Rheonix COVID-19™ MDx Assay

℞ ONLY

For *in vitro* diagnostic use
Catalog Number KCCOV19-192

For use only with the Rheonix Encompass MDx® Workstation

For Use Under an Emergency Use Authorization (EUA) Only

Instructions for Use

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M26991 Rev L 06/30/2021

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Product Name

Rheonix COVID-19™ MDx Assay

Intended Use

The Rheonix COVID-19™ MDx Assay is an endpoint RT-PCR assay intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs, oropharyngeal (throat) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal washes, nasal aspirates and bronchoalveolar lavage (BAL) fluid from individuals who are suspected of COVID-19 by their healthcare provider.

The Rheonix COVID-19 MDx Assay is also for use with saliva specimens collected without preservatives in a sterile tube in a healthcare setting from individuals who are suspected of COVID-19 by their healthcare provider.

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

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

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information. Negative results for SARS-CoV-2 RNA from saliva should be confirmed by testing of an alternative specimen type, if clinically indicated.

The Rheonix COVID-19 MDx Assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of PCR and *in vitro* diagnostic procedures.

The Rheonix COVID-19 MDx Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

Summary and Explanation

Information below derived from the US Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) websites.

https://www.cdc.gov/coronavirus/2019-ncov/index.html

https://www.who.int/health-topics/coronavirus#tab=tab_1

Coronaviruses are a group of viruses found in humans and other mammals. They are enveloped, single-stranded, positive sense RNA viruses. The novel 2019 coronavirus, now referred to as SARS-CoV-2 is a beta coronavirus similar to MERS-CoV and SARS-CoV and causes respiratory illness referred to as COVID-19 disease. Symptoms include fever, cough and shortness of breath and may appear 2-14 days after exposure. The majority of people will exhibit mild or moderate respiratory symptoms and will recover without specific treatment. Some patients have presented with acute respiratory infection symptoms during the early stage of disease and it has been identified that individuals with underlying health conditions including diabetes, cardiovascular disease, chronic respiratory disease and cancer are more likely to progress to serious disease.

As cases began spreading around the world, on January 30, 2020, the World Health Organization (WHO) declared the outbreak a public health emergency of international concern. On January 31, 2020 the US Health and Human Services Department declared a public health emergency for the United States. Finally, on March 11, 2020, the WHO declared SARS-CoV-2 a global pandemic.

Principles of the Procedure

The Rheonix COVID-19 MDx Assay is an *in vitro* diagnostic test capable of detecting the presence of SARS-CoV-2 RNA in saliva specimens, nasopharyngeal swabs, oropharyngeal (throat) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal washes, nasal aspirates and bronchoalveolar lavage (BAL) fluid samples. The assay uses proprietary Rheonix CARD® cartridge technology that provides a microfluidic network complete with pumps, valves, and reaction chambers for automated assay performance. Each CARD cartridge provides assay chambers for four separate clinical specimens or control samples. The Rheonix Encompass MDx Workstation can simultaneously process 6 CARD cartridges, for a total of up to 24 samples (22 specimens and 2 external controls), per test run. All residual liquids are contained within

the device and discarded with the Rheonix COVID-19 MDx Assay consumables, thus optimizing work flow and minimizing cross contamination.

After clinical specimens are obtained using saliva, nasopharyngeal swabs, oropharyngeal (throat) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal washes and nasal aspirates samples, they are transferred into sample tubes containing Rheonix 4X Sample Buffer where cell lysis begins. Once loaded into the CARD cartridge, additional cell lysis and RNA purification steps automatically take place. To detect SARS-CoV-2 RNA PCR amplification of nucleic acid sequences corresponding to one target site called N1 of the nucleocapsid protein gene sequence is completed. In all cases, the target gene is amplified in the presence of biotin-tagged primers and the resulting amplicons denatured and flowed over the low-density array of capture probes contained within the CARD cartridge. Following incubation with streptavidin conjugated horseradish peroxidase and substrate, color precipitated spots are detected and analyzed via the onboard image capture system and results provided by the workstation's software for the target microorganism (Figure 1 and Table 1).

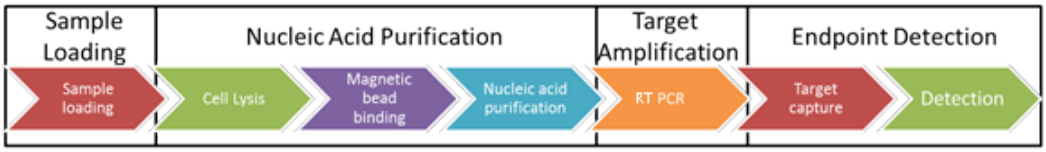

Figure 1. Flowchart for Rheonix COVID-19 MDx Assay. All steps, performed automatically on the Encompass MDx workstation are shown.

Table 1. Overview of results and the corresponding symbols generated by Workstation software

Symbol on run report	Symbol meaning
+	Positive (POS) result with the COVID-19 MDx assay
-	Negative (NEG) result with the COVID-19 MDx assay
!	Error (ERR)
?	Indeterminate (IND)

Materials Provided/Assay Kit Case Components

The Rheonix COVID-19 MDx case can be divided into 3 parts, the assay kit, the PCR mix (Pack B), and the accessories kit. Specific components provided are outlined below. The Assay Kit Case contains 8 assay kits and each assay kit has sufficient volume of reagents to analyze up to 24 samples (22 specimens and 2 external controls) in a single run. Each assay kit contains two packs of COVID-19 CARD Packs (with 3 cartridges in each pack) and one COVID-19 Reagent Pack

(Pack A). Each PCR Mix Pack B Case contains 8 PCR mix strips and each strip has sufficient volume of reagents to analyze up to 24 samples (22 specimens and 2 external controls) in a single run. The assay accessories kit contains bulk Sample tubes, Sample tube caps, barcoded labels and 4X Sample Buffer. Each case has the capacity, to run a total of 192 tests. Kit components are stored at room temperature (15 °C to 30 °C) and -15 °C to -20 °C as directed on the packaging.

- COVID-19 CARD Packs of 3 cartridges each (15 °C to 30 °C)
- COVID-19 Reagent Pack (Pack A) (15 °C to 30 °C)
- COVID-19 MDx PCR Mix, Pack B (-15 °C to -20 °C)
- 4X Sample Buffer
- Sample tubes
- Sample tube caps
- Barcode labels for Sample tubes

COVID-19 CARD Pack

Each COVID-19 CARD Pack consists of three CARD cartridges in a Tyvek®-sealed plastic tray (Figure 2). Each assay kit contains two CARD Packs.

Figure 2. The Rheonix COVID-19 MDx Assay cartridge Pack. Left: Pack of three CARDs with pack cover in place. Right: Pack of three CARDS with pack cover removed.

COVID-19 Reagent Pack (Pack A)

The COVID-19 Reagent Pack (Pack A) contains six sealed reagent tube strips (Figure 3). The COVID-19 Reagent Pack is sealed with a Tyvek cover. See Table 2 for a detailed description of the contents of the Reagent Pack.

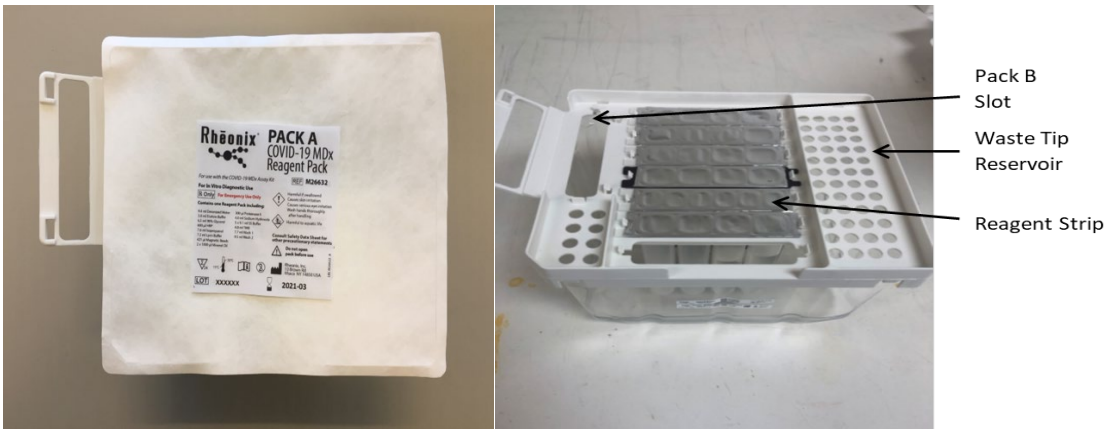

Figure 3. Rheonix COVID-19 MDx Assay Pack A. Left: Pack A after being removed from assay kit box and with its Tyvek lid in place. Right: Pack A with the lid removed and various aspects of the pack called out on the right.

Table 2. Contents of Rheonix COVID-19 MDx Reagent Pack A. The contents (number and volume, where appropriate) are shown.

Reagent	Amount	Unit of Measure	Quantity
Deionized Water	9.4	mL	1
Elution Buffer	3.0	mL	1
96% Glycerol	6.5	mL	1
HRP	700	µL	1
Isopropanol	7.6	mL	1
Lysis Buffer	7.2	mL	1
Magnetic Beads	430	µL	1
Mineral Oil	1000	µL	2
Proteinase K	350	µL	1
Sodium Hydroxide	4.0	mL	1
SS Buffer	9.1	mL	5
TMB	4.8	mL	1
Wash 1	7.7	mL	1
Wash 2	9.5	mL	1
MM2 Process Control*	350	µL	1

**This reagent is included in the reagent pack but not used in the Rheonix COVID-19 MDx Assay.*

The Reagent Pack also contains an empty strip slot running perpendicular to the other six tube strip slots for securing the COVID-19 PCR Mix strip (Pack B) into the Reagent Pack via a hinged latch. Each Reagent Pack can perform testing for up to 24 samples. Store the COVID-19 Reagent Pack B in an upright position.

COVID-19 PCR Mix (Pack B)

Each COVID-19 PCR Mix (Pack B) strip is supplied in a resealable bag. The PCR Mix tube strip contains the PCR Mix (used to amplify SARS-CoV-2 cDNA and controls) (Figure 4).

Figure 4. **Rheonix COVID-19 MDx Assay PCR Mix, Pack B.** Pack B ready for insertion into Pack A (Figure 3), at its designated location.

The plastic tabs at either end of the COVID-19 PCR Mix strip are keyed to ensure correct insertion and orientation into COVID-19 Reagent Pack A.

The PCR Mix strip is sufficient to analyze up to 24 samples.

Materials Required/Recommended, but not Included

The following items are not provided, but required/recommended:

a) Rheonix Encompass MDx® Workstation (Rheonix Catalog Number RNXMDX)

i. The software version used in the validation study consisted of:

User Interface: 1.0.0.76

EncompassCore: 1.6.0.1

SGB FW: 1.2.5

MCB FW: 1.0.4

HB FW: 1.2.3

RTSB FW: 2.0.1

Camera Job: Cov1.1.2

COVID-19 MDx Assay: X3

b) External controls:

i. Positive Controls that can be used with the Rheonix COVID-19 MDx Assay:

- Commercially available, inactivated SARS-CoV-2 (Catalog Number 0810587CFHI-0.5 mL) at 5X LoD (i.e., 3125 genomic equivalents/mL) from ZeptoMetrix, Buffalo, NY (www.zeptometrix.com, Phone number 1-800-274-5487).
- Other commercially available positive controls can be used provided they are spiked at 3125 genomic equivalents/mL and validated by the laboratory.
- For the purposes of diluting controls, use the Certificate of Analysis provided by the vendor to determine appropriate dilution.

ii. Negative Controls that can be used with the Rheonix COVID-19 MDx Assay:

- Commercially available control from IDT (www.idt.com, phone number 1-800-328-2661): Hs_RPP30 Positive Control (IDT Catalog Number 10006626).

c) Disposable gloves
d) Lab coat
e) Safety glasses
f) Sodium hypochlorite
g) Lint Free wipes (for cleaning of instrument)
h) Spill kit to safely clean up spills of potentially hazardous materials
i) Axygen 1000 µL tips (Axygen/TTF-1000-C-HTR-S; VWR/89040-092)
j) 15 ml conical tube for collection of saliva sample (Laboratory Product Sales, Rochester, NY, Catalog L232161), or equivalent

Warnings and Precautions

- The Rheonix COVID-19 MDx Assay is intended for Emergency Use Only.
- Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner
- The Rheonix COVID-19 MDx Assay is for *in vitro* Diagnostic Use and must be performed using the Rheonix Encompass MDx workstation within the appropriate operating conditions (Table 3).

Table 3. Rheonix Encompass MDx Workstation Operating Conditions

ENVIRONMENTAL CONDITIONS – OPERATION	
Temperature	18 °C-30 °C / 64 °F-86 °F
Humidity	20 to 85% non-condensing
Altitude	1600 m maximum

- The Rheonix COVID-19 MDx Assay has not been FDA cleared or approved, but has been authorized by FDA under an Emergency Use Authorization (EUA) for use in laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
- The Rheonix COVID-19 MDx Assay has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens
- The Rheonix COVID-19 MDx Assay 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 authorization is terminated or revoked sooner.
- The Rheonix COVID-19 MDx Reagent Pack contains guanidine hydrochloride, a chaotropic agent that is widely used for purification of nucleic acids. In case of skin contact with these reagents, remove contaminated clothing and wash the affected area with soap and water. In case of eye contact, flush eyes thoroughly with water for at least 15 minutes. Consult a physician.
- Do not use expired kits.
- Do not use the kit if the seal to the outer box is broken upon arrival.
- Do not use any kit components that display damage or broken seals.
- Do not mix reagents from one kit with reagents from another kit.
- The laboratory should perform routine environmental monitoring to minimize the risk of cross contamination.
- All specimens should be handled by operators as if they are infectious and in accordance with safe laboratory procedures.
- Operators should wear protective clothing and disposable gloves. The Rheonix Encompass MDx workstation's User Interface (UI) provides instructions on when to change disposable gloves during the testing procedure. Strict adherence to the instructions is required to protect the operator and reduce possible erroneous results.
- Thoroughly wash hands after performing the tests.
- There is no need to pipette any reagents.
- Do not eat, drink, smoke, chew in areas where specimens or kits are being used.
- Dispose of all consumable test components and waste in accordance with local, state and/or federal regulations.

Sample Collection

The specimens tested with the Rheonix COVID-19 MDx Assay must be run using the Rheonix sample tubes, or equivalent and the Rheonix 4X Sample Buffer (Rheonix Catalog Number M26365) provided with the kit as follows:

- Collect respiratory specimens according to standard collection technique.
    - Prior to transferring the sample to the Rheonix sample tube, add 330 µL of Rheonix 4X Sample Buffer for samples containing 1 mL (in this case the final volume of the combined sample and Rheonix 4X Sample Buffer must be at least 1.3 mL) or add 1 mL of Rheonix 4X Sample Buffer for samples containing 3 mL.
    - The final concentration of Rheonix Sample Buffer must be 1X for each sample.
    - Note: If sample is collected in a buffer already containing guanidine, there is no need to add the Rheonix 4X Sample Buffer.
    - Assure that the final volume is a minimum of 1.3 mL to allow for repeat analysis if required.
    - Cap each tube tightly prior to placing into the Specimen Rack which is subsequently placed into its location in the Rheonix Encompass MDx Workstation.
- Saliva Sample Collection:
    - Saliva specimens must be self-collected in a healthcare setting under the supervision of a healthcare provider.
    - Avoid coughing prior to harvesting of the sample.
    - No eating, drinking, smoking, chewing of gum or brushing teeth for at least 30 minutes prior to sampling.
    - No dental work or examination performed 24 hours prior to saliva collection.
    - Remove the screw cap from the 15 mL conical tube and collect 3 mL of saliva (approximately 2-3 spitting events) in the conical tube.
    - Ensure liquid (not bubbles) reaches the 3 mL line on the sterile 15 mL conical tube.
    - Replace the screw cap.
    - The conical tube should be given to the healthcare worker:
        - 1 mL of Rheonix 4X Sample Buffer should be added immediately, or
        - The conical tube should be transported to a laboratory where 1 mL of Rheonix 4X Sample Buffer is subsequently added within 4-6 hours
    - A minimum of 1.3 mL volume should be transferred to a Rheonix Sample tube.
    - Cap each tube tightly prior to placing into the Specimen Rack which is subsequently placed into its location in the Rheonix Encompass MDx Workstation.

Sample Transport and Storage

- Transportation of collected specimens must comply with all applicable regulations for the transport of etiological agents.
- Respiratory specimens:

    - Following collection and transfer of the respiratory specimen into the sample tube, testing should be completed as soon as possible for best results.
    - If immediate testing is not possible samples can be stored at 2 °C – 8 °C for up to 14 days.
    - Ensure Rheonix 4X Sample buffer is added to samples prior to storage.
    - If testing is not performed as outlined above, a new patient sample should be obtained.
- Saliva samples:
    - Following collection and transfer of the saliva sample into the sample tube, testing should be completed as soon as possible for best results.
    - If immediate testing is not possible saliva samples can be stored at room temperature for up to 14 days.
    - Ensure Rheonix 4X Sample buffer is added to samples prior to storage.
    - If testing is not performed as outlined above, a new patient sample should be obtained.
- Sample Reruns
    - In the event a respiratory specimen needs to be reanalyzed, the test should be repeated as soon as possible. Respiratory specimens should be stored at 2 °C – 8 °C for up to 14 days.
    - In the event a saliva sample needs to be reanalyzed, the test should be repeated as soon as possible. Saliva samples should be stored at room temperature for up to 14 days.
    - **Ensure Rheonix 4X buffer is added to samples prior to storage**.

Instructions for Use

- **Caution:** In order to reduce the chance for contamination, do not open assay consumables (CARD Pack, Reagent Pack A and Reagent Pack B) until prompted by the UI.
- The assay must be run on the Rheonix Encompass MDx workstation.
- Assay run time is less than 5 hours.
- Gloves and other appropriate personal protective equipment should be worn at all times during preparation and running of the assay. The touch screen can be manipulated while wearing gloves.
- **Test results must be reported in accordance with local, state, and federal regulations.**

The following instructions provide the primary steps for conducting the COVID-19 Assay on the Encompass MDx workstation. For detailed information on Encompass MDx workstation operation, refer to *Encompass MDx Workstation Operator Manual.*

Assay Steps

1. Ensure the Encompass MDx workstation has been cleaned according to the instructions provided in the *Encompass MDx Workstation Operator Manual*.
2. Start the UI Software by powering on the Encompass MDx workstation. The power on switch is located in the rear, lower left corner of the workstation.
3. Log in when prompted by the workstation.
4. To begin a new run, select "New Run" from the home screen.
5. Load 22 samples plus 1 positive and 1 negative external control into a cleaned Encompass MDx Sample Rack. Consult the Operator Manual for rack cleaning instructions.
   a) Control samples can be loaded in any position in the sample rack.
6. Ensure that the sample tubes are inserted into the rack such that the barcodes are centered in the open side of the rack and visible to the operator.
7. Place the sample rack containing the test samples into the workstation deck as instructed by the UI. Push down gently.
   a) **Do not force the rack into the workstation deck.** If met with resistance upon loading, the sample rack may be in the incorrect orientation.
8. Select "Confirm".
9. After pressing "Confirm," follow the UI prompts and load CARD cartridges.
   Caution: Before proceeding to load remaining consumables, change your gloves.
10. Remove CARD Packs from the kit using the touch points as shown in Figure 5.
    Caution: Do not touch the array covers on the CARD cartridges.

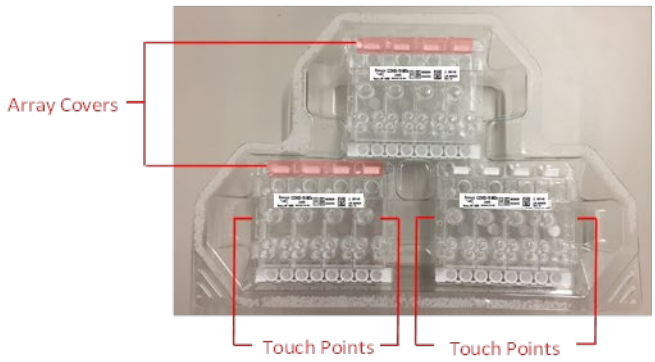

Figure 5. COVID-19 CARD Touch Points and Array Covers. Handle the CARD cartridges at the highlighted touch points and do not touch the Array Covers (highlighted in red).

11. Load CARD cartridges with the PCR tube strip facing operator and close clamps.

a) A test run requires 6 CARD cartridges to be loaded, for a total of up to 24 samples, per test run.

12. **Mix the reagent Pack.** Prior to removing the Tyvek® lid from Reagent Pack A, grasp the pack and use your gloved hands to invert the entire pack three times to mix.
13. Remove the lid from COVID-19 Reagent Pack (Pack A).

    Caution: Do not use if any foil sealant is compromised or the reagent strips are not secured in place upon opening.

    Caution: Do not remove reagent strips from the reagent pack or touch the foil sealant covering the reagent strips.

    Caution: Do not use Reagent Packs leftover from a previous run.
14. Remove COVID-19 PCR Mix (Pack B) from its resealable bag. Record the lot number in the log book (provided with the instrument).
15. Insert Reagent Pack B into Reagent Pack A by securing with plastic tab on Reagent Pack A.
16. Load the fully assembled Reagent Pack (now consisting of Reagent Pack A and Reagent Pack B) onto the workstation deck.
17. Place the two tip tubs onto the workstation deck as indicated by the UI.

    Caution: Ensure that at least one of the tip boxes contains a full rack of 96 Axygen tips before continuing.
18. When prompted, manually close the workstation door to initiate the Pre-Run Checks.
19. Touch "Next" on the "Pre-Run Checks Successful" screen and then touch "Start" on the "Run Monitor" screen to start the assay.
20. When the test is complete, the UI displays the "Test Complete" screen:
    a) To review the results in greater detail, touch the "Review" button.
    b) A color code indicates the individual results, with green indicating a negative result, red indicating a positive result, blue indicating an indeterminate result, and yellow indicating an error has occurred during the test procedure.
21. Test results can either be printed or sent to a USB.

    Note: All data are saved on the Encompass MDx Workstation unless removed by an administrator.
22. Once finished with the results, unload the deck by removing all samples and used consumables. Dispose of the consumables into properly-labeled biohazard trash receptacles; process trash in accordance with all institutional practices and local, state, and federal regulations.

Quality Controls

The Rheonix COVID-19 Assay includes one internal control. In addition, a laboratory must also run external controls. External controls are not provided by Rheonix.

- Reference Spots (RS) – Three reference spots are included on each microarray. The locations of these spots permit the camera to properly align itself during image analysis. In addition, the Rheonix Encompass MDx Workstation uses information from the image analysis to confirm that all necessary detection reagents performed properly during the performance of the assay.

- Internal Control – Detection of human RNase P acts as an internal control for each sample. Each sample is tested in the presence of RNase P (RP) specific primers, which are part of the PCR master mix. The presence or absence of the control will be confirmed via detection by amplification and specific probe capture on the microarray. The internal control will control for three important aspects of the assay: (1) specimen integrity, (2) properly performed processing, including extraction, and (3) PCR. The Rheonix Encompass MDx workstation's software requires that the internal control yield a positive result in order for a test specimen to be scored as negative for the target being analyzed. Due to the potential for competition from the authentic target present in the specimen, the internal control result may be positive or negative, since high concentrations of target in the clinical specimens may compete with the control and yield a negative internal control result. The internal control passes if it meets the acceptance criteria in the software algorithm.

- External Control – External positive and negative controls must be included with every COVID-19 MDx Assay run.

  a) A positive template control is needed to assure that all assay steps perform properly. This must consist of 3125 genomic equivalents/mL. At least one Positive Control should be analyzed per run. The Positive Control should yield a positive result for the N1 target. Zeptometrix inactivated SARS-CoV-2 virus should be spiked at 3125 genomic equivalents/mL in negative NP swab matrix. Refer to the Certificate of Analysis provided with the inactivated virus to determine the concentration of the stock.
  b) A "no viral template" (negative) control is needed to assure that truly negative samples are correctly scored as "Negative" for the presence of SARS-CoV-2 RNA. At least one negative control should be included in each run of the workstation. Negative Control should yield a negative result for the N1 target and a positive result for RP.
     a. Since the Hs_RPP30 Positive Control (IDT) is supplied at 200,000 copies/µL, we recommend that 1 µL be diluted in 2.0 mL (i.e., 100,000 copies/mL). Collection media should be mixed 3:1 with the Rheonix 4X Sample Buffer as the matrix.

Meaning of Error or Indeterminate Codes

An ERROR result will be displayed by the Encompass MDx Workstation if any of the following occurred during the performance of the assay:

- The RS detection failed.
- The assay was aborted due to workstation or consumable failure.
- Insufficient data were collected.

An INDETERMINATE result will be displayed if any of the following occurred during the performance of the assay:

- Both N1 target and internal control (RNase P) are negative.
- An error in the intensity or quality of one or more spots on the integrated DNA array is determined by the algorithm.

Results/Test Interpretation

First, the validity of the assay should be established by the user, based on the performance of the external controls and the guidelines established in Table 4. Once the validity of the assay has been established, the valid (positive and/or negative) patient results may be reported, according to Table 5.

Positive test results must be reported in accordance with local, state, and federal regulations.

Rheonix COVID-19 MDx Assay Controls – External (Positive and Negative) and Internal and Reference Spot Controls.

Signal intensity values have been established that delineate the upper and lower thresholds used by the workstation's software to determine the presence or absence of SARS-CoV-2 viral sequences defined by the N1 targets. Using the positive and negative controls as described in the *Quality Controls* section valid results are noted below:

- Positive Control – the signal intensity of the positive control sample must be greater than 25.00 intensity units for N1.
- Negative Control – the signal intensity of the negative control sample must be less than 25.00 intensity units for N1 target and greater than 25.00 intensity units for RNase P.
- Array Control – the signal intensity of all three reference spots must be greater than the predetermined level to assure that the colorimetric portion of the assay performed as expected. In addition, the specific location of the hybridization spots resulting from the Reference Spot Controls also assures

that the DNA microarrays have been properly inserted during manufacturing.

Examination and Interpretation of Patient Specimen Results

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and sample validity established following the criteria in Table 4. The user does not interpret any numerical test values since the workstation's software automatically acquires the signal intensity of the various hybridization spots on the integrated DNA array and interprets the results based upon the software's predetermined algorithm.

The possible results obtained by the Rheonix COVID-19 MDx are as provided in Table 5. Valid Positive or Negative results should be acted upon according to current medical practice regarding suspected COVID-19 infections. Specimens with indeterminate (IND) results and system errors (ERR) should be retested with the Rheonix COVID-19 MDx Assay using the original specimen, if available. If not available, a new specimen sample should be obtained. Possible error codes that will result in invalid results are shown in Table 6.

Table 4. Validity of patient test results from the Rheonix COVID-19 MDx Assay based on interpretation of External Controls

Patient Test Result [1]	Control Result			
	Positive: Pass	**Positive: Fail**	**Positive: Pass**	**Positive: Fail**
	Negative: Pass	**Negative: Pass**	**Negative: Fail**	**Negative: Fail**
POS	Valid	Valid	Invalid	Invalid
NEG	Valid	Invalid	Valid	Invalid

[1]*Result displayed by the Encompass MDx Workstation*

Table 5. Interpretation of patient and External Control results from the Rheonix COVID-19 MDx Assay

Result Reported [1]	Interpretation [2]	External Control	
		Positive	Negative
POS	Positive for SARS-CoV-2 RNA; report result	Pass or Fail	Pass
NEG	Negative for SARS-CoV-2 RNA; report result	Pass	Pass or Fail
IND	Indeterminate: no detectable signal for the N1 or RNase P targets; retest required	Pass or Fail[3]	Pass or Fail[3]
ERR	Error: system error; retest required	Pass or Fail[3]	Pass or Fail[3]

[1]Result displayed by the Encompass MDx Workstation

*[2]The validity of patient test result must be determined manually after review of the results obtained with the External Controls according to the algorithm shown in **Table 4**.*

[3]Regardless of the results obtained for the External Controls, if the Workstation reports either an IND or ERR code, the specimens must be retested.

Table 6. Description of possible error codes

Code	Cause
E01	Reference spots on filter invalid
E02	Bubble check on filter failed
E03	Spacing check on filter failed
E04	Angle check on filter failed
E05	Quality checks on filter failed
N02	Indeterminate: standard deviation for target spots is above threshold
N03	Indeterminate: No target spots and no internal control spots
N04	Indeterminate: The intensity of one or more target spots is indeterminate

Limitations

- The Rheonix COVID-19 MDx Assay may only be performed using the Rheonix Encompass MDx workstation using clinical specimens that have been collected as per testing lab procedures or following vendor instructions.
- The performance of the Rheonix COVID-19 MDx Assay was established using contrived nasopharyngeal swab specimens. Anterior nasal swabs, mid-turbinate nasal swabs, nasal washes, nasal aspirates, bronchoalveolar lavage (BAL) fluid and saliva are also considered acceptable specimen types for use with the Rheonix COVID-19 MDx Assay.
- Validation studies for non-saliva respiratory specimens were performed using BD Universal Viral Transport media (www.bd.com, phone number 201-847-6800) and UTM (Copan Universal Transport Medium, www.copanusa.com, phone number: (800)-216-4016). Compatibility with other specimen collection media and/or transport media has not been evaluated. Please contact Rheonix technical support with questions. Use of

this assay is limited to personnel who have been trained in the procedure. Failure to follow the instructions provided in this package insert may cause erroneous results.

- Reliable results are dependent on adequate specimen collection. Because the collection and transport system does not allow for microscopic assessment of specimen adequacy, training of clinicians in proper specimen collection techniques is necessary. Please refer to the *Sample Collection* guidelines for more information.
- Careful compliance with the instructions in this package insert is necessary to avoid erroneous results.

Conditions of Authorization for the Laboratory

The Rheonix COVID-19 MDx Assay Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients and authorized labeling are available on the FDA website:

https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas

However, to assist clinical laboratories running the Rheonix COVID-19 MDx Assay, the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[1] using the Rheonix COVID-19 MDx Assay will include with result reports of the Rheonix COVID-19 MDx 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 Rheonix COVID-19 MDx Assay will perform the Rheonix COVID-19 MDx Assay as outlined in the Rheonix COVID-19 MDx Assay 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 Rheonix COVID-19 MDx Assay are not permitted.

C. Authorized laboratories that receive the Rheonix COVID-19 MDx Assay must notify the relevant public health authorities of their intent to run the test prior to initiating testing.

D. Authorized laboratories using the Rheonix COVID-19 MDx Assay will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories will collect information on the performance of the test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Rheonix Inc.

Customer Technical Support 1-844-RHEONIX (1-844-743-6649) 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 test must be appropriately trained in 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. Rheonix Inc., its authorized distributor(s), and authorized laboratories using the Rheonix COVID-19 MDx Assay 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, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests" as "authorized laboratories."

Non-Clinical Performance Evaluation

Limit of Detection (LoD) (Analytical Sensitivity)

Nasopharyngeal swab

The Limit of Detection (LoD) was established by evaluating a dilution series of inactivated SARS-CoV-2 virus in pre-screened negative nasopharyngeal swabs matrix using the protocol described below.

The preliminary LoD was determined by evaluating a total of 5 different concentrations of inactivated SARS-CoV-2 virus, diluted in pooled NP swab matrix around the presumed LoD. Each replicate was independently processed through the entire automated process and the preliminary LoD was determined to be in the range of 312 to 625 genomic equivalents/mL or less. In order to be conservative, however, we established the preliminary LoD at 625 genomic equivalents/mL and confirmed this LoD by analyzing a total of 22 replicates of pooled NP swabs spiked with inactivated SARS-CoV-2 spiked at 625 genomic equivalents/mL (Table 7 below). Based on the manner in which the Rheonix EncompassMDx Workstation processes the NP swabs (i.e., 200 µL of sample loaded into the assay cartridge and then 6 µl of the final 30 µl of purified RNA transferred into the RT-PCR reaction), 625 genomic equivalents/mL is

equivalent to 25 genomic equivalents/reaction. Based on these studies, the LoD of the Rheonix COVID-19 MDx Assay was estimated to be 625 genomic equivalents/mL (Table 8).

Table 7. Preliminary Limit of Detection of the Rheonix COVID-19 MDx Assay

Concentration of SARS-CoV-2		Number Tested	Number Positive	Percent Positive
$TCID_{50}$/mL	Genomic equivalents/mL*			
3.14×10^{-3}	**78**	**4**	1	25%
6.28×10^{-3}	**156**	**5**	3	60%
1.26×10^{-2}	**312**	**5**	5	100%
2.52×10^{-2}	**625**	**5**	5	100%
5.04×10^{-2}	**1250**	**2**	2	100%

**Dilutions were based on $TCID_{50}$ values. Based on qPCR analysis, the genomic equivalents were shown to be 3.5 x 10^9 genome equivalents/mL (performed by ZeptoMetrix, Buffalo, NY).*

Table 8. Confirmation of LoD for Rheonix COVID-19 MDx Assay

Concentration of SARS-CoV-2		Number Tested	Number Positive	Percent Positive
$TCID_{50}$/mL	Genomic equivalents/mL			
2.52×10^{-2}	**625**	**22**	**22**	**100%**

Saliva Specimen

The Limit of Detection (LoD) was established by evaluating a dilution series of NATtrol™ control (NATtrol™ SARS-Related Coronavirus 2 (SARS-CoV-2) Stock, Catalog Number NATSARS(COV2)-ST, ZeptoMetrix (Buffalo, NY)) in saliva.

The preliminary LoD was determined by evaluating a total of 5 different concentrations of NATtrol SARS-CoV-2. Each replicate was independently processed through the entire automated process. The LoD was confirmed by analyzing a total of 20 replicates at the preliminary LoD concentration. Based on these studies, the LoD of the Rheonix COVID-19 MDx Assay was estimated to be 625 genomic equivalents/mL for saliva specimens.

A complete LoD study (preliminary and confirmatory) was repeated in nasopharyngeal swab matrix using the NATtrol SARS-CoV-2 in parallel with saliva specimen matrix. The analytical sensitivity of the Rheonix COVID-19 MDx Assay is estimated to be 625 genomic equivalents when testing for the presence of SARS-CoV-2 in either saliva or NP swabs.

Table 9. Limit of Detection of the Rheonix COVID-19 MDx Assay When Testing Saliva Specimens.

Concentration of SARS-CoV-2 (Genomic Equivalents/mL)	Number Tested	Number Positive	Percent Positive
625	20	20	100%

Inclusivity (Analytical Sensitivity)

SARS-CoV-2 sequences were obtained from both the GISAID (38199 full-length sequences as of August 28th 2020) and NCBI (11663 full length sequences as of September 2nd 2020) dedicated databases. Neither geographic nor time restrictions were placed on either of the searches of the databases. Full-length sequences from both datasets were analyzed against the N1 primers and probe used in the Rheonix COVID-19 MDx assay. Table 10 shows that 98.087% of the combined 49862 full length sequences demonstrate 100% identity with the probe, as well as 99.773% and 99.585% of the combined full length sequences demonstrating 100% identity with the forward and reverse primers, respectively. Table 10 also shows the percentage of sequences with 1, 2 or 3 mismatches with the primer and probe sequences. The majority of mismatched sequences contain single base polymorphisms. In general, the target sequences of for the Rheonix COVID-19 MDx Assay were shown to be well conserved and the locations of those mismatches with the primer and probe that are known exist are not predicted to have an adverse effect on test performance.

Table 10. SARS-CoV-2 sequence analysis: Number of 100% identity and mismatches (percent of total). MM: mismatch

Name	0 MM	1 MM	2 MM	3 MM	Total
Forward_nCoV-N1	49749 (99.773)	111 (0.223)	1 (0.002)	1 (0.002)	49862
Reverse_nCoV-N1	49655 (99.585)	205 (0.411)	2 (0.004)	0	49862
2019-nCoV_N1 Probe	48908(98.087)	952 (1.909)	2 (0.004)	0	49862

Cross Reactivity (Analytical Specificity)

Cross reactivity studies were performed on all organisms noted (Table 11), with each microorganism subjected to *in silico* analysis with all combinations of primer pairs present in the Rheonix COVID-19 MDx Assay. The Rheonix COVID-19 MDx Assay uses a subset of the primers developed by the CDC and utilized in the CDC developed 2019-Novel Coronavirus (2019-nCoV) Real-time RT-PCR assay. The Rheonix COVID-19 MDx Assay uses the primers generated for the human RNase P gene as sample control, and for the N1 target region of the SARS CoV-2 nucleocapsid protein gene sequence. The sequences of the four primers are shown in Table 12 and all potential primer combinations are shown in Table 13. All potential primer combinations were subjected to several analyses using the National Center for Biotechnology Information (NCBI) primer analysis tool Primer-BLAST (Basic Local Alignment Search Tool). In one analysis, only the potential cross-reacting organisms shown in Table 11 were tested with all combinations. In the second analyses, all combinations were tested against the complete non-redundant nucleotide collection database. To ensure the potential of any off target being detected, parameters are set wide (e.g. aggressively) to identify any possible target, even if

implausible, would be detected. For example, the melting temperature parameters for the search are set between 40 °C and 77 °C, and a high allowance for mismatches. All analysis demonstrated the expected detection of SARS-CoV-2 and the human RNase P mRNA and gene with the correct corresponding primer pair. As expected, based on the current understanding of how SARS CoV-2 evolved, there is a significantly high degree of homology (97.2% identity) with the similar bat and pangolin virus. Contamination of the swab samples with bat or pangolin virus presents negligible risk and thus false positives due to these homologies are not expected. It should also be noted that the primers for the human RNase P gene demonstrate identity with the predicted sequences of several primates. The risk of the swabs being contaminated with exotic primate sequences presents negligible risks as well and therefore is not a concern for the assay. These data confirm that no potential off-targets could be amplified with any combination of the primers in the assay resulting in either the potential of false positives, or competition with the actual desired targets.

Table 11. In silico Analysis of Potentially Cross-Reactive Organisms

Other high priority pathogens from the same genetic family	High priority organisms likely in the circulating area	High priority organisms, including organisms commonly found in the clinical matrix	Organisms to be analyzed for non-blood clinical specimens
Human coronavirus 229E	Adenovirus (e.g. C1 Ad. 71)	Influenza C	*Escherichia coli*
Human coronavirus OC43	Human Metapneumovirus (hMPV)	Parechovirus	*Lactobacillus spp.*
Human coronavirus HKU1	Parainfluenza virus 1-4	*Candida albicans*	*Bacillus spp.*
Human coronavirus NL63	Influenza A & B	*Corynebacterium diphtheria*	*Clostridium spp.*
SARS-coronavirus	Enterovirus (e.g. EV68)	*Legionella non-pneumophila*	*Enterobacter spp.*
MERS-coronavirus	Respiratory syncytial virus	*Bacillus anthracis* (Anthrax)	*Enterococcus spp.*
	Rhinovirus	*Moraxella catarrhalis*	*Fusobacterium spp.*
	Chlamydia pneumoniae	*Neisseria elongata*	*Bacteroides spp.*
	Haemophilus influenzae	*Neisseria meningitidis*	*Bifidobacterium spp.*
	Legionella pneumophila	*Pseudomonas aeruginosa*	*Ruminococcus spp.*
	Mycobacterium tuberculosis	*Staphylococcus epidermidis*	
	Streptococcus pneumoniae	*Leptospira spp.*	
	Streptococcus pyogenes	*Chlamydia psittaci*	
	Bordetella pertussis	*Coxiella burneti* (Q-Fever)	
	Mycoplasma pneumoniae	*Staphylococcus aureus*	
	Pneumocystis jirovecii (PJP)		
	Candida albicans		
	Pseudomonas aeruginosa		
	Staphylococcus epidermidis		
	Streptococcus salivarius		

Table 12. Primer Sequences used in the Rheonix COVID-19 MDx Assay

Primer	Sequence (5' – 3')
RNAp For	AGATTTGGACCTGCGAGCG
RNAp Rev	GAGCGGCTGTCTCCACAAGT
N1 For	GACCCCAAAATCAGCGAAAT
N1 Rev	TCTGGTTACTGCCAGTTGAATCTG

Table 13. All Possible Primer Combinations in the RT-PCR Master Mix

	PRIMER 1			
	RNAp For	RNAp Rev	N1 For	N1 Rev
PRIMER 2	RNAp For	RNAp For	RNAp For	RNAp For
	RNAp Rev	RNAp Rev	RNAp Rev	RNAp Rev
	N1 For	N1 For	N1 For	N1 For
	N1 Rev	N1 Rev	N1 Rev	N1 Rev

Interfering Substances

The main objective of this study is to investigate the potential for interference resulting from medically relevant concentrations of various interfering substances ("interferents") that could potentially be present in respiratory specimens evaluated by the Rheonix COVID-19 MDx Assay.

A total of 12 potentially interfering substances that could be present in the NP swab specimens were evaluated at concentrations selected to be medically relevant. The testing was performed by analyzing pooled NP swabs (previously screened with the Rheonix COVID-19 MDx and shown to be negative) under the following conditions:

- Each potentially interfering substance was first separately evaluated in pooled NP swab to determine if the presence of the interfering substance would cause a "negative" sample to yield a "positive" result.
- Each potentially interfering substance was then separately evaluated in the presence and absence of inactivated SARS-CoV-2 virus at 3X the established LoD of the Rheonix COVID-19 MDx Assay.
- Testing was performed in triplicate.
- A single operator performed the testing on a single Encompass MDx workstation.
- A single external positive control run at 5X LoD and a single and a single negative control (pre-tested pooled negative NP swab) was run at least once/day.
- When tested in the absence of inactivated SARS-CoV-2 virus, a substance will be considered an interfering substance if one or more of the two replicates yields a positive result for SARS-CoV-2 RNA.

Statistical Methods

In the absence of target organisms, the percent of "positive" results in the presence of interfering substances was calculated. In the presence target organisms and interfering substances, the percent of "positive" results was calculated.

Acceptance Criteria

For a substance to be classified as non-interfering, the results displayed in Table 14 are expected. Any other combination of results will classify the substance as interfering.

Table 14. Anticipated Interference Study Results

Test combination	Anticipated Results
Matrix alone	Negative for all replicates for SARS-CoV-2 RNA
Matrix, plus interferent	Negative for all replicates for SARS-CoV-2 RNA
Matrix, plus SARS-CoV-2 RNA	Positive for all replicates for SARS-CoV-2 RNA
Matrix, plus SARS-CoV-2 RNA and interferent	Positive for all replicates for SARS-CoV-2 RNA

Accordingly, when tested in the absence of the SARS-CoV-2 RNA, a substance is considered an interfering substance if one or more of the replicates yields a positive signal.

Similarly, when tested in the presence of SARS-CoV-2 RNA, a substance is considered an interfering substance if one or more of the replicates yields a negative signal.

The results of the interference study are reported in Table 15. The concentration of potentially interfering substance that was tested did not demonstrate interference (of either a negative sample being converted to a positive result by the presence of the substance or a positive sample being converted to a negative result by the presence of the substance).

Table 15. Endogenous Interference Study Results

		Results detected*	
Product tested	Concentration tested	Interferent + matrix	SARS-CoV-2+Interferent + matrix
		Neg (x3)	Pos (x3)
Mucin: bovine submaxillary gland, type I-S	1mg/mL	3	3
Blood (human)	1%	3	3
Saline nasal spray	10% v/v	3	3
Afrin nasal spray (Oxymetazoline HCl 0.05%)	10% v/v	3	3
Flonase allergy relief (Fluticasone furoate)	500 ng/mL	3	3

Zicam cold remedy (Luffa operculata)	10% v/v	3	3
Children's allergy relief, (Loratadine 5mg/mL)	100 ng/mL	3	3
Vicks Vapocool sore throat (Benzocaine 5%, menthol 1%)	2.5% v/v	3	3
Oseltamivir phosphate	500 ng/mL	3	3
Mupirocin	500 ng/mL	3	3
Tobramycin	500 ng/mL	3	3
Biotin	3,500 ng/mL	3	3

**In all cases the expected results were obtained under each of the listed conditions*

FDA SARS-CoV-2 Reference Panel Testing

Nasopharyngeal Swabs

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to corroborate the LoD. The extraction method used was exactly as described in this Package Insert and was performed on the Rheonix Encompass MDx® Workstation that automatically performs all sample extraction, amplification and detection steps in a closed system. The results are summarized in Table 16.

Table 16. Summary of LoD Confirmation Result Using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal Swab	$1.8x10^3$ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL: RNA NAAT detectable units/mL
N/A: Not Applicable
ND: Not Detected

Saliva

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to corroborate the LoD. The extraction method used was exactly as described in this Package Insert and was performed on the Rheonix Encompass MDx® Workstation that automatically performs all sample extraction, amplification and detection steps in a closed system. The results are summarized in Table 17.

Table 17: Summary of LoD Confirmation Result Using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Saliva	$2.0x10^3$ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL: RNA NAAT detectable units/mL
N/A: Not Applicable
ND: Not Detected

Clinical Evaluation

Nasopharyngeal Swabs

The clinical evaluation of the Rheonix COVID-19 MDx Assay was performed by analyzing contrived samples that consisted of 30 reactive and 30 nonreactive NP swabs. Each swab was obtained from a single, unique subject and spiked with various concentrations of SARS-CoV-2 RNA as shown in Table 18. A total of 30 unspiked (negative) NP samples and 30 contrived NP samples were tested.

Table 18. Composition of Contrived Clinical Specimens Used in Clinical Evaluation Studies

Specimen Type	Number of Specimens	Spike level	Expected Test Result
Low LoD	20	2X LoD	≥95% Positive
Intermediate LoD	5	5X LoD	100% Positive
High LoD	5	10X LoD	100% Positive
Negative	30	None	100% Negative

The specimens were collected in BD Universal Viral Transport with 3 ml media and shipped to Rheonix's facility in Ithaca, NY for spiking and testing. One ml of Rheonix 4X Sample Buffer was added to each tube prior to analysis.

Qualitative test results are reported together with the associated assay metric expressed in term of Intensity Units (Table 19 and Table 20).

Table 19. Clinical Evaluation of Unspiked NP Swab: Results Obtained Using the Rheonix COVID-19 MDx Assay

Sample ID	Spike Level	Qualitative*	Intensity Units	
			RNase P Target	N1 Target
1	None	Negative	149.932	-1.635
2	None	Negative	166.74	-11.371
3	None	Negative	163.847	6.495
4	None	Negative	175.114	0.834
5	None	Negative	182.435	-0.922
6	None	Negative	184.31	-2.214
7	None	Negative	151.872	-2.88
8	None	Negative	176.078	-7.626
9	None	Negative	166.339	-3.92
10	None	Negative	185.055	-1.79
11	None	Negative	169.528	-6.595
12	None	Negative	168.64	0.294
13	None	Negative	170.468	-4.116
14	None	Negative	192.41	-2.67
15	None	Negative	167.623	3.499
16	None	Negative	182.884	0.797
17	None	Negative	173.286	5.897
18	None	Negative	158.428	-0.317
19	None	Negative	186.174	-3.248
20	None	Negative	182.289	-4.13
21	None	Negative	184.611	-5.518
22	None	Negative	175.881	-10.286
23	None	Negative	187.694	0.373
24	None	Negative	182.555	0.119
25	None	Negative	190.94	8.301
26	None	Negative	196.368	1.753
27	None	Negative	190.455	0.411
28	None	Negative	157.149	-2.131
29	None	Negative	186.288	0.802
30	None	Negative	142.583	-4.811

** Intensity readings less than 25.00 units are scored as "Negative" while intensity readings greater than 25.00 are scored as "Positive".*

Table 20. Clinical Evaluation of Spiked NP Swab: Results Obtained Using the Rheonix COVID-19 MDx Assay

Sample ID	Spike Level	Qualitative*	Intensity Units	
			RNase P Target	N1 Target
1	2 X LoD	Pos	171.287	158.333
2	2 X LoD	Pos	163.705	67.499
3	2 X LoD	Pos	159.723	138.684
4	2 X LoD	Pos	156.037	128.951
5	2 X LoD	Pos	111.669	117.139
6	2 X LoD	Pos	170.692	119.603
7	2 X LoD	Pos	172.921	161.551
8	2 X LoD	Pos	197.868	168.628
9	2 X LoD	Pos	164.422	143.409
10	2 X LoD	Pos	147.253	149.632
11	2 X LoD	Pos	158.49	156.341
12	2 X LoD	Pos	167.307	145.039
13	2 X LoD	Pos	144.464	134.459
14	2 X LoD	Pos	164.925	116.614
15	2 X LoD	Pos	119.043	39.519
16	2 X LoD	Pos	146.027	124.338
17	2 X LoD	Pos	163.178	127.369
18	2 X LoD	Pos	163.137	150.677
19	2 X LoD	Pos	163.691	137.36
20	2 X LoD	Pos	178.737	153.44
21	5 x LoD	Pos	177.602	177.92
22	5 x LoD	Pos	196.724	188.258
23	5 x LoD	Pos	188.573	199.827
24	5 x LoD	Pos	190.925	185.071
25	5 x LoD	Pos	161.391	171.233
26	10 x LoD	Pos	208.951	180.55
27	10 x LoD	Pos	125.543	109.55
28	10 x LoD	Pos	189.218	189.913
29	10 x LoD	Pos	154.301	180.131
30	10 x LoD	Pos	169.465	182.442

** Intensity readings less than 25.00 units are scored as "Negative" while intensity readings greater than 25.00 are scored as "Positive".*

Analysis of the contrived samples gave rise to expected results (Table 21).

Table 21. Results Obtained When Analyzing Contrived Specimens

Specimen Type	Number of Specimens	Spike level	Number Positive or Negative	Percent Correct
Low LoD	20	2X LoD	20 Positive	100%
Intermediate LoD	5	5X LoD	5 Positive	100%
High LoD	5	10X LoD	5 Positive	100%
Negative	30	None	30 Negative	100%

Saliva

A clinical study was performed to evaluate the use of saliva as a specimen type for detection of SARS-CoV-2 in patients who are suspected of COVID-19 by their healthcare provider. Two separate IRBs oversaw the studies and samples were collected at two different study sites.

The protocol was designed to obtain paired, de-identified nasopharyngeal swabs and saliva specimens in order to compare the positive and negative agreement between these two clinical matrices when tested using the Rheonix COVID-19 MDx Assay. Paired nasopharyngeal swab and saliva specimens were obtained from 79 individuals suspected of COVID-19 by their health care provider. Each paired set of specimens was obtained from a unique subject. Participants in the study were classified as symptomatic or asymptomatic. Paired specimens from patients with previous known positive results for SARS-CoV-2 were obtained no later than 5 days after the date on which their positive test result was obtained.

Collection of NP swabs

All NP swabs were collected by a health care provider in NP collection devices (BD or Copan Diagnostics) or in a UTM validated by the study site.

Collection of Saliva Specimens

3 mL of saliva specimens were collected into sterile 15 mL conical tubes under the observation of a healthcare provider. The conical tube was then given to the healthcare worker who either immediately added 1 mL of Rheonix 4X Buffer or transported the conical tube to a laboratory where 1 mL of Rheonix 4X Buffer was subsequently added within 4-6 hours.

Results from Evaluation of Paired Specimens

The performance of the Rheonix COVID-19 MDx Assay was evaluated by comparing the results obtained from the saliva specimens to those obtained with the paired nasopharyngeal specimens. A summary of the results of the study is presented in Table 22.

Table 22. Clinical Performance of the Rheonix MDx Assay Evaluating Saliva Specimens with Matched Nasopharyngeal Specimens

		Results from Nasopharyngeal Swab Specimens		
		Positive	**Negative**	**Total**
Results from Saliva Specimens	**Positive**	44	0	44
	Negative	1	34	35
	Total	45	34	79
Positive Agreement		97.8% (44/45); 88.4%-99.6%		
Negative Agreement		100.0% (34/34); 89.9%-100%		

**Note: all paired negative specimens were obtained from asymptomatic subjects*

Sample stability

Study Objective

The objective of this study was to establish the duration that nasopharyngeal swabs and saliva specimens can be stored under recommended conditions (after immediate addition of Rheonix 4X Sample Buffer or and after a delay of up to 6 hours for saliva specimens.

Study Design

Specimen stability studies were performed for both the NP swabs and the saliva specimens in Rheonix Sample buffer. In both cases, pooled negative matrix, mixed with Rheonix Sample buffer, was spiked with 1X LoD (i.e., 625 genomic equivalents/mL) of inactivated SARS-CoV-2 virus (NATtrol™ SARS-Related Coronavirus 2 (SARS-CoV-2) Stock, Catalog Number NATSARS(COV2)-ST, Zeptometrix, Buffalo, NY)), and then 20 replicates of each specimen type were tested over a 16 day period (testing at time zero, 7, 9, 14 and 16 days). Since a 14-day stability claim was the goal, 16 days represents a time frame 10% longer than the planned claim. The saliva specimens were stored at room temperature (25 °C) while the NP swabs were stored at 2-8 °C.

In order to support up to a 6 hour waiting period before addition of the 4X Rheonix Sample Buffer post-collection of saliva specimens, 21 replicates of inactivated SARS-CoV-2 at 1875 copies/mL (3x LoD) were tested at different timepoints (baseline, 24 hours, 48 hours, 72 hours) after storage at either room temperature or 4 °C.

Study Results

Saliva Specimens

As noted in Table 23, all tested time points for saliva specimens stored at 25 °C (i.e., room temperature) through 16 days of storage returned at least 95% positive results.

Table 23. Stability of Saliva Specimens When Stored at Room Temperature after Immediate Addition of Rheonix 4X Sample Buffer

Days Stored	Number of Samples Tested	Number of Samples that Yielded Positive Results	Percent Positive Results
0	20	20	100%
7	20	20	100%
9	20	20	100%
14	20	20	100%
16	20	19	95%

As noted in Table 24, all tested time points for saliva specimens stored at either room temperature or 4 °C through 72 hours before Rhenoix 4X Sample Buffer addition returned 100% positive results.

Table 24. Stability of Saliva Specimens when Rheonix 4X Sample Buffer was not Immediately Added after storage at Room Temperature and 4 °C

SARS-CoV-2 in saliva matrix at 1875 copies/mL	Time prior to adding 4X Rheonix Buffer	Number of Positive Results	
		Room Temp	4°C
	0 hrs	12/12	12/12
	24 hrs	3/3	3/3
	48 hrs	3/3	3/3
	72 hrs	3/3	3/3

NP Swab specimens

As noted in Table 25, all tested time points for nasopharyngeal swabs stored at 2-8°C through 16 days of storage returned at least 95% positive results.

Table 25. Stability of NP Swab Specimens When Stored at 2-8 °C after immediate addition of Rheonix Sample Buffer.

Days Stored	Number of Samples Tested	Number of Samples that Yielded Positive Results	Percent Positive Results
0	20	20	100%
7	20	20	100%
9	20	19	95%
14	20	19	95%
16	20	19	95%

The above results support the stability of both saliva specimens stored at 25 °C and nasopharyngeal swab specimens stored at 2-8 °C for 14 days.

Labeling Symbology

Symbol	Title of symbol	Explanatory Text	Standard Reference
	Manufacturer	Indicates the medical device manufacturer	ISO 15223-1:2016 Reference no. 5.1.1
	Date of Manufacture	Indicates the date when the medical device was manufactured	ISO 15223-1:2016 Reference no. 5.1.3 ISO 7000:2014 Reference no. 2497
	Consult instructions for use	Indicates the need for the user to consult the instructions for use	ISO 15223-1:2016 Reference no. 5.4.3 ISO 7000:2014 Reference no.1641
	Temperature limits	Indicates the temperature limits to which the medical device can be safely exposed.	ISO 15223-1: 2016 Reference number 5.3.7 ISO 7000:2014 Reference no. 0632
	Do not use if damaged	Indicates a medical device that should not be used if the package has been damaged or opened	ISO 15223-1: 2016 reference number 5.2.8 ISO 7000:2014 Reference no. 2606
	Single use only; do not reuse	Indicates a medical device that is intended for one use, or for use on a single patient during a single procedure	ISO 15223-1:2016 Reference no. 5.4.2 ISO 7000:2014 Reference no. 1051

	Caution, consult accompanying documents	Indicates the need for the user to consult the instructions for use for important cautionary information	ISO 15223-1:2016 Reference no. 5.4.4 ISO 7000:2014 Reference no. 0434
	Contains sufficient contents for <n> tests	Indicates the total number of IVD tests that can be performed with the IVD	ISO 15223-1:2016 Reference no. 5.5.5 ISO 7000:2014 Reference no. 0518
	*In vitro* diagnostic medical device	Indicates a control material that is intended to verify the performance characteristics of another medical device	ISO 15223-1:2016 Reference no. 5.5.2 ISO 7000:2014 Reference no. 2494
	Prescription use only	Caution: Federal law (USA) restricts this device to sale by or on the order of a licensed healthcare practitioner	21 CFR 801.109
	Catalog Number	Indicates the manufacturer's catalogue number to identify the medical device	ISO 15223-1:2016 Reference no. 5.1.6 ISO 7000:2014 Reference no. 2493
	Lot Number	Indicates the manufacturer's batch code to identify the batch or lot	ISO 15223-1:2016 Reference no. 5.1.5 ISO 7000:2014 Reference no. 2492
	Environmental or aquatic toxicity	Indicates a potential of environmental or aquatic toxicity	US: 29 CFR 1910:1200 (HCS) EU: Regulation 1272/2008/EC GHS 09
	Use by	Indicates the date after which the medical device is not to be used	ISO 15223-1:2016 Reference no. 5.1.4 ISO 7000:2014 Reference no. 2607
	Skin Irritation, category 2 Eye Irritation, category 2	Indicates a potential for health risk to the user of the medical device	US: 29 CFR 1910:1200 (HCS) EU: Regulation 1272/2008/EC GHS 07

Intellectual Property

The Assay is covered by the following patents:

The Rheonix COVID-19 MDx Assay is covered by the following patents (US patents referenced unless otherwise noted):

Workstation: US 7,976,795; US 8,101,428; US 8,383,039; US 8,609,039; US 9,151,701; CN 102906573; JP 6058399; JP 6104327; US 9,096,890; US 8,986,614; US9,102,979; US 9,328,381; US 9,556,478; AU 2011221244

CARD Cartridges: US 7,608,160; US 7,832,429; US 7,837,821; US 7,959,875; US 8,057,629; US 8,293,053; US 8,323,586; US 8,512,502; US 8,535,020; US 8,646,482; US 8,715,446; US 8;715;447; US 8,763,641; AU 2006320916; AU 2007207681; CN 101282789; EP 1,706,467 (CH, DE, FR, GB, IT & SE); IN 255971; JP 4,516,606; JP 4,939,541; JP 5,250,425; JP 5,323,747; IN 262645; EP 2,520,367 (CH, DE, FR, GB, IT & SE); US 9,638,338; CN 101495236; IN 281148; AU 2007207681; US 8,372,355; US 8,778,280; US 9,134,207; US 9,132,398; CN 101903104; JP 5,523,327; IN 277018;