PhoenixDx® ***2019-nCoV***

qualitative RT-PCR-based detection of SARS-CoV-2

INSTRUCTIONS FOR USE

For Use under Emergency Use Authorization

For *In Vitro* Diagnostic Use
R_X Only

IVD

50 Tests

REF PCCSKU15261

Rx Only

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1) INTENDED USE

PhoenixDx® 2019-nCoV is a real-time Qualitative RT-PCR-based test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasal, nasopharyngeal and oropharyngeal swabs and BAL 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, to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. SARSCoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude 2019-nCoV 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 PhoenixDx 2019-nCoV is intended for use by qualified and trained healthcare professionals or clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The PhoenixDx 2019-nCoV is only for use under the Food and Drug Administration's Emergency Use Authorization.

2) PHOENIXDX® DETECTION SYSTEM

2.1) Explanation of the Test/Principles of the Procedure

The PhoenixDx® 2019-nCoV test is based on conventional RT-PCR technology including extraction and purification of the RNA genome of SARS-CoV-2 followed by reverse transcription to cDNA and PCR amplification and detection of the target sequences. The test is run on the BIO-RAD CFX96-IVD platform. Nucleic acid from patient samples and controls are extracted in parallel using the RTA Viral Nucleic Acid Isolation Kit. Nucleic acid is released by the lysis reagent and bound to the silica columns. Unbound substances and impurities, such as denatured protein, cellular debris and potential PCR inhibitors, are removed with subsequent wash steps and purified nucleic acid is eluted silica columns with elution buffer. External controls (positive and negative) are processed in the same way with each run.

Selective amplification of target nucleic acid from the sample is achieved by the use of target-specific forward and reverse primers and probes specific to the SARS-CoV-2 envelope gene (E-gene) and the polymerase gene (RdRP) https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045). The RdRP gene target is detected by one prob, unique to SARS-CoV-2. Additionally, a conserved region in the structural protein envelope E-gene was chosen also for the pan-Sarbecovirus detection. The pan-Sarbecovirus detection sets will also detect SARS-CoV-2 virus. Due to the intrinsic mutation rate of RNA viruses, it is possible that mutations in the target sequence occur and accumulate over time leading to false-negative results. PhoenixDx 2019-nCoV mitigates this risk by using 2 different target sequences for SARS-CoV-2.

Selective amplification of the RNase P Internal Control cDNA is achieved by the use of non-competitive, sequence specific forward and reverse primers and a probe which have no homology with the coronavirus genome. A thermostable DNA polymerase enzyme is used for amplification.

The PhoenixDx® 2019-nCoV master mix contains detection probes for the two SARSCoV-2 targets and one for the internal RNase P gene. Each of the targets is amplified in a separate reaction. Probes are each labeled with fluorescent dyes that act as a reporter. Each probe also has a second dye which acts as a quencher. When not bound to the target sequence, the fluorescent signals of the intact probes

are suppressed by the quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5' to 3' exonuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly. Each reporter dye is measured at defined wavelengths, which enables simultaneous detection and discrimination of the amplified coronavirus targets.

2.2) MATERIALS PROVIDED

Table 1:

PRODUCT	SIZE	SKU
PHOENIXDX® 2019-NCOV	50 rxn / 20 µl	PCCSKU15259

QUANTITY AND VOLUME	COMPONENT
1 x 100 µl	PhoenixDx® **Enzyme Mix**
1 x 500 µl	PhoenixDx® **E gene Mix**
1 x 500 µl	PhoenixDx® **RdRP Mix**
1 x 500 µl	PhoenixDx® **RNase P Mix**
1 x 50 µl	2019-nCoV Target Positive Control **(TPC)**
2 x 1 ml	Nuclease Free **dH2O**

*Each of the colors above corresponds to the color-coded vials in the test kit.

2.3) ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

- RTA Viral RNA Extraction Kit as extraction Kit (RTA Laboratories, Cat #09010100)
- BioRad CFX-96 IVD marked instrument with BioRad CFX Manager Software version 3.0
- BioRad CFX-96 IVD nuclease free 96 well plates: Hard-Shell Thin-Wall 96-Well Skirted PCR Plates (BIO-RAD, Cat#: HSP-9655)
- BioRad sealing tape: Microseal 'B' Adhesive Seals, optically clear (BIO-RAD, Cat#: MSB-1001)
- Adjustable pipettes & fitting filtered pipette tips
- Appropriate PPE & workspaces for working with potentially infectious samples
- Surface decontaminants such as DNAZap (Life Technologies), DNA Away (Fisher)
- Scientific), RNAse Away (Fisher Scientific), 10% bleach (1:10 dilution of commercial 5.25-6.0% sodium hypochlorite)
- Nuclease-free tubes / strips / plates to prepare dilutions and master-mixes, such as Eppendorf colorless 1.5 ml Microtubes, Cat. No. Z606340
- Suitable storage options for reagents and specimen (4°C, -20°C, -70°C)

2.4) STORAGE

- Store all components at -20°C and avoid repeated freeze and thaw cycles.
- Protect the 2X qPCR mastermixes from light as prolonged exposure can diminish the performance of the fluorophores.
- If the kit components have been damaged during transport, contact Procomcure Biotech. Do not use as performance may be compromised.
- Keep reagents separate from sample material to avoid contamination.
- Do not use after the designated expiry date

3) SPECIMEN STORAGE AND HANDLING

Only the following specimens were validated with use of the PhoenixDx 2019-nCoV test:

- Respiratory specimens including nasal, nasopharyngeal and oropharyngeal swabs, bronchioalveolar lavage.
- Swab specimens should be collected only on swabs with a synthetic tip (such as polyester or Dacron®) with aluminum or plastic shafts. Swabs with calcium alginate or cotton tips with wooden shafts are not acceptable.
- Specimens can be stored at 4°C for up to 48 hours after collection.
- If a delay in extraction is expected, store specimens at -70°C or lower.
- Extracted nucleic acids should be stored at -70°C or lower if storage is needed.

⚠ Do not vortex specimens as this will fragment the RNA and lead to failure of the **PHOENIXDX® 2019-NCOV** assays.

Do not use specimens if:

- they were not kept at 2-4°C (≤ 2 days) or frozen at -70°C or below.
- they are insufficiently labelled or lack documentation.
- they are not suitable for this purpose (see above for validated sample types).
- the specimen volume is insufficient (i.e., a minimum volume of 150 ul of sample is needed for testing).

4) WARNINGS

4.1) BIOSAFETY

- For in vitro diagnostic use"
- For Prescription Use Only (Rx only)
- For use under an Emergency Use Authorization (EUA) only
- Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (2019-nCoV) https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical- specimens.html
- Wear appropriate personal protective equipment (e.g. gowns, powder-free gloves, eye protection) when working with clinical specimen.
- Specimen processing should be performed in a certified class II biological safety cabinet following biosafety level 2 or higher guidelines.
- Biosafety in Microbiological and Biomedical Laboratories 5th edition available at http://www.cdc.gov/biosafety/publications/.
- The use of **PHOENIXDX® 2019-NCOV** is restricted to trained laboratory personnel only.
- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplifications reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon). Workflow in the laboratory should proceed in a unidirectional manner.

- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.

4.2) SAMPLE PREPARATION / NUCLEIC ACID EXTRACTION

- The performance of RT-PCR assays strongly depends on the amount and quality of sample template RNA. It is strongly recommended to qualify and validate RNA extraction procedures for recovery and purity before testing specimens.
- The validated nucleic acid extraction system used in combination with **PHOENIXDX DETECTION KITS** is the RTA Viral RNA Isolation Kit (RTA Laboratories).
- Store and keep residual specimens and extracted nucleic acids at -70°C.
- Only thaw the number of specimen extracts that will be tested in a single day.
- Do not freeze/thaw extracts more than once before testing as each freeze/thaw cycle will decrease the RNA quality.

4.3) REACTION SETUP

- Make sure that all necessary equipment and devices are suitable, calibrated and functional before starting a RT-PCT runs.
- Decontaminate equipment and workspace and prepare everything needed for testing.
- Thaw all components of PhoenixDx 2019-nCoV on ice and mix gently but thoroughly to ensure even distribution of components. Collect liquid at the bottom of the tube with a quick spin.
- In an area separate from the PCR area, dilute the provided Target Positive Control (TPC) as follows: TPC is to be diluted 1:5 in nuclease-free dH2O. To prepare a working dilution for 3 runs, take 6 µl of TPC and add 24 µl of nuclease-free dH2O to make a final volume of 30 µl. Do not dilute the entire TPC at once.

1. For each of the targets (i.e., E, RdRP and RNase P), prepare enough master mix for all planned reactions (n) according to your sample size. Each target is amplified separately. Therefore, for each mastermix 1 negative control must be included. Additionally, for both SARS-CoV-2 targets (E and RdRP) the TPC positive control must be included. It is recommended to prepare master mix for 2 additional reactions to compensate for pipetting inaccuracies. Find the total volume by multiplying the volume per reaction (see table below) by n+2 (“n” being the number of total samples including controls). Prepare 3 master mixes, i.e., one for each of the following targets: E, RdRP, and RNase P. When calculating the pipetting volumes for each mastermix, please use the volume table below. We recommend calculating 2 additional reactions for pipetting errors. For example, for 10 samples + 1 positive control + 1 negative control, the volumes should be multiplied by 14 (12+2). Then prepare the mastermix for each target, E, RdRP and RNase P. Aliquot 15 µl of each mastermix into separate wells and add 5 µl of sample /negative control onto the mastermix. Each patient specimen will have 3 separate wells. The TPC should be added only to the E and RdRP mastermixes.

   The pipetting amounts for a single reaction as given below, multiply reagents highlighted in yellow with n+2 for the Mastermixes:

Table 2:

COMPONENT	VOLUME
PhoenixDx® Enzyme Mix	0.4 µl
PhoenixDx® 2X qPCR Mastermix (E / RdRP / RP)	10 µl
Nuclease-free dH2O	4.6 µl
Total Master Mix	15µl
Isolated sample RNA / TPC	5 µl

2. Distribute 15 µL of the master mix to each well of your PCR plate.

3. Transfer the Mastermix Plate to a separate workspace to add the sample material. Preparing reagents and final reaction setup in separate workspaces helps to avoid contamination of equipment and reagents with sample material.

4. Prepare negative reactions first and seal them before handling positive samples. It is recommended to only bring potentially positive sample material and the included target positive control to the workspace once the NTC is prepared and sealed.

5. Add 5 µl Sample or Control to the respective sample and control wells and seal the plate. Keep reactions on ice until transferring them to the PCR device.

Table 3: Example pipetting scheme for the distribution of master mixes with the individual assay mixes.

	1	2	3	4	5	6	7	8	9	10	11	12
A	E gene	E gene	E gene	E gene	E gene	E gene	E gene	E gene	E gene	E gene	E gene	E gene
B	RdRP	RdRP	RdRP	RdRP	RdRP	RdRP	RdRP	RdRP	RdRP	RdRP	RdRP	RdRP
C	RNase P	RNase P	RNase P	RNase P	RNase P	RNase P	RNase P	RNase P	RNase P	RNase P	RNase P	RNase P
D												
E												
F												
G												
H												

Table 4: Example pipetting scheme for the addition of samples. The bottom half of the plate could be used for replicates with an identical setup.

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	TPC
B	NTC	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	TPC
C	NTC	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	TPC
D												
E												
F												
G												
H												

a. Setting up RT-PCR Program:

1. **Switch on the PCR detection system BioRad CFX-96 with BioRad CFX Manager Software version 3.0 or higher and program it with the following thermal protocol:**

Table 5:

STEP	CYCLES	TEMPERATURE	DURATION
Reverse Transcription	1	45°C	10 minutes
Initial Denaturation	1	95°C	10 minutes
Amplification	45	95°C	15 seconds
		58°C[1]	45 seconds

2. **Transfer the RT-PCR plate to the PCR device, then cycle according to the program described above. The instrument and software only use default settings for running and analyzing the samples; no additional programming or adjustments (beyond the basic reaction program above) are made by the end user.**

b. Data Collection and Analysis

By the end of the thermal protocol the data is collected automatically by the integrated software of BioRad CFX-96 IVD. Software version 3.0 or higher.

Enable Data Collection for FAMTM. Not required in BioRad CFX-96 IVD set Passive Reference to **ROXTM.**

c. Clean up Reaction after run

Once the run is finished, do not open the reaction tubes to avoid contamination and discard according to local guidelines and regulations. Do not autoclave as this may contaminate laboratory equipment with amplicons.

5) QUALITY CONTROL

The PhoenixDx 2019-nCoV kit contains an NTC, a RP, and a TPC. The quality control of each study should be performed according to the criteria provided below:

- **NTC:** dH2O control (NTC) taken through extraction and PCR is run for each Mastermix must not give a positive Ct for any assay. If they do, sample results cannot be reported as the reaction was contaminated with sample RNA / DNA. Decontaminate equipment and workspace and repeat all sample reactions.
- **Internal Control (RP):** All reactions containing samples must give positive Ct values for the internal RNase P (RP) target. The Ct values should be ≤35 cycles. Failure to amplify the RNase P within 35 Ct values indicates inadequate RNA extraction or loss of RNA isolate due to RNAse contamination. A sample result without RNase P amplification cannot be interpreted and needs to be repeated.
- **TPC:** Both, E and RdRP targets must be observed with Ct values of ≤35 cycles for the TPC control to be valid. If the Ct value is > Ct 35 or not all SARS-CoV-2 targets are tested positive, PCR was compromised. Check the reaction setup and PCR device settings and repeat the reactions. If any of the targets in the positive control is negative the run is invalid.

6) RESULT INTERPRETATION

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. For both, controls and patient specimens the threshold for RNase P to be called positive is a Ct of ≤35 For the SARS-CoV-2 targets E and RdRP to be called positive the cutoff is a Ct of ≤35.

6.1) PhoenixDx 2019-nCoV Test Controls – Positive, Negative, and Internal:

If any control does not perform as described above, run is considered invalid and all specimens must be repeated from extraction step after a root cause is identified and eliminated.

Avoid repeated freeze thaw cycles, aliquot the components of the kit if the contents of the kit will be used for more than 3 times.

Table 6: Expected Performance of PhoenixDx 2019-nCoV Test Controls

Control Type	External Control Name	E	RdRP	RP (IC)	Expected Ct values
Positive Control	TPC	+	+	-	≤35 Ct for E, RdRP and RP (IC) targets
Negative Control	NTC	-	-	-	≤35 Ct

If any of the above controls does not exhibit the expected performance as described, the assay may have been improperly set up and/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run, perform a root cause analysis and re-test after the root cause has been eliminated.

6.2) Examination and Interpretation of Patient Specimen Results:

- **For a sample to be considered positive** for SARS-CoV-2, both targets (E / RdRP) AND the RNase P target must give positive Ct values of ≤35. If the RNase P target fails to amplify within ≤Ct 35, but both SARS-2 specific targets are amplified, the sample is considered valid positive.
- **For a sample to be considered negative** for SARS-CoV-2, none of the two SARS-CoV-2 targets (E / RdRP) must give positive Ct values AND the RNase P must give a Ct value ≤35 cycles to ensure that sample material of suitable quality was present.
- **A sample result is invalid if the detection** of RNase P in the sample fails and the sample also fails to show amplification of both SARS-CoV-2 targets (E and RdRP) within ≤35 Ct. Invalid results cannot be interpreted. These samples should be repeated from extraction step. If both SARS-CoV-2 targets are detected in the sample in the absence of the RNase P target, the sample is valid positive (see above).

Table 7: Result Interpretation of PhoenixDx 2019-nCoV (Samples)

E GENE	RDRP	RNase P (IC)	Interpretation	Report	Actions
-	-	+	Only the target sequence for the internal Control was amplified. The sample is considered **negative for SARS-CoV-2.**	Negative	Report results
+	+	+	Both target sequences for SARS-CoV-2, and the internal Control were amplified. The sample is considered positive for SARS-CoV-2.	Positive	Report results
-	+	+	SARS-CoV-2 specific RdRP target sequence is detected, and sample is considered positive for SARS-CoV-2. A positive SARS-CoV-2 RdRP result and a negative Sarbecovirus (E-gene) result is suggestive of low concentration of viral RNA, or mutation in the target region of Sarbeco sequence.	Positive	Report results
+	-	+	A negative RdRP result and a positive E result is suggestive of low concentration of viral RNA, a mutation in the SARS-CoV-2 specific RdRP target sequence, or an infection with other Sarbecovirus (e.g., SARS-CoV or some other Sarbecovirus previously unknown to infect humans).	Presumptive positive	Repeat test once. If sample is repeat reactive with identical result additional confirmative testing consistent with public health guidelines must be conducted
-	-	-	PCR was inhibited, results are invalid.	Invalid	Sample is repeated once. If the result is again invalid, it is reported to the sender as invalid and collection of a new sample is recommended.

7) LIMITATIONS

- For reliable results, it is essential to adhere to the guidelines given in this manual. Changes in reaction setup or cycling protocol may lead to failed experiments and is not allowed under the Emergency Use Authorization.
- Spontaneous mutations within the target sequence may result in failure to detect the target sequence. While this risk is mitigated in the test's design, if failure to detect the target is expected it is recommended to test the specimen with a different test that detects different target sequences from the SARS-CoV-2 genome.
- Results must always be interpreted in consideration of all other data gathered from a sample. Interpretation must be performed by personnel trained and experienced with this kind of experiment.
- Users should be trained to perform this assay and competency should be documented
- Testing of nasal swabs even if collected by a healthcare provider is limited to patients with symptoms of COVID-19.
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions.
- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

8) CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

The PhoenixDx® 2019-nCoV Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd.

However, to assist clinical laboratories using the PhoenixDx® 2019-nCoV (referred to as "your product" in the conditions below), the relevant Conditions of Authorization are listed below and are required to be met by laboratories performing the EUA test:

A. Authorized laboratories[1] using your product will include with result reports of your product, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

B. Authorized laboratories using your product will use your product as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.

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

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

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

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

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

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

9) PERFORMANCE DATA

9.1) NON-CLINICAL PERFORMACE EVALUATION

a. Limit of Detection (LoD) - Analytical Sensitivity:

The LoD study established the lowest concentration of SARS-CoV-2 (genome copies(cp)/mL) that can be detected by the PhoenixDx 2019-nCoV test at least 95% of the time. The preliminary LoD was established by testing 10-fold dilutions of a positive patient sample quantitated by Droplet Digital PCR (QX200 Droplet Digital PCR System, BioRad) extracted and tested according to the instructions for use. The extracted RNA was tested in triplicate by PhoenixDx 2019-nCoV. The tentative LoD was determined to be 100 cp/mL.

The LoD was then confirmed by testing 20 replicates of nasopharyngeal swab matrix with SARS-CoV-2 RNA from the quantified human specimen at 100 cp/mL, and 50 cp/mL.

Table 8: SARS-CoV-2 - Confirmatory LoD

Target Level	Valid replicates tested	E-Gene			RdRP			RNase P		
		n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate
100 cp/mL	20	20	33.50	100%	20	34.04	100%	20	34.03	100%
50 cp/mL	4	9	34.78	45%	13	34.84	65%	4	36.14	20%

Of the samples at 50 cp/mL only 4 replicates were valid based on the Ct cutoff of 35 for the RNase P internal control gene. The final LoD for the PhoenixDx 2019-nCoV is 100 copies/mL which is the lowest concentration at which equal or above 95% of replicates were detected (i.e., 24/24 for the RdRP gene and 24/24 for the E gene).

b. Inclusivity (Analytical Sensitivity):

Primer/probe inclusivity was evaluated by BLASTn analysis against 389 publicly available SARS-CoV-2 sequences in the Betacoronavirus database on April 5, 2020. The Primers E_Sarbeco_F1, E_Sarbeco_R2, RdRP_SARSr-F2, RdRP_SARSr-R1 and probes E_Sarbeco_P1 and RdRP_SARSr-P2 exhibited 100% homology with all the available sequences.

The Primers and Probes from WHO were used during the studies. Please refer to the link below: https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045).

c. Cross-reactivity (Analytical Specificity):

Wet Testing

In this study, the specificity of the PhoenixDx 2019-nCoV Kit was evaluated by testing the organisms listed in table 1 below. 9 reference organisms and 11 clinical specimens were tested in the absence of SARS-CoV-2 RNA. The potential cross-reactive organisms were tested at concentrations between 1x103 – 1x105 copies/ml. Exact concentrations for the cross reactants was not available. Cross-reactivity with other coronaviruses cell culture supernatants containing human coronaviruses (HCoV)-229E, -NL63, -OC43, and -HKU1 as well as MERS-CoV were tested in all three assays.

For the not cultivable HCoV-HKU1, supernatant from human airway culture was used. Virus RNA concentration in all samples was determined by specific real-time RT-PCRs and in-vitro transcribed RNA standards designed for absolute viral load quantification.

Samples were extracted by RTA Viral RNA Isolation Kit according to RTA Viral RNA Isolation Kit Handbook. Starting sample volumes were 150 µl and elution volumes were 50 µl. Then, PCR reactions were setup by PhoenixDx 2019-nCoV Kit Real Time PCR Kit according to PhoenixDx 2019-nCoV Kit Handbook. BIO-RAD CFX96-IVD Real-Time PCR Detection System was used for amplification, detection and analysis. Amplification Ct values of the study are provided in table 1 below. PhoenixDx 2019-nCoV Kit do not show any cross-reactivity with other potential cross-reactive markers at the tested concentration for the organisms listed in the table 1.

Table 9: Potential cross-reactive markers tested in the study.

Sample	Source	Sample ID	Replicates Detected/Total	Result
Human Adenovirus	NIBSC	16/324	0/3	Negative
Parainfluenza virus	ATCC	VR-93	0/3	Negative
Influenza A	ATCC	VR-95	0/3	Negative
Influenza A H5N1	ATCC	VR-1609	0/3	Negative
Influenza A H1N1	ATCC	VR-1672	0/3	Negative
Influenza A H3N2	ATCC	VR-822	0/3	Negative
Influenza A H7N7	ATCC	VR-1641	0/3	Negative
Influenza B	ATCC	VR-101	0/3	Negative
Parainfluenza 1	ATCC	VR-94	0/3	Negative
Parainfluenza 2	ATCC	VR-92	0/3	Negative
Parainfluenza 3	ATCC	VR-93	0/3	Negative
Parainfluenza 4	ATCC	VR-579	0/3	Negative
Human Metapneumovirus (hMPV)	ATCC	VR-3250SD	0/3	Negative
Human Enterovirus V71	ATCC	VR-1432	0/3	Negative
Human respiratory syncytial virus	ATCC	VR-154	0/3	Negative
Human Coronavirus NL63	ATCC	VR-3263SD	0/3	Negative
Human Coronavirus HKU1	ATCC	VR-3262SD	0/3	Negative
Human Coronavirus 229E	ATCC	VR-740	0/3	Negative
Betacoronavirus 1 OC43	ATCC	VR-1558D	0/3	Negative
MERS Coronavirus	ATCC	VR-3248SD	0/3	Negative
TPC			0/3	20,91
NTC			0/3	Negative

d. In Silico Analysis:

BLAST analysis showed no homology with primers and probes of the PhoenixDx 2019-nCoV Kit for the organisms listed in the table below.

The in-silico analysis for possible cross-reactions with all the organisms listed in Table 2 was conducted by mapping primers in PhoenixDx 2019-nCoV Real Time PCR Kit individually to the sequences downloaded from NCBI database. No potential cross reactivity was observed with analyzed pathogens.

Table 10: In Silico Analysis for Primers and Probes EXAMPLE BLAST Results

Pathogen	Strain	GenBank Accession #	% Homology Forward E Primer	% Homology Reverse E Primer	% Homology E Probe	% Homology Forward RdRP Primer	% Homology Reverse RdRP Primer	% Homology RdRP Probe
SARS-CoV-2	Wuhan seafood market pneumonia virus isolate Wuhan-Hu-1, complete genome	NC_045512.2	100%	100%	100%	100%	100%	100%
Human coronavirus 229E	Human coronavirus 229E strain 229E/human/USA/932-72/1993, complete genome	KF514432.1	<50%	<50%	<50%	<50%	<50%	<50%
	Human coronavirus 229E strain 229E/human/USA/933-40/1993, complete genome	KF514433.1	<50%	<50%	<50%	<50%	<50%	<50%
Human coronavirus OC43	Human coronavirus OC43 strain OC43/human/USA/971-5/1997, complete genome	KF530099.1	<50%	<50%	<50%	<50%	<50%	<50%
	Human coronavirus OC43 isolate LRTI_238, complete genome	KX344031.1	<50%	<50%	<50%	<50%	<50%	<50%
Human coronavirus HKU1	Human coronavirus HKU1 strain HKU1/human/USA/HKU1-18/2010, complete genome	KF430201.1	<50%	<50%	<50%	<50%	<50%	<50%
	Human coronavirus HKU1 isolate SI17244, complete genome	MH940245.1	<50%	<50%	<50%	<50%	<50%	<50%
Human coronavirus NL63	Human coronavirus NL63 strain NL63/human/USA/905-25/1990, complete genome	KF530113.1	<50%	<50%	<50%	<50%	<50%	<50%
	Human coronavirus NL63 strain NL63/human/USA/891-4/1989, complete genome	KF530114.1	<50%	<50%	<50%	<50%	<50%	<50%
SARS-coronavirus	SARS coronavirus CUHK-AG01, complete genome	AY345986.1	100%	100%	100%	100%	100%	52%
	SARS coronavirus A022, complete genome	AY686863.1	100%	100%	100%	100%	100%	52%
MERS-Coronavirus	Middle East respiratory syndrome-related coronavirus strain HCoV-EMC, complete genome	MH013216.1	<50%	<50%	<50%	<50%	78%	<50%

Adenovirus	Human adenovirus type 1, complete genome	AC_000017.1	<50%	<50%	<50%	<50%	<50%	<50%
Human Metapneumovirus (hMPV)	Human metapneumovirus strain HMPV/Homo sapiens/PER/FPP00726/2011/A, complete genome	KJ627437.1	<50%	<50%	<50%	<50%	<50%	<50%
Parainfluenza 1	Human parainfluenza virus 1 isolate NM001, complete genome	KX639498.1	<50%	<50%	<50%	<50%	<50%	<50%
Parainfluenza 2	Human parainfluenza virus 2 isolate VIROAF10, complete genome	KM190939.1	<50%	<50%	<50%	<50%	<50%	<50%
Parainfluenza 3	Human parainfluenza virus 3 strain HPIV3/AUS/3/2007, complete genome	KF530243.1	<50%	<50%	<50%	<50%	<50%	<50%
Parainfluenza 4	Human parainfluenza virus 4a isolate HPIV4_DK(459), complete genome	KF483663.1	<50%	<50%	<50%	<50%	<50%	<50%
Influenza A	Influenza A virus (A/New York/PV305/2017(H1N1)) segment 2 polymerase PB1 (PB1) gene, complete cds; and nonfunctional PB1-F2 protein (PB1-F2) gene, complete sequence	MH798556.1	<50%	<50%	<50%	<50%	<50%	<50%
Influenza B	Influenza B virus (B/Nicaragua/8689_13/2017) segment 2 polymerase PB2 (PB2) gene, complete cds	MK969560.1	<50%	<50%	<50%	<50%	<50%	<50%
Enterovirus	Human enterovirus 68 isolate EV68_NL_201013421 VP1 protein gene, partial cds	JF896312.1	<50%	<50%	<50%	<50%	<50%	<50%
Respiratory syncytial virus	Respiratory syncytial virus strain B/WI/629-Q0190/10, complete genome	JN032120.1	<50%	<50%	<50%	<50%	<50%	<50%
Rhinovirus	Human rhinovirus 14, complete genome	NC_001490.1	<50%	<50%	<50%	<50%	<50%	<50%
Chlamydia pneumoniae	Chlamydia pneumoniae genome assembly PB2, chromosome : 1	NZ_LN847241.1	<50%	77%	50%	<50%	<50%	52%
Haemophilus influenzae	Haemophilus influenzae PittGG, complete genome	CP000672.1	<50%	59%	<50%	<50%	<50%	<50%
Legionella pneumophila	Legionella pneumophila strain Philadelphia_1_CDC, complete genome	CP015928.1	<50%	54%	50%	59%	50%	56%
Mycobacterium tuberculosis	Mycobacterium tuberculosis DNA, complete genome, strain: HN-506	AP018036.1	<50%	63%	50%	59%	<50%	<50%
Streptococcus pneumoniae	Streptococcus pneumoniae strain D39V chromosome, complete genome	CP027540.1	<50%	<50%	54%	<50%	50%	56%
Streptococcus pyogene	Streptococcus pyogenes MGAS8232, complete genome	AE009949.1	53%	59%	<50%	<50%	50%	64%
Bordetella pertussis	Bordetella pertussis strain B3921, complete	CP011448.1	<50%	63%	<50%	<50%	<50%	52%

	genome							
Mycoplasma pneumoniae	Mycoplasma pneumoniae strain 14-637 chromosome, complete genome	CP039772.1	<50%	54%	<50%	<50%	<50%	<50%
Pneumocystis jirovecii	Pneumocystis jirovecii isolate SW7_full mitochondrion, complete genome	MH010446.1	<50%	<50%	<50%	<50%	<50%	<50%
Candida albicans	Candida albicans strain L757 mitochondrion, complete genome	NC_018046.1	<50%	<50%	<50%	<50%	<50%	<50%
Pseudomonas aeruginosa	Pseudomonas aeruginosa UCBPP-PA14, complete genome	CP000438.1	50%	77%	<50%	59%	<50%	<50%
Staphylococcus epidermidis	Staphylococcus epidermidis strain SP3 16S ribosomal RNA gene, partial sequence	KY750253.1	<50%	<50%	<50%	<50%	<50%	<50%
Streptococcus salivarius	Streptococcus salivarius strain LAB813 chromosome, complete genome	CP040804.1	65%	54%	<50%	59%	50%	<50%

e. Endogenous Interference Substances Studies:

We tested potential endogenous interference substances which may interfere with PCR using the PhoenixDx 2019-nCoV. The substances were tested at the concentrations indicated in the table below. UTM was spiked with the substances indicated below. The sampled matrixes RNA was extracted using the RTA RNA Viral Isolation kit. The extracted RNA was tested in triplicates using the PhoenixDx 20190-nCoV.

In the table below, the results show that the PCR was not affected by the potential endogenous interfering substances.

Table 11: Interference Study

Potential Interfering Substance	Conc.	Positive Samples		Negative Samples
		Viral Strain Level	Results	Results
Blood (human)	2.5% v/v	2.5X LoD	3/3	0/3
Afrin Original nasal spray	15% v/v	2.5X LoD	3/3	0/3
Basic Care allergy relief nasal spray (Gluococorticoid)	5% v/v	2.5X LoD	3/3	0/3
GoodSense All Day Allergy, Cetirizine HCl Tablets 10 mg	1mg/mL	2.5X LoD	3/3	0/3
Cepacol Sore Throat (benzocaine/menthol lozenges)	5 mg/mL	2.5X LoD	3/3	0/3

9.2) CLINICAL PERFORMANCE EVALUATION

Clinical Nasal, nasopharyngeal, oropharyngeal and BAL specimens were obtained from an Austrian government laboratory that characterized the samples a negative for SARS-CoV-2 by an FDA authorized SARS-CoV-2 test. They were collected from patients with signs and symptoms of an upper respiratory infectionand by qualified personnel according to the package insert of the collection device the Copan swabs and Copan UTM. Specimens were handled as described in the package insert of the collection device and were stored frozen until use. Samples were tested to be negative also

for common upper respiratory tract infections. The following samples were obtained: 30 oropharyngeal, 10 nasal, 30 nasopharyngeal swabs and 30 bronchoalveolar lavage (BAL) specimens. Aliquots of the samples were extracted and tested in a blinded manner together with the positive spiked samples described below and according to the PhoenixDx 2019-nCoV Instructions for Use using the BIO-RAD CFX96-IVD Real-Time PCR Detection System for amplification, detection and analysis.

A second aliquots of the negative samples described above was tested in a contrived clinical study. Positive samples were generated by spiking the negative aliquots of the 30 NP swabs and 30 BALs with a quantified clinical specimen positive for SARS-CoV-2 (see LoD above) at 1.5X LOD (20 samples) and 2X LOD (5 samples) and 80X LOD (5 samples) SARS-CoV-2 RNA. Positive specimens were tested in a blinded manner with the negative specimen.

The negative percent agreement was calculated based on the result obtained from the prior testing at the government laboratory. None of the 100 SARS-CoV-2 negative clinical specimens gave positive test result for SARS-CoV-2. Diagnostic specificity of PhoenixDx 2019-nCoV is 100 % (see combined performance tables below).

The positive percent agreement was calculated based on the agreement of the PhoenixDx 2019-nCoV result with the expected spiked results in NP swabs and BALs are shown below.

Table 12: Clinical Performance of the PhoenixDx 2019-nCoV Kit against the expected results (spiking status) in NP swab specimens

Sample Concentration	**n**	**Target 1 (E Gene)**		**Target 2 (RdRP Gene)**		**RNase P**	
		% positive (two-sided 95% CI)	Mean Ct	% positive (two-sided 95% CI)	Mean Ct	% positive	Mean Ct
NASOPHARYNGEAL-SWABS							
150 c/mL 1.5X LoD	20	100 (80.6 – 99.9)	33.8	100 (80.6 – 99.9)	34.3	100 (80.6 – 99.9)	26.1
200 c/mL 2X LoD	5	100 (65.8 – 99.9)	33.9	100 (65.8 – 99.9)	34.2	100 (65.8 – 99.9)	26.1
8000c/mL 80X LoD	5	100 (65.8 – 99.9)	25.0	100 (65.8 – 99.9)	25.2	100 (65.8 – 99.9)	25.5
Negative	30	0 (n/a)	N/A	0 (n/a)	N/A	100 (88.7 - 100)	25.4
Positive Percent Agreement: 30/30 = 100% (95% CI: 88.7% - 100%) **Negative Percent Agreement: 10/10 = 100% (95% CI: 72.1% - 100%)**							

NASAL-SWABS							
Negative	10	0 (n/a)	N/A	0 (n/a)	N/A	100 (72.1 – 99.9)	26.5
OROPHARYNGEAL SWABS							
Negative	30	0 (n/a)	N/A	0 (n/a)	N/A	100 (88.7 - 100)	25.7
Negative Percent Agreement (Nasal Swabs): 10/10 = 100% (95% CI: 72.1% - 100%) **Negative Percent Agreement (Oropharyngeal Swabs): 10/10 = 100% (95% CI: 72.1% - 100%)**							
BAL							
150 c/mL **1.5X LoD**	20	100 (80.6 – 99.9)	33.2	100 (80.6 – 99.9)	33.8	100 (80.6 – 99.9)	25.8
200 c/mL **2X LoD**	5	100 (65.8 – 99.9)	33.4	100 (65.8 – 99.9)	33.7	100 (65.8 – 99.9)	25.9
8000 c/mL **80X LoD**	5	100 (65.8 – 99.9)	24.4	100 (65.8 – 99.9)	24.7	100 (65.8 – 99.9)	26.0
Negative	30	0 (n/a)	N/A	0 (n/a)	N/A	100 (88.7 - 100)	26.2
Positive Percent Agreement: 30/30 = 100% (95% CI: 88.7% - 100%) **Negative Percent Agreement: 30/30 = 100% (95% CI: 88.7% - 100%)**							

10) SYMBOL DEFINITION (MANUAL & PACKAGING)

Rx Only	*Prescription Only*
IVD	*In vitro diagnostic*
Σ	Contains sufficient for <n> tests
REF	Catalogue Number
	Manufacturer
LOT	Batch Code
	Temperature Limit
	Use-by Date
	Consult instructions for use

11) ORDERING INFORMATION / TECHNICAL ASSISTANCE

For ordering information, contact Trax Management Services Inc:

Trax Management Services Inc
2900 Atoll Dr.
Lewis Center, OH 43035
Tel: +1 833 KIT TEST (+1 833 548 8378)
info@traxconnects.com

For questions or technical support, contact Procomcure Biotech:

Procomcure Biotech GmbH
Breitwies 1
5303 Thalgau, Austria
Tel: +43 6229 39608
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