the PCR reaction are incorporated and dried within the PCR well. An Internal Control is incorporated into each PIE to verify RNA transcription/amplification/detection steps including the verification of potential inhibitory substances as well as reagent failure. A Microfluidic Control is also incorporated into each PIE to verify the fluidic properties of the PIE. The amplified products are detected in real-time using target-specific TaqMan® chemistry-based probes. No operator intervention is necessary once a PIE is loaded into the Revogene.

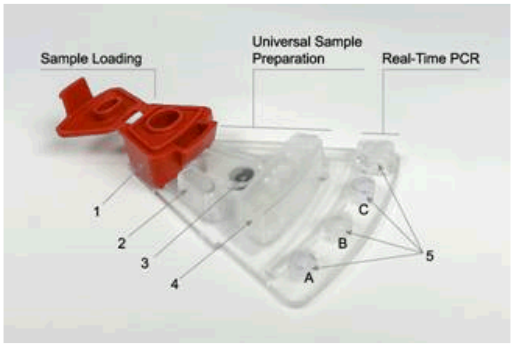

Figure 1. Top View of a PIE.
1: Sample Loading Chamber, 2: Overflow Chamber,
3: Homogenization Chamber containing the Microfluidic Control, 4: Dilution/Lysis Chamber,
5: Three (3) PCR Wells (A to C from left to right) and one (1) Waste Chamber (at the right end).

The Revogene can process from one (1) up to eight (8) samples simultaneously in the same run. The carousel must contain eight (8) PIEs to maintain thermodynamic balance within the run. During the run and at run completion, the results are computed by the system from measured fluorescent signals and embedded calculation algorithms. Results are displayed on the touchscreen and may be printed or transferred to a USB flash drive or transmitted to the laboratory information system.

An Early Positive Result Outcome (E-PRO) feature provides a positive result if the signal from the SARS-CoV-2 target reaches a predetermined threshold before the full PCR cycles have been completed. With E-PRO, a positive result could be obtained as early as 47 minutes after run start for samples with a high viral load. For negative samples, the time to result is approximately 85 minutes.

REAGENTS AND MATERIALS PROVIDED

Revogene SARS-CoV-2 kit contains sufficient reagents and materials to process 24 samples. Each kit contains the following materials:

1. Twenty-four (24) **Disposable Transfer Tools (DTT):** Plastic pipette with minimal and maximal volume marks for transferring the sample into the PIE.
2. Twenty-four (24) individual pouches, each pouch containing one (1) **Revogene SARS-CoV-2 microfluidic cartridge (PIE):** Integrated device which comprises dried reagents allowing sample process and rRT-PCR steps for transcription, amplification and detection of SARS-CoV-2 RNA. Each PIE contains an Internal Control (IC), IC-specific primers and probe, SARS-CoV-2 target-specific primers and probes, a Microfluidic Control, dNTPs, buffer and DNA polymerase displaying reverse transcriptase activity.

MATERIALS REQUIRED BUT NOT PROVIDED

- Revogene® Instrument (cat# 610210)
- Revogene® System RNA Software Upgrade Kit[5] (cat# 610240; must be installed prior to running the Revogene SARS-CoV-2 assay. Please contact Technical Support at 1-800-343-3858 to ensure the appropriate software has been installed on your instrument.
- Disposable powderless gloves
- MOCK PIE(s) (cat# 610208; optional)
- Positive external control (for example: viral transport media or saline spiked with well characterized SARS-CoV-2 virus)
- Negative external control (viral transport media or saline)

WARNING AND PRECAUTIONS

1. For *in vitro* diagnostic (IVD) use under Emergency Use Authorization only.
2. For prescription use only.
3. Positive results are indicative of presence of SARS-CoV-2 RNA.
4. This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
5. Laboratories within the United States and its territories are required to report all test results to the appropriate public health authorities.
6. This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.
7. The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3 (b)(1), unless the declaration is terminated, or authorization is revoked sooner.
8. The Revogene SARS-CoV-2 assay can only be used on the Revogene instrument.
9. Always handle specimens as if they are infectious and in accordance with Good Laboratory Practices such as those described in Biosafety in Microbiological and Biomedical Laboratories[6] and in Clinical and Laboratory Standards Institute (CLSI) Document M29-A4[7]. Only personnel proficient in handling infectious materials and the use of Revogene SARS-CoV-2 should perform this procedure.
10. If infection with SARS-CoV-2 is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions. Refer to the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19)[8] for more information. https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html
11. Wear disposable powderless gloves while handling specimens and thoroughly wash hands afterwards.
12. Do not use the kit if the label that seals the outer box is broken upon arrival.
13. Do not use PIEs if the protective pouches are open or broken upon arrival.

14. Each single-use DTT and PIE are used to process one (1) specimen. Do not reuse DTT or PIE.
15. The PIE contains dried reagents. The protective pouch should not be opened until ready to perform the test.
16. Dispose of unused reagents and waste in accordance with country, federal, provincial, state and local regulations.
17. Do not open or break apart the PIE after use. The cap and the seals in the PIE prevent contamination with amplification products and/or infectious particles.
18. Do not use a PIE that has been dropped, shaken or inverted after the specimen has been loaded as this may cause invalid results.
19. Do not use a kit that has passed its stated expiration date.
20. Do not refrigerate the loaded PIE.
21. Each run must be performed with eight (8) PIEs in the Revogene carousel to maintain thermodynamic and mechanical balance within the run. Place MOCK PIEs or assay PIEs loaded with viral transport media or saline in empty positions if less than eight (8) specimens are tested.
22. Do not modify assay reagents, assay protocols, or instrumentation.

HAZARD AND PRECAUTIONARY STATEMENTS

There are no known hazards associated with this product.

STORAGE AND STABILITY

1. Swab specimens collected in VTM/UTM can be stored at 2-8 C for up to seven (7) days before being tested with the Revogene SARS-CoV-2 assay. Specimens collected in CDC media, MicroTest™ M4RT® (Remel) or saline may be stored up to 72 hours at 2-8 C before use.
2. Store the Revogene SARS-CoV-2 kit at 2-8 C. The expiration date is indicated on the kit box label.
3. Do not open a pouch until ready to perform testing. Use the PIE within one (1) hour after opening the pouch.

SPECIMEN COLLECTION

Nasopharyngeal, anterior nasal, mid-turbinate nasal, or oropharyngeal swab specimens should be collected according to standard technique and placed in 3 mL of viral transport media. Patient samples that have visible blood in the collection media after addition of the patient specimen should not be used, as whole blood may interfere with the detection of positive samples.

Refer to the Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19)[8] https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html.

INSTRUCTION FOR USE

PIE PREPARATION FOR PATIENT SPECIMEN TESTING

NOTE 1: One (1) pouch is required for each specimen to be tested.
NOTE 2: Process one (1) specimen at a time.
NOTE 3: The loaded PIE should be tested within one (1) hour of opening the protective pouch.

1. Mix the vial containing the specimen by inversion five (5) times before loading into the PIE.
2. Unseal the pouch containing the PIE, removing it from the pouch.
3. Place the PIE on a flat surface.
4. Using a DTT, aspirate the specimen by squeezing the entire bulb. The liquid level in the DTT must be anywhere between the two (2) marks (**Figure 2**). If the liquid level is not between the two (2) marks, discharge the specimen volume completely into the specimen tube by squeezing the entire bulb and repeat step 4.
5. Insert the tip of the DTT vertically into the sample loading chamber of the PIE and discharge completely the specimen (Figures 3A and 3B). Make sure not to touch the outer edges or the bottom of the sample loading chamber with the DTT. In case of contact with the outer edge or the bottom of the sample loading chamber, load a new PIE.
6. Close the cap of the PIE tightly. Do not refrigerate the loaded PIE. Make sure that only one (1) PIE is open at once. The PIE should not be inverted or shaken after the patient specimen has been added.
7. Prepare all additional specimens for testing by repeating steps 1 to 6 then proceed to step 1 of the **Revogene System Operation** section.

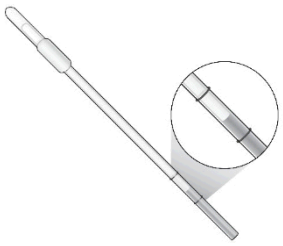

Figure 2.
Representation of an Appropriate Sample Level Using the Disposable Transfer Tool (DTT).

Figure 3A. Adequate Insertion Angle of the DTT into the Sample Loading Chamber of the PIE

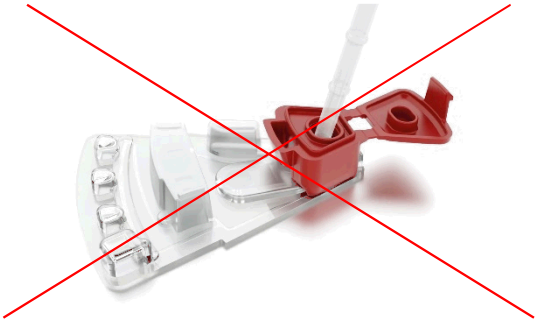

Figure 3B. Inadequate Insertion Angle of the DTT into the Sample Loading Chamber of the PIE

REVOGENE SYSTEM OPERATION

NOTE 1: A maximum of eight (8) specimens can be processed simultaneously in a single run using the Revogene.
NOTE 2: Each run must be performed with eight (8) PIEs in the Revogene. When less than eight (8) specimens are processed, the empty places must be filled with MOCK PIEs*.
NOTE 3: Refer to the Revogene Operator's Manual[9] for further information regarding Revogene set-up and operation.
NOTE 4: Refer to Section - Materials Required But Not Provided for software requirements.

1. Ensure the Revogene is powered on.
2. Log in by entering the **<Username>** and **<Password>** and tap **<Login>**. The main menu will appear automatically.
3. Tap **<Setup Run>**.
4. Enter the specimen identification using either the barcode scanner or manual entry. Manual entry can be done by tapping the **pencil** icon of the **<Scan or Enter Sample ID>** line.
5. Enter the PIE barcode using the Revogene barcode scanner. Gently position the PIE almost vertically in front of the scanner. Alternatively, PIE barcode may be entered manually (tap the **pencil** icon of their respective lines). Handle the PIE carefully without dropping, shaking or inverting it.
6. (Optional) Tap the **pencil** icon of the **<Add Comments>** line and type to add a comment.
7. Insert the PIE into the Revogene, at any position of the carousel. The software will automatically associate specimen to the correct PIE.
8. Confirm that the PIE is inserted into the instrument by tapping **<OK>** on the **<insert PIE into instrument>** line and repeat steps 4 to 8 for all specimens. If less than eight (8) PIEs are being tested, load MOCK PIEs* in the carousel remaining positions. No scan is required when inserting MOCK PIEs into the Revogene.
9. When all PIEs and MOCK PIEs (when necessary) are inserted into the instrument, tap **<Next>**.
10. If you have entered MOCK PIEs into the carousel, check the box to confirm that they were inserted.
11. Scan the retention ring and place it on the carousel.
12. Gently rotate carousel around to check all PIEs are loaded correctly with no friction or resistance to rotation. Close the instrument lid with both hands and hold on until you hear the lock activate. Then gently pull up on the lid to guarantee that the lock is engaged.
13. Initiate the test run by tapping **<Start>**.

*If MOCK PIEs are not available, use unused assay PIEs filled with un-inoculated viral transport media or saline (blank). An unused PIE, which was used as a balance within the instrument, can no longer be used for patient samples.

VIEWING AND EXPORTING RESULTS

NOTE 1: Refer to the Revogene Operator's Manual[9] for further information regarding the acquisition of test results.

1. **During the run**
   a. If the lights indicating run progression on the Revogene lid start to blink, this is a notification for an Early-Positive Result Outcome (E-PRO). An icon will also appear on the title bar of the screen. The **E-PRO** icon includes the "+" symbol and a number representing the number of positive results available at this time. The number will increase if additional positive results become available.
   b. Enter **<Username>** and **<Password>** and tap **<Login>** if the user's session has logged-out.
   c. Tap the **E-PRO** icon.
   d. Results from the current run are automatically listed on the screen. For each specimen line, there will be either a positive symbol or an in-progress symbol. The in-progress symbol, consisting of a rotating animation, is displayed until a positive result is obtained or the run is completed.
   e. Select the desired specimen by tapping on its position number, then tap either on the positive result symbol or on the Report button to open the interim report.
   f. (Optional) Tap **<Export>** and save the interim report where appropriate (e.g., USB flash drive). Once the results screen has been consulted, the E-PRO notifications will disappear. New E-PRO notifications will appear upon the detection of an additional early positive result. Any specimen result displayed in the interim report is final and will remain unchanged in the final assay result report.

2. At the End of the Run

a. Once the run is completed, the lid opens automatically.
b. Enter **<Username>** and **<Password>** and tap **<Login>** if the user's session has logged-out.
c. Tap **Results** icon.
d. Results from the last run are automatically listed on the screen.
e. Select specimens for which results report(s) has (have) to be exported:
   - All specimens can be selected simultaneously by checking the box on the upper left corner of the screen.
f. Tap **<Report>** to see the specific result for each specimen. Refer to the **Results Interpretation** section to confirm if any additional action needs to be taken according to the result obtained.
g. Tap **<Export>** and save where appropriate (e.g., USB flash drive or via connectivity option).
h. Alternatively:
   - Tap **<Search>** to find a specific specimen and its result.
   - Tap **< Run Report >** to group the results of multiple samples together in a single report if they were processed in the same run.
i. Remove the retention ring and retain for future use.
j. Remove all the assay PIEs and MOCK PIEs from the Revogene after each run:
   - Dispose of the assay PIEs according to your institution's standard practices.
   - Do not discard MOCK PIEs as they can be reused.

QUALITY CONTROL

Quality control procedures monitor the accuracy and precision of the analytical process. Each laboratory must establish the number, type and frequency of testing control materials per applicable regulations or accrediting agencies. The procedure described below may be employed, if appropriate, based on local policies and procedures.

INTERNAL CONTROL

The PIE contains an Internal Control (IC) that verifies RNA transcription/amplification/detection, nucleic acid amplification inhibition and assay reagents failure.

MICROFLUIDIC CONTROL

The PIE contains a Microfluidic Control (MFC) to verify the fluidic properties of the PIE.

EXTERNAL CONTROLS

Clinical laboratories are responsible for running quality control material when using the Revogene SARS-CoV-2 assay. External Controls should be run in accordance with laboratory protocols, local regulatory requirements, or accrediting organizations as applicable. For example, Positive and Negative External Controls should be tested with each new lot and/or shipment of reagents. Viral transport media or saline can be used as the Negative Control. The Positive External Control must be a a commercially available control. This control should be prepared at a concentration that is known to be detectable by this assay; for example, not more the 5X LoD (e.g., at approximately 22800 copies/mL). A positive external control that is not more that 5X LoD is recommended to ensure the instrument and assay are detecting the viral RNA at clinically relevant concentrations.

REPEAT TESTING PROCEDURE

UNEXPECTED POSITIVE OR UNEXPECTED NEGATIVE RESULT FOR AN EXTERNAL CONTROL

When an unexpected Negative result is obtained for the Positive External Control or when an unexpected Positive result is obtained for the Negative External Control, the run is invalid. A repeat test of the External Controls must be performed with new External Control materials as described in the **External Controls** section. Handling and preparation techniques should be reviewed.

In addition, a repeat test must be performed for all specimens included in the run.

Load new PIEs using the remaining specimens within the timeframe defined in the **Storage and Stability** section. Follow the **PIE Preparation** section from step 1, then follow the **Revogene System Operation** section.

INDETERMINATE OR UNRESOLVED RESULT FOR A SPECIMEN

When an Indeterminate (IND) or an Unresolved (UNR) result is obtained for a specimen, a repeat test from the corresponding specimen must be performed within the specified timeframe described in the **Storage and Stability** section.

If an IND/UNR result is obtained on the repeat test, it is recommended that a second specimen be requested and the test be repeated on the new specimen. If an IND/UNR result is obtained on the new specimen, please contact Technical Support.

INDETERMINATE OR UNRESOLVED RESULT FOR AN EXTERNAL CONTROL

When an Indeterminate (IND) or an Unresolved (UNR) result is obtained for an External Control, the run is invalid. A repeat test of the External Controls must be performed.

In addition, a repeat test must be performed for the specimens included in the same run within the specified timeframe defined in the **Storage and Stability** section.

Load new PIEs using the corresponding External Controls and specimen(s). Follow the **PIE Preparation** section from step 1, then follow the **Revogene System Operation** section.

RESULTS INTERPRETATION

The results are computed by the Revogene from measured fluorescent signals and embedded calculation algorithms and are available on the "Results" window. Possible reported results are listed below.

Sample	Symbol displayed on user screen	Reported Result	Interpretation
Positive External Control		Positive	Valid Positive External Control result.
		Negative	A Positive External Control that yields a negative result is indicative of a specimen handling/preparation problem. **The run is invalid**. Review the specimen handling/preparation technique. Repeat testing must be performed (refer to the **Repeat Testing Procedure** section).
		Unresolved	Incorrect Positive External Control result. **The run is invalid.** Repeat testing must be performed (refer to the **Repeat Testing Procedure** section).
		Indeterminate	Incorrect Positive External Control result. **The run is invalid.** Repeat testing must be performed (refer to the **Repeat Testing Procedure** section).
		In progress	Results not available yet.
Negative External Control		Positive	A Negative External Control that yields a positive result is indicative of a specimen handling/preparation problem or contamination event. **The run is invalid**. Repeat testing must be performed (refer to the **Repeat Testing Procedure** section).
		Negative	Valid Negative External Control result.
		Unresolved	Incorrect Negative External Control result. **The run is invalid.** Repeat testing must be performed (refer to the **Repeat Testing Procedure** section).
		Indeterminate	Incorrect Negative External Control result. **The run is invalid.** Repeat testing must be performed (refer to the **Repeat Testing Procedure** section).
		In progress	Results not available yet.

Sample	Symbol displayed on user screen	Reported Result	Interpretation
Patient Specimen		Positive	SARS-CoV-2 target RNA detected.
		Negative	SARS-CoV-2 target RNA not detected.
		Unresolved	Amplification/detection failure of the Internal Control as well as for the SARS-CoV-2 target RNA. Could be caused by inhibitory specimens or reagent failure. Repeat testing must be performed (refer to the **Repeat Testing Procedure** section).
		Indeterminate	No reportable result due to possible Revogene detection error during the assay processing, the data analysis, or due to an error code, a microfluidic failure or if the run is interrupted by the user. Repeat testing must be performed (refer to the **Repeat Testing Procedure** section).
		In progress	Results not available yet.

LIMITATIONS OF THE PROCEDURE

1. Performance of the Revogene SARS-CoV-2 assay has been established with nasopharyngeal swab specimens collected in 3 mL of viral transport media, only. Testing with oropharyngeal, anterior nasal, and mid-turbinate nasal swabs is also acceptable, although performance with these specimen types has not been established with the Revogene SARS-CoV-2 assay.
2. The Revogene SARS-CoV-2 assay should not be run together with any other Revogene assay.
3. Positive results are indicative of the presence of SARS CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.
4. 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.
5. Erroneous test results may occur from improper specimen collection, handling or storage, technical error, specimen mix-up or because the specimen does not contain a sufficient viral load for detection by the assay. Careful compliance with the instructions of these Instructions for Use, the Revogene Operator's Manual9 and established guidelines is necessary to avoid erroneous results.
6. Contamination or unexpected positive results may occur if a PIE cap is incorrectly closed or if a droplet has been dropped on the edges of the sample loading chamber.
7. Whole blood or Tobramycin may interfere with the Revogene SARS-CoV-2 assay when either of these substances is present in a nasopharyngeal swab specimen at a concentration of > 0.22% (v/v) or > 2.16 mg/mL, respectively.
8. Salinex® nasal spray or Oseltamivir may interfere with the Revogene SARS-CoV-2 assay when either of these substances is present in a nasopharyngeal swab specimen at a concentration of > 0.22% (v/v) or > 0.013 mg/mL, respectively.
9. Based on *in silico* analysis, there is a risk for the Revogene SARS-CoV-2 assay to detect a bat coronavirus sequence. The risk is considered low as this organism should normally not be found in a human sample.
10. The performance of this test was established based on the evaluation of a limited number of clinical specimens. The clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

11. As with any molecular test, mutations within the target regions of SARS-CoV-2 could affect primer and/or probe binding resulting in failure to detect the presence of virus.
12. The performance of this device has not been assessed in a population vaccinated against COVID-19.
13. Revogene SARS-CoV-2 assay has not been evaluated for patients receiving intranasally administered influenza vaccine.

Conditions of Authorization for Labs

The Revogene SARS-CoV-2 assay Letter of Authorization, along with the authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas. However, to assist clinical laboratories using the Revogene SARS-CoV-2 assay, the relevant Conditions of Authorization are listed below.

A. Authorized laboratories[1] using the Revogene SARS-CoV-2 assay must include with result reports of the Revogene SARS-CoV-2 assay, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

B. Authorized laboratories using the Revogene SARS-CoV-2 assay must use Revogene SARS-CoV-2 assay as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to perform the Revogene SARS-CoV-2 assay are not permitted.

C. Authorized laboratories that receive the Revogene SARS-CoV-2 assay must notify the relevant public health authorities of their intent to run the test prior to initiating testing.

D. Authorized laboratories using the Revogene SARS-CoV-2 assay must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories must collect information on the performance of the Revogene SARS-CoV-2 assay and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Meridian (via email: MBI-TechService@meridianbioscience.com) 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.

F. All laboratory personnel using the Revogene SARS-CoV-2 assay 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.

G. Meridian Biosciences, its authorized distributor(s) and authorized laboratories using the Revogene SARS-CoV- 2 assay must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

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

PERFORMANCE CHARACTERISTICS

CLINICAL EVALUATION

The performance of the Revogene SARS-CoV-2 assay was evaluated against an EUA authorized highly sensitive SARS-CoV-2 RT-PCR assay (Comparator) in a head-to-head comparison using frozen samples from retrospectively collected nasopharyngeal swab (NPS) specimens in 3 mL of viral transport media.

A total of 43 SARS-CoV-2 positive and 43 SARS-CoV-2 negative de-identified NPS samples, collected from patients suspected of COVID-19 by their healthcare provider, were included in the study and tested with Revogene SARS-CoV-2 assay and Comparator Method in a randomized and blinded fashion. The sample population included in the study represents a broad clinical range of SARS-CoV-2 viral loads including low positives based on Ct values of the Comparator.

RESULTS

Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were calculated from the results of these 86 retrospective NPS samples as shown in **Table 1**. The positive samples showed a 97.7% (95% CI: 87.9% - 99.6%) agreement with the Comparator results, and the negative samples showed 97.7% (95% CI: 87.9% - 99.6%) agreement. The clinical performance of the Revogene SARS-CoV-2 assay against the Comparator Method is presented in **Table 1**.

Table 1. Revogene SARS-CoV-2 Clinical Comparison with an EUA authorized SARS-CoV-2 rRT-PCR assay

		Comparator Result		
		Positive	**Negative**	**Total**
Revogene SARS-CoV-2 assay	**Positive**	42	1	43
	Negative	1	42	43
	Total	43	43	86
Positive Percent Agreement		97.7% [95% CI: 87.9% - 99.6%]		
Negative Percent Agreement		97.7% [95% CI: 87.9% - 99.6%]		

The overall initial unresolved rate was 0.0% (0/86). The overall initial indeterminate rate was 0.0% (0/86). No repeat testing was required.

OMICRON (BA.1) LINEAGE ASSESSMENT

Due to the emergence of the Omicron (BA.1) variant lineage and mutations associated with it which affected the original assay, a modification was made to the Revogene SARS-CoV-2 assay reagents. In 2022, performance of the modified assay was evaluated in comparison to a highly sensitive EUA authorized SARS-CoV-2 real-time RT-PCR assay (Comparator) to demonstrate performance against Omicron based variant lineages. The study consisted of a head-to-head comparison of the modified assay with the comparator using previously frozen leftover deidentified nasopharyngeal swab (NPS) specimens collected with Universal Transport Media (UTM) or Universal Viral Transport (UVT) system. The clinical specimens used were collected when the Omicron variant was the dominant lineage within the United States (dates: January 10, 2022 - May 9, 2022).

In addition to comparison with the RT-PCR comparator, all samples with sufficient viral titer were sequenced to confirm the presence of the mutation(s) that would have negatively impacted the performance of the previous assay formulation and to demonstrate the modifications to the assay resolves the prior limitation.

A total of 30 positive and 44 negative de-identified NPS samples, collected from patients suspected of COVID-19 by their healthcare provider, were included in the study and tested with the Revogene SARS-CoV-2 assay and the Comparator method. The sample population included in the comparison represents a broad clinical range of SARS-CoV-2 viral loads including low positives based on the Ct values of the comparator.

Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were calculated from the results of these 74 retrospective NPS samples as shown in **Table 2**. The positive samples showed a 96.7% (95% CI: 83.3% - 99.4%) agreement with the comparator results, and the negative samples showed 100% (95% CI: 92.0% - 100%) agreement.

Table 2. Revogene® SARS-CoV-2 Clinical Comparison with an EUA authorized SARS-CoV-2 rRT-PCR assay against Omicron Variant Lineage Confirmed Samples.

		Comparator Result		
		Positive	**Negative**	**Total**
Revogene SARS-CoV-2 assay	**Positive**	29	0	29
	Negative	1	44	45
	Total	30	44	74
Positive Percent Agreement		96.7% [95% CI: 83.3 - 99.4%]		
Negative Percent Agreement		100% [95% CI: 92.0 – 100%]		

ANALYTICAL PERFORMANCE CHARACTERISTICS

ANALYTICAL SENSITIVITY (LIMIT OF DETECTION)

The Limit of Detection (LoD) for the Revogene SARS-CoV-2 assay was determined as the lowest concentration of SARS-CoV-2 RNA where the detection rate is consistently ≥ 95%. It was determined using quantified SARS-CoV-2 heat-inactivated culture fluid (USA-WA1/2020 strain, ZeptoMetrix Corporation, 0810587CFHI) diluted into pooled negative nasopharyngeal (NP) matrix over six (6) dilutions ranging from 0.9 to 14.1 $TCID_{50}$/mL. The preliminary LoD was determined by testing each concentration with a total of 12 replicates across three (3) lots of Revogene SARS-CoV-2 assay reagents. The LoD was confirmed using two (2) preparations of SARS-CoV-2 diluted into pooled negative nasopharyngeal (NP) matrix over three (3) dilutions ranging from 3.52/14.1 $TCID_{50}$/mL. A total of 20 replicates for each concentration was tested across three (3) lots of Revogene SARS-CoV-2 reagents (**Table 3**). The LoD of the Revogene SARS-CoV-2 assay is 7 $TCID_{50}$/mL in VTM which is equivalent to 4,575 copies/mL in VTM.

Table 3. LoD Confirmation Results for the Revogene SARS-CoV-2 Assay

Concentration ($TCID_{50}$/mL of VTM)	**Positive/Total Results**			
	Pooled NP Matrix 1	**Pooled NP Matrix 2**	**Overall Positive/ Total Results (% positivity)**	**Mean Ct values**
14.1	10/10	10/10	20/20 (100%)	34.2
7.0	10/10	10/10	20/20 (100%)	35.3
3.5	9/10	9/10	18/20 (90.0%)	36.5
0	0/10	0/10	0/20 (0.0%)	N/A

After the Revogene SARS-CoV-2 assay was modified in 2022, the LoD was reassessed using samples prepared with the same viral USA-WA1/2020 strain material. A total of 20 replicates each were evaluated at the same concentrations tested in the previous study (**Table 3**). The LoD of the modified assay was confirmed to be the same as the original assay formulation (7.0 TCID50 mL or 4,575 copies/mL in VTM).

INCLUSIVITY

The inclusivity of Revogene SARS-CoV-2 was evaluated using *in silico* analysis of the assay primers and probes to confirm the detection of the N gene of SARS-CoV-2 strains. The *in silico* analysis was performed on all sequences available as of May 6, 2021 in the National Center for Biotechnology Information (NCBI) database and as of May 4, 2021 in the Global Initiative on Sharing All Influenza Data (GISAID) database. A total of 1 724 784 sequences were identified.

Of the 1 724 784 sequences, 50 908 sequences were excluded either because they presented ambiguous nucleotides in the regions targeted by the primers and probes of the Revogene SARS-CoV-2 assay, were incomplete or were not isolated from human specimen. Of the remaining 1 673 876 sequences, 98.22% (1 644 150 sequences) are 100% homologous to the Revogene SARS-CoV-2 primers and probe(s) and 3 835 (0.23%) sequences had one or more critical mismatches that could impact the revogene SARS-CoV-2 test performance. Overall, the *in silico* analysis revealed that 99.77% of the SARS-CoV-2 sequences assessed are deemed to have a high probability of being detected by the Revogene SARS-CoV-2 assay.

A subsequent inclusivity study was conducted to evaluate emerging Omicron lineages. The study used published sequence data from March 22, 2022 to April 21, 2022, including 117 772 sequences from the NCBI and 162 455 sequences from GISAID. The Omicron (BA.1) variant was the dominant variant lineage at this time, occurring at a frequency of greater than 95% in the circulating population of the US. Of these sequences, 13,786 were excluded from the analysis either because they harbored ambiguous nucleotides in the region targeted by the primers and probes of the Revogene SARS-CoV-2 assay or were incomplete. Of the 266 441 remaining sequences, 98.74% (263 078 sequences) are 100% homologous to the forward primer, at least one of the reverse primers and at least one of the probes. A risk analysis of the mutations affecting the remaining 3363 sequences indicated that 985 were highly improbable to show successful detection and 2378 had no significant predicted effect on performance. Overall, the *in silico* analysis revealed that 99.63% of the SARS-CoV-2 sequences assessed are deemed to have a high probability of being detected by the Revogene SARS-CoV-2 assay.

CROSS-REACTIVITY

The cross-reactivity of the Revogene SARS-CoV-2 assay was assessed through bench testing using a diverse set of commensal and pathogenic microorganisms, which are listed in **Table 4**. The study included nine (9) bacteria, two (2) yeasts, 13 infectious or inactivated viruses (**Table 4**). Two (2) bacteria, three (3) viruses, and human genomic DNA were tested as extracted or synthetic DNA or RNA. Each analyte was tested in triplicate (3) across three (3) Revogene SARS-CoV-2 reagent lots. Under the conditions of the study, none of the 32 non-targeted analytes tested were reactive with the Revogene SARS-CoV-2 assay.

The cross-reactivity with primers and probes of the Revogene SARS-CoV-2 assay was evaluated by an *in silico* analysis performed on sequences contained in the NCBI database as of May 5, 2021 for the analytes listed in **Table 4** as well as for coronavirus target found in bats and civets. The sequence #MN996532 (Bat coronavirus RATG13) presents ≥ 90% homology with primers and probes of the Revogene SARS-CoV-2 assay. No other sequences were found to have significant level of homology with the Revogene SARS-CoV-2 primers and probes.

Table 4. Cross-Reactivity Results with the Revogene SARS-CoV-2 Assay

Analyte	Identification	Concentration	Positive Results/Total
Bacteria and Yeasts			
Bordetella pertussis	ATCC® 9797™	1.20 x 10^8 CFU/mL	0/3
Candidas albicans	ATCC® 10231™	5.98 x 10^6 CFU/mL	0/3
Chlamydophila pneumoniae	ATCC® VR-1435™	8.79 x 10^6 IFU/mL	0/3
Haemophilus influenzae	ATCC® 33533™	1.53 x 10^8 CFU/mL	0/3
Legionella pneumophila	ATCC® 33152™	1.36 x 10^7 CFU/mL	0/3
Mycobacterium tuberculosis (genomic DNA)	ATCC® 25618DQ™	2.08 x10^5 cp/mL	0/3
Mycoplasma pneumoniae (genomic DNA)	ATCC® 15531D™	2.11 x 10^5 cp/mL	0/3
Pseudomonas aeruginosa	ATCC® 35554™	7.45 x 10^7 CFU/mL	0/3
Staphylococcus epidermidis	ATCC® 14990™	3.78 x 10^7 CFU/mL	0/3
Streptococcus pneumoniae	ATCC® 49619™	4.23 x 10^7 CFU/mL	0/3
Streptococcus pyogenes	ATCC® 12344™	8.50 x 10^7 CFU/mL	0/3
Streptococcus salivarius	ATCC® 13419™	6.88 x 10^7 CFU/mL	0/3
Pneumocystis jirovecii (PJP) (formerly known as Pneumocystis carinii)	ATCC® PRA-159™	6.10 x 10^6 nuclei/mL	0/3
Viruses, Human DNA, and Negative NP matrix pool			
Human Coronavirus 229E	0810229CFHI	1.00 x 10^5 $TCID_{50}$/mL	0/3
Human Coronavirus OC43	0810024CFHI	1.00 x 10^5 $TCID_{50}$/mL	0/3
Human Coronavirus NL63	0810228CFHI	1.00 x 10^5 $TCID_{50}$/mL	0/3
Human Coronavirus HKU1(synthetic RNA)	ATCC® VR-3262SD™	2.26 x 10^5 cp/mL	0/3
NATtrol™ Coronavirus-SARS Strain 2003-00592	NATSARS-ST	Undiluted*	0/3
MERS-Coronavirus (genomic RNA)	NR-45843	4.31 x 10^5 cp/mL	0/3
Enterovirus 68 (genomic RNA)	ATCC® VR-1826DQ™	4.64 x 10^5 cp/mL	0/3
Rhinovirus 1A	0810012CFN	1.00 x 10^5 $TCID_{50}$/mL	0/3
Adenovirus C1 Ad. 71	ATCC® VR-1™	1.34 x 10^6 $TCID_{50}$/mL	0/3
Human Metapneumovirus (hMPV-16 Type A1)	0810161CF	1.93 x 10^5 $TCID_{50}$/mL	0/3
Parainfluenza virus type 1	ATCC® VR-94™	9.77 x 10^5 $TCID_{50}$/mL	0/3
Parainfluenza virus type 2	0810504CF	1.34 x 10^5 $TCID_{50}$/mL	0/3
Parainfluenza virus type 3	0810016CF	2.07 x 10^6 $TCID_{50}$/mL	0/3
Parainfluenza virus type 4A	0810060CF	2.07 x 10^6 $TCID_{50}$/mL	0/3
Influenza A H1N1 (Swine/Iowa/15/30)	ATCC® VR-333™	5.43 x 10^7 $CEID_{50}$/mL	0/3
Influenza B Nevada/03/2011 (Victoria Lineage)	NR-44023	1.71 x 10^7 $CEID_{50}$/mL	0/3
Respiratory Syncytial Virus (RSV) A2/Australia/1961	ATCC® VR-1540™	3.36 x 10^6 PFU/mL	0/3
Human genomic DNA	N/A	2.54 x 10^5 cp/mL	0/3
Pooled nasopharyngeal negative matrix	N/A	N/A	0/3

* Highest concentration available. Concentration is unknown.

MICROBIAL INTERFERENCE

NATtrol™ Coronavirus-SARS strain 2003-00592, a non-targeted analyte for which the *in silico* analysis has revealed a sequence homology ≥ 80% with the Revogene SARS-CoV-2 probes, was tested for microbial interference. In addition, the potential microbial interference of pooled nasopharyngeal matrix collected from patients symptomatic for respiratory infection but negative for the SARS-CoV-2 target was also evaluated. One (1) heat-inactivated strain of SARS-CoV-2 (USA-WA1/2020, ZeptoMetrix Corporation, 0810587CFHI) was tested at a load of 3xLoD in VTM (21 $TCID_{50}$/mL or 13 725 cp/mL of VTM) in the absence of non-targeted analyte (control without interference), as well as in combination with each individual non-targeted analyte. Each sample type (SARS-CoV-2 strain with/without potential interferents) was tested in triplicate with three (3) Revogene SARS-CoV-2 reagent lots. Neither of the analytes tested interfered with detection of SARS-CoV-2.

Table 5: Results for the Control and Analytes tested with the Revogene SARS-CoV-2 Assay

Analyte	Concentration	Positive Results/Total
NATtrol™ Coronavirus-SARS Strain 2003-00592	Undiluted*	3/3
Pooled nasopharyngeal negative matrix	N/A	3/3
Control without interference	3xLoD (21 $TCID_{50}$/mL or 13 725 cp/mL of VTM)	3/3

* Highest concentration available. Concentration is unknown.

INTERFERING SUBSTANCES

Thirteen (13) potentially interfering substances (endogenous and exogenous) that may be found in nasopharyngeal swab (NPS) specimens were evaluated. For each substance, three (3) replicates were tested in negative NP matrix in the presence of the SARS-CoV-2 strain (USA-WA1/2020 strain, ZeptoMetrix Corporation, 0810587CFHI) (at three (3) times its LoD, i.e., 21.09 $TCID_{50}$/mL or 13 725 cp/mL of VTM) and three (3) SARS-CoV-2-negative replicates (negative NP matrix only) were also tested. Nine (9) substances did not show interference with the Revogene SARS-CoV-2 assay when tested at their potentially highest concentration that could be found in a NPS specimen. Four (4) substances, including whole blood, Salinex® nasal spray, Oseltamivir, and Tobramycin did interfere with detection of SARS-CoV-2, the internal control (IC) or the Microfluidic Control (MFC) when tested at their initial concentrations. These substances showed no reportable interference with the Revogene SARS-CoV-2 assay when tested at 0.22% (v/v) for whole blood and Salinex® nasal spray, or at 0.013 mg/mL and 2.16 mg/mL for Oseltamivir and Tobramycin respectively (**Table 6**), The highest concentration at which the potentially interfering substances do not show interference on the Revogene SARS-CoV-2 assay is shown in **Table 6**.

Table 6: Overview of the Endogenous and Exogenous Interfering Substances Concentrations without Interference on the Revogene SARS-CoV-2 Assay

Substance	Commercial Name	Composition / Active Ingredient(s)	Concentration in VTM without Interference observed[1]	Concentration in VTM with Interference observed
Blood	Whole blood	Glucose, hormones, enzymes, ions, iron, etc.	0.22 % (v/v)	2.2 % (v/v)[2]
Mucin	Mucin from bovine submaxillary glands	Purified mucin protein	2.2 % (w/v)	n/a
Nasal sprays	Dristan® nasal spray	Oxymetazoline	2.2 % (v/v)	n/a
	Salinex® nasal spray	Sodium chloride with preservatives	0.22 % (v/v)	2.2 % (v/v)[3]
	Neo-Synephrine®	Phenylephrine hydrochloride	2.2 % (v/v)	n/a
Nasal corticosteroids	Triamcinolone	Triamcinolone	2.2 % (v/v)	n/a
	Flonase® spray	Fluticasone	2.2 % (v/v)	n/a
	Budesonide	Budesonide (glucocorticoid)	133.3 µg/mL	n/a
Nasal gel / Homeopathic allergy and analgesic	Zicam® allergy relief nasal gel	Luffa opperculata, sulfur, Galphimia glauca, Histaminum hydrochloricum	2.2 % (w/v)	n/a
Throat lozenges	Cepacol® Extra Strength	Benzocaine, Menthol	2.2 % (w/v)	n/a
Anti-viral drugs	Zanamivir	Zanamivir	3.3 mg/mL	n/a
	Oseltamivir	Oseltamivir phosphate	0.013 mg/mL	0.13 mg/mL[3]
Antibacterial, systemic	Tobramycin	Tobramycin sulfate	2.16 mg/mL	21.6 mg/mL[4]

v/v: Volume/Volume; w/v: Weight/Volume; n/a: not applicable

[1] 3/3 positive replicates yielded expected results. 3/3 negative replicates yielded expected results.

[2] One false negative obtained out of 3 positive replicates. 3/3 negative replicates yielded expected results.

[3] One false positive obtained out of 3 negative replicates. 3/3 positive replicates yielded expected results.

[4] One indeterminate due to an error code and two false negatives obtained out of 3 positive replicates. 3/3 negative replicates yielded expected results.

E-LABELING

Documentation related to this product can be accessed online at www.meridianbioscience.com/pi. Additionally, paper copies are available free of charge upon request by contacting your local distributor or via the phone number listed on the kit box.

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	Use-by date		Humidity Limitation
LOT	Batch Code	Rx Only	Prescription Use Only
IVD	In vitro diagnostic medical device		Do not use if package is damaged
CE	CE Mark		Keep away from sunlight
REF	Catalog number	PIE	Revogene Test Device
	Consult Instructions for use	SBT	Sample Buffer Tube
	Manufacturer	LOOP	Disposable Transfer Loop
Σ	Contains sufficient for <n> tests	DTT	Disposable Transfer Tool
	Temperature limit	24x	Contains 24 Disposable Transfer Loops (DTL)
EC REP	Authorized representative in the European Community	EUA	For Emergency Use Authorization Only
	Do not reuse		Revogene Test Device
	Keep dry		
#x	Contains # pouches: 1 Disposable Transfer Tool (DTT), 1 Sample Buffer Tube (SBT), 1 PIE		

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