PhoenixDx® ***SARS-CoV-2 Multiplex***

for invitro diagnostic use

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

INSTRUCTIONS FOR USE

For Use under Emergency Use Authorization

For *In Vitro* Diagnostic Use
Rx Only

50 Tests

REF **PCCSKU15262**

v 2.0

Rx Only

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NOTICE TO PURCHASER

The purchase of this product includes a limited, non-transferable immunity from suit under patent claims for using only this amount of product solely in performance of diagnostic services for human in vitro diagnostics, including reporting results of purchaser's activities for a fee or other commercial consideration, and also for the purchaser's own internal research. No right under any other patent claim is conveyed expressly, by implication, or by estoppel. This product is for laboratory use only.

LICENSE AGREEMENT FOR THE PHOENIXDX® MANUAL:

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:
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1) INTENDED USE

The PhoenixDx SARS-CoV-2 Multiplex is a real-time RT-PCR-based test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in upper respiratory specimens (such as nasal, mid-turbinate, 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, that meet the 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 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 SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The PhoenixDx SARS-CoV-2 Multiplex 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 SARS-CoV-2 Multiplex 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 SARS-CoV-2 Multiplex test is based on conventional RT-PCR technology including extraction and purification of the nucleic acid genome of SARS-CoV-2 from the patient sample followed by PCR amplification and detection. The test is run on Thermo Fisher QS1, Qiagen Rotor-Gene Q, Applied Biosystems ABI 7500 Fast Real time PCR Dx, DNA Technologie DTPrime5, and Analytik Jena qTower3G platforms. Nucleic acid from patient samples and controls are extracted in parallel using the RTA Viral Nucleic Acid Isolation Kit, Qiagen QIAamp MinElute Virus Spin kit, or Roche High Pure Viral RNA Kit, Thermo Fisher MagMax, or SphaeraMag DNA/RNA 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 conserved regions of the ORF1ab and N genes for SARS-CoV-2.

Selective amplification of RNA Internal Control 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 SARS-CoV-2 Multiplex master mix contains detection probes for the two SARS-CoV-2 targets and one for the internal RNase P. Probes are each labelled 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 01: Kit Content

Quantity and Volume	Component
1 x 50 µl	PhoenixDx® **RT Enzyme Mix**
1 x 750 µl	PhoenixDx® **SARS-COV-2 MULTIPLEX Mix**
1 x 200 µl	SARS-COV-2 MULTIPLEX **TPC**

2.3) Additional Materials Required but not Provided

RNA Extraction Kits:

The following nucleic acid extraction kits are validated for use with this test:

- RTA Viral RNA Extraction Kit
- RTA Laboratories catalogue number 09010100
- Qiagen QIAamp MinElute Virus Spin Kit Cat No./ID: 57704
- Roche High Pure Viral RNA Kit Cat. No. 11858882001
- Procomcure SphaeraMag DNA/RNA Isolation Kit Cat. No. PCCSKU16001
- Thermo Fisher MagMax Viral/Pathogen Nucleic Acid Isolation Kit (MVP I) cat. No. A48310

Instruments:

The following Realtime PCR instruments are validated for use with this test:

- BioRad CFX-96 IVD marked instrument
- Thermo Fisher QS1
- Qiagen Rotor-Gene Q
- Applied Biosystems ABI 7500 Fast Real time PCR Dx
- DNA Technologie DTPrime5
- Analytik Jena qTower3G

Materials:

- 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)
- Qiagen PCR Tubes, 0.2 ml Cat No./ID: 981008 or Strip Tubes and Caps, 0.1 ml Cat No./ID: 981103
- Thermofisher MicroAmp Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL, Cat. No. 4366932
- Thermofisher MicroAmp Optical Adhesive Film, Cat. No. 4311971
- 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
- Nuclease-free dH2O

2.4) Reagent Storage

- Store all kit components at -20°C and avoid repeated freeze and thaw cycles.
- Protect the PhoenixDx® SARS-CoV-2 Multiplex Mix 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

3.1) Specimens

Only the following validated specimens should be used for testing:

- Upper respiratory specimens such as nasal, mid-turbinate, nasopharyngeal and oropharyngeal swabs, and lower respiratory specimens such as bronchioalveolar lavage, tracheal aspirates and sputum
- 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 recommended as they may contain substances that inactivate some viruses and inhibit PCR testing and should only be used if dacron or rayon swabs are not available.

3.2) Specimens - Handling and Storage

- Specimens can be stored at 4°C for up to 72 hours after collection.
- If a delay in extraction is expected, store specimens at -70°C or lower.
- Do not freeze and thaw samples more than once before testing them.
- Extracted nucleic acids should be stored at -70°C or lower and (if re-testing is expected) stored in aliquots. Avoid multiple freeze thaw cycles for samples and extracted RNA.
- Clinical specimens must be considered potentially infectious and treated accordingly.
- Do not use specimens if:

- they were not kept at the indicated storage condition (i.e., 2-4° C (⩽ 4 days) or frozen at -70° C or below).
- they are insufficiently labelled or lack documentation.

⚠ Do not vortex specimens as this will fragment the RNA and lead to failure of the **PHOENIXDX® SARS-COV-2 MULTIPLEX** assays.

4) WARNINGS

4.1) GENERAL

- For in vitro diagnostic use
- For Prescription Use Only (Rx only)
- For use under an Emergency Use Authorization (EUA) only
- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet the requirements to perform high complexity tests.
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

4.2) BIOSAFETY

- Wear appropriate personal protective equipment (e.g. gowns, powder-free gloves, eye protection) when working with clinical specimens.
- Specimen processing should be performed in a certified class II biological safety cabinet following biosafety level 2 or higher guidelines.
- For more information on Sample Collection and Handling, refer to:
- Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (SARS-COV-2) https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html
- Biosafety in Microbiological and Biomedical Laboratories 5th edition available at http://www.cdc.gov/biosafety/publications/.
- The use of PhoenixDx® SARS-COV-2 MULTIPLEX and data evaluation is restricted to trained laboratory personnel only.
- Good laboratory practice is essential for optimal performance of this assay. Special care must be taken avoid contamination of the components of the kit. All reagents must be closely monitored for impurities and contamination. Discard suspicious reagents according to local guidelines and regulations.
- 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.

4.3) Contamination Prevention

- 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 where positive control materials and samples are handled in separate areas from RT-PCR reagents.
- Sample extractions need to be performed in a separate area from RT-PCR Setup

5) Sample Preparation / Nucleic Acid Extraction

- The performance of RT-PCR assays strongly depends on the amount and quality of sample template RNA. The following extraction methods were validated for adequate performance with the PHOENIXDX® SARS-COV-2 MULTIPLEX using manual extraction and should be performed according to the manufacturer's instructions for use:
  - RTA Viral Nucleic Acid Isolation Kit
  - Sphaera Mag DNA/RNA Isolation kit
  - Qiagen QIAamp MinElute Virus Spin kit
  - Thermo Fischer MagMax
  - Roche High Pure Viral RNA Kit
- For frozen samples or frozen extracted RNA, only thaw the number of specimen extracts that will be tested in a single day.
- Do not freeze/thaw extracted RNA more than once before testing as each freeze/thaw cycle can decrease the RNA quality. For optimal results, use directly.

6) Setting up the RT-PCR Reaction:

6.1) Prepare Reagents

- 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 SARS-CoV-2 Multiplex 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.
- The TPC is supplied in a formulation ready to use without prior dilution
- Prepare enough master mix for all planned reactions (n) according to the sample size of your batch/run, including one negative control and one TPC positive control for each Mastermix. It is recommended to prepare master mix for 2 additional reactions to account for pipetting inaccuracies. Find the total volume by multiplying the volumes per reaction (provided in Table 02 below) by n+2 ("n" being the number of total samples including controls). When calculating the pipetting volumes for each mastermix, please use the volume table below. For example, for 10 samples + 1 positive control + 1 negative control, the volumes should be multiplied by 14 (12 samples/control +2 additional). Aliquot 16 µl of the mastermix into separate wells and add 4 µl of sample /negative control onto the mastermix.

Table 02: Reagent Volumes per Reaction

COMPONENT	VOLUME
PhoenixDx® RT Enzyme Mix	1 µl
PhoenixDx® SARS-COV-2 MULTIPLEX Mix	15 µl
isolated sample RNA / TPC / NTC	4 µl / 4 µl / 4 µl dH_2O

6.2) SETTING UP THE PCR PLATE

1) Distribute 16 µL of the master mix to each well of your PCR plate.
2) Transfer the Master mix 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.
3) Add 4 µl of the TPC to the corresponding reaction well and continue with the potentially positive sample material.
4) Add 4 µl of nuclease-free water as the NTC control to the corresponding reaction well and seal the plate. Keep reactions on ice until transferring them to the PCR device.

Example pipetting scheme for the distribution of master. Please see below:

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

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

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

5) Transfer the reactions to the PCR device, then cycle according to these guidelines:

Table: 03: PCR Program

STEP	CYCLES	TEMPERATURE	DURATION
Reverse Transcription	1	50°C	5 minutes
Initial Denaturation	1	95°C	5 minutes
Amplification	40	95°C	5 seconds
		60°C[1]	30 seconds

[1]**NOTE:** Enable Data Collection for FAM™ (for virus detection) and HEX/VIC (for Internal control; IC). When using low-ROX or high-ROX cyclers set Passive Reference to ROX, for cyclers without ROX normalization, continue with default baseline normalization.

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.

7) QUALITY CONTROL

Each run of samples must include the following Quality Control materials:

- **NTC (no template control):** A "no template" (negative) control (NTC) is included in each run and taken through the full sample processing procedure starting with extraction. It is needed to monitor for potential contamination of reagents or samples with nucleic acid containing the target sequence and it should be added once for each PCR run. The NTC consists of molecular grade dH2O.

- **Internal Control (IC):** An internal control is needed to monitor for correct processing of each sample, adequate amount and quality of amplifiable material in the sample, and the presence of inhibitory substances in the sample. The internal control consists of the endogenous human RNAse P gene, which is extracted together with the viral RNA from each patient specimen.

- **TPC**: A positive template control (TPC) is needed to control functionality of all RT-PCR enzymes and reagents in the test kit. The positive control consists of synthetic DNA plasmid for N and ORF1ab genes as well as RNase P. The positive control only controls for the RT-PCR reaction but not for the extraction.

8) RESULT INTERPRETATION

8.1) Examination and Interpretation of Quality Controls:

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 as well as for the SARS-CoV-2 targets (N and ORF1ab) to be called positive is a Ct of ≤35.

Table 04: Expected Performance of PhoenixDx SARS-CoV-2 Multiplex Test Controls

Control Type	External Control Name	Virus (N Gene & ORF1 ab)	IC (RNase P)	Expected Ct values
Positive Control	TPC	+	+	≤35 Ct
Negative Control	NTC	-	-	≥35 Ct

- NTC (dH2O controls) must not give a Ct value for any assay. If they do, the reaction was contaminated with amplifiable RNA or DNA templates. Decontaminate equipment and workspace and repeat the reactions. Also, check for device-derived artifacts or falsely placed threshold. If a contamination persists, use fresh reagents.

- For the TPC a positive Ct in the FAM™ channel (for SARS-CoV-2) and in the HEX Channel (for RNase P) must be observed. The Ct value for the TPC should be < 35 cycles. The IC

must not give a signal when using the TPC. If the Ct value does not correspond to the expected value or not all assays are tested positive, PCR was compromised. Check the reaction setup and PCR device settings and repeat the reactions. Repeated freeze and thaw cycles of the TPC can compromise its quality resulting in late Ct values.

If any of the above controls do 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.

8.2) Examination and Interpretation of Patient Specimen Results:

A sample result is invalid if the detection of RNase P (HEX/VIC channel) in the sample fails and the sample also fails to show amplification of SARS-CoV-2 targets (N / ORF1ab in the FAM channel) within ≤35 Ct. Invalid results cannot be interpreted. Check reaction setup and device settings and repeat the RNA extraction if necessary. These samples should be repeated from the extraction step.
Note: Failure to amplify the negative human extraction control may indicate inadequate RNA extraction or loss of RNA isolate due to RNAse contamination. Late Ct values for the IC may indicate a low RNA quality / amount in the extract.

- For a sample to be considered positive for SARS-CoV-2, the SARS-CoV-2 targets (FAM™ channel) must give a positive Ct value. Amplification of the IC in the HEX/VIC channel is expected around Ct 22-29. The IC may fail to amplify in the presence of high virus titers. Therefore, a sample with positive amplification of SARS-CoV-2 is positive even in the absence of RNase P amplification (IC).

- For a sample to be considered negative for SARS-COV-2, the SARS-CoV-2 assays in the FAM™ channel must not give a positive Ct value. The IC must give a positive Ct value in the HEX/VIC channel (Ct 22-29) for these samples to ensure that sample material of suitable quality was present.

Table 05: Interpretation of sample results with PhoenixDx® SARS-CoV-2 Multiplex

SARS-CoV-2	IC	INTERPRETATION
+	+	**SARS-CoV-2** target sequences were detected. The sample is considered positive for SARS-CoV-2 in the presence of IC amplification. Signal for SARS-CoV-2 and RP is expected for the TPC reaction.
+	-	**SARS-CoV-2** target sequences were detected. The sample is considered positive for SARS-CoV-2 in the absence of IC amplification. Signal for SARS-CoV-2 and RP is expected for the TPC reaction.
/	+	Only the target sequence for the **IC** was amplified. The sample is considered negative for SARS-CoV-2.
/	/	Sample result is invalid*. Sample needs to be retested. If the re-test result is also invalid, it is then recommended to do a recollection of a new sample from the patient to perform the test again using the RT-PCR method.

*invalid sample results can be generated due to insufficient amounts or quality of sample RNA or due to inhibitory substances within the sample or RNA extract. Sample retesting can be attempted from the RT-PCR only, however, re-extraction of the sample is needed if the RT-PCR is repeat invalid. Re-extraction can be attempted if left over sample material if adequately stored. If no additional material is available or if the re-extracted sample is still invalid a new sample needs to be requested.

9) 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
- This test has been validated with samples from patients suspected of COVID-19 by their healthcare provider.
- 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.
- When processing samples with insufficient volume, samples can be diluted with nuclease-free water to enable processing but will affect the sensitivity negatively.
- 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.

10) CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

The PhoenixDx SARS-CoV-2 Multiplex Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labelling are available on the FDA website: https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization.

However, to assist clinical laboratories using the PhoenixDx SARS-CoV-2 Multiplex (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.

11) NON-CLINICAL PERFORMANCE EVALUATION

11.1) LIMIT OF DETECTION (LOD) - ANALYTICAL SENSITIVITY:

The LoD study established the lowest concentration of SARS-CoV-2 (genome copies(cp)/µl) that can be detected by the PhoenixDx SARS-CoV-2 Multiplex test at least 95% of the time. Negative patient (Nasopharyngeal Swab) samples were spiked with viral genomic RNA (Twist Bioscience, Cat #102019) at a concentration of 5 000 cp/µl; 500 cp/µl; 50 cp/µl; 5 cp/µl; 0.5 cp/µl and 0.05 cp/µl. Patient material was screened negative using PhoenixDx SARS-CoV-2 Kit. The samples were extracted using the different Isolation kits and analyzed with Applied Bioscience 7500 Fast Instrument (Software: Applied Biosystems 7500 Software v2.3). For each isolation kit, the tentative LoD and confirmatory LoD was determined. Every sample was run through the extraction process. Replicas were generated by spiking multiple patient samples with RNA and full extraction of all replicas. The results are summarized below. Tentative LoD was determined to be at 50 cp/µl with all extraction kits. As real patient material was used for the spiking human RNA can be found in all target levels and RNase P gives a positive result in all samples.

Table 06: Tentative LoD study with different extraction methods on the ABI7500 Fast

Target Level	Valid results	N-gene ORF1ab-gene			RNase P		
		N positive	Mean Ct	Detection Rate	N positive	Mean Ct	Detection Rate
SphaeraMag DNA/RNA Isolation kit							
5 000 cp/µL	3	3	26.77	100%	3	23.93	100%
500 cp/µL	3	3	30.60	100%	3	24.87	100%
50 cp/µL	3	3	32.63	100%	3	24.40	100%
5 cp/µL	3	2	34.02	66.6%	3	24.23	100%
0.5 cp/µL	3	0	NA	0%	3	24.47	100%
0.05 cp/µL	3	0	NA	0%	3	25.20	100%
Negative	3	0	NA	0%	3	23.80	100%

Target Level	Valid results	N-gene ORF1ab-gene			RNase P		
		N positive	Mean Ct	Detection Rate	N positive	Mean Ct	Detection Rate
RTA Viral Isolation Kit							
5 000 cp/µL	3	3	26.78	100%	3	25.97	100%
500 cp/µL	3	3	29.67	100%	3	25.33	100%
50 cp/µL	3	3	33.48	100%	3	25.47	100%
5 cp/µL	3	0	>35	0%	3	24.80	100%
0.5 cp/µL	3	0	NA	0%	3	23.37	100%
0.05 cp/µL	3	0	NA	0%	3	23.53	100%
Negative	3	0	NA	0%	3	24.20	100%
Target Level	**Valid results**	**N-gene ORF1ab-gene**			**RNase P**		
		N positive	Mean Ct	Detection Rate	N positive	Mean Ct	Detection Rate
Roche High Pure Viral RNA Kit							
5 000 cp/µL	3	3	26.41	100%	3	25.20	100%
500 cp/µL	3	3	29.76	100%	3	24.30	100%
50 cp/µL	3	3	33.16	100%	3	24.23	100%
5 cp/µL	3	1	34.36	33.3%	3	24.50	100%
0.5 cp/µL	3	0	NA	0%	3	24.23	100%
0.05 cp/µL	3	0	NA	0%	3	25.53	100%
Negative	3	0	NA	0%	3	23.50	100%
Target Level	**Valid results**	**N-gene ORF1ab-gene**			**RNase P**		
		N positive	Mean Ct	Detection Rate	N positive	Mean Ct	Detection Rate
Qiagen QIAamp MinElute Virus Spin kit							
5 000 cp/µL	3	3	25.73	100%	3	24.11	100%
500 cp/µL	3	3	29.53	100%	3	24.34	100%
50 cp/µL	3	3	32.41	100%	3	24.00	100%
5 cp/µL	3	0	>35	0%	3	24.34	100%
0.5 cp/µL	3	0	NA	0%	3	23.25	100%
0.05 cp/µL	3	0	NA	0%	3	24.50	100%
Negative	3	0	NA	0%	3	23.69	100%
Target Level	**Valid results**	**N-gene ORF1ab-gene**			**RNase P**		
		N positive	Mean Ct	Detection Rate	N positive	Mean Ct	Detection Rate
Thermo Fischer MagMax							
5 000 cp/µL	3	3	26.17	100%	3	24.04	100%
500 cp/µL	3	3	30.12	100%	3	24.80	100%
50 cp/µL	3	3	33.08	100%	3	24.76	100%
5 cp/µL	3	0	>35	0%	3	23.90	100%
0.5 cp/µL	3	0	NA	0%	3	25.44	100%
0.05 cp/µL	3	0	NA	0%	3	23.65	100%
Negative	3	0	NA	0%	3	23.33	100%

Target Level	Valid results	N-gene ORF1ab-gene			RNase P		
		N positive	Mean Ct	Detection Rate	N positive	Mean Ct	Detection Rate
GE Healthcare Sera-Xtracta Virus/Pathogen Kit							
5 000 cp/µL	3	3	26.40	100%	3	24.03	100%
500 cp/µL	3	3	29.18	100%	3	24.34	100%
50 cp/µL	3	3	32.99	100%	3	24.76	100%
5 cp/µL	3	0	>35	0%	3	24.25	100%
0.5 cp/µL	3	0	NA	0%	3	24.82	100%
0.05 cp/µL	3	0	NA	0%	3	25.75	100%
Negative	3	0	NA	0%	3	25.38	100%

The tentative LoD was determined with all extraction kits to be 50 cp/µl. The confirmatory LoD Study was performed in the same manner as the tentative LoD study above at the previously determined tentative LoD of 50 copies/mL as well as at 25 copies/mL. The results can be found in the Table below. The LoD was determined with all extraction kits to be 50 cp/µl.

Table 07: Confirmatory LoD study with different extraction methods on the ABI7500 Fast

Target Level	Valid results	N-gene ORF1ab-gene			RNase P		
		N positive	Mean Ct	Detection Rate	N positive	Mean Ct	Detection Rate
SphaeraMag DNA/RNA Isolation kit							
50 cp/µL	20	20	32.15	100%	20	26.08	100%
25 cp/µL	20	9	34.39	45%	20	26.19	100%
Target Level	**Valid results**	**N-gene ORF1ab-gene**			**RNase P**		
		N positive	Mean Ct	Detection Rate	N positive	Mean Ct	Detection Rate
RTA Viral Isolation Kit							
50 cp/µL	20	19	32.66	95%	20	24.43	100%
25 cp/µL	20	8	33.96	40%	20	24.60	100%
Target Level	**Valid results**	**N-gene ORF1ab-gene**			**RNase P**		
		N positive	Mean Ct	Detection Rate	N positive	Mean Ct	Detection Rate
Qiagen QIAamp MinElute Virus Spin kit							
50 cp/µL	20	19	32.50	95%	20	23.93	100%
25 cp/µL	20	6	34.42	30%	20	24.24	100%

Target Level	Valid results	N-gene ORF1ab-gene			RNase P		
		N positive	Mean Ct	Detection Rate	N positive	Mean Ct	Detection Rate
Roche High Pure Viral RNA Kit							
50 cp/µL	20	19	32.72	95%	20	24.45	100%
25 cp/µL	20	8	33.89	40%	20	24.50	100%
Target Level	**Valid results**	**N-gene ORF1ab-gene**			**RNase P**		
		N positive	Mean Ct	Detection Rate	N positive	Mean Ct	Detection Rate
Thermo Fischer MagMax							
50 cp/µL	20	20	32.77	100%	20	24.13	100%
25 cp/µL	20	8	33.33	40%	20	24.40	100%
Target Level	**Valid results**	**N-gene ORF1ab-gene**			**RNase P**		
		N positive	Mean Ct	Detection Rate	N positive	Mean Ct	Detection Rate
GE Healthcare Sera-Xtracta Virus/Pathogen Kit							
50 cp/µL	20	20	32.95	95%	20	24.17	100%
25 cp/µL	20	10	33.56	50%	20	24.53	100%

The LoD was determined with all extraction kits on the ABI 7500 Fast to be 50 cp/µl.

As all RNA extraction methods show the same LoD (50cp/µl) SphaeraMag DNA/RNA Isolation kit was chosen to validate the different rt-PCR instruments shown below because it has the largest elution volume (worst case scenario). The tentative LoD for the different instruments was determined with a narrower range finding based on the previously established LoD for the ABI 7500 Fast Instrument with the SphaeraMag DNA/RNA isolation kit using 0.5 x LoD; 1 x LoD; 1.5 x LoD and 2 x LoD. Each replicate was individually extracted and tested. The results are shown below.

Table 08: Tentative LoD study with different Instruments using the extraction SphaeraMag DNA/RNA isolation kit

Target Level	Valid results	N-gene ORF1ab-gene			RNase P		
		N positive	Mean Ct	Detection Rate	N positive	Mean Ct	Detection Rate
DNA Technologie DTPrime5							
2 x LoD (100 cp/µL)	5	5	28.76	100%	5	24.58	100%
1,5 x LoD (75 cp/µL)	5	5	30.49	100%	5	24.59	100%
1 x LoD (50 cp/µL)	5	5	31.55	100%	5	23.56	100%
0,5 x LoD (25 cp/µL)	5	3	34.01	60 %	5	24.38	100%
Negative	5	0	NA	0%	5	23.77	100%

Target Level	Valid results	N-gene ORF1ab-gene			RNase P		
		N positive	Mean Ct	Detection Rate	N positive	Mean Ct	Detection Rate
Thermo Fisher QS1							
2 x LoD (100 cp/µL)	5	5	29.45	100%	5	24.45	100%
1,5 x LoD (75 cp/µL)	5	5	30.62	100%	5	23.81	100%
1 x LoD (50 cp/µL)	5	5	32.03	100%	5	24.57	100%
0,5 x LoD (25 cp/µL)	5	3	32.80	60%	5	23.83	100%
Negative	5	0	NA	0%	5	23.89	100%
Target Level	**Valid results**	**N-gene ORF1ab-gene**			**RNase P**		
		N positive	Mean Ct	Detection Rate	N positive	Mean Ct	Detection Rate
Analytik Jena qTower3G							
2 x LoD (100 cp/µL)	5	5	28.02	100%	5	24.42	100%
1,5 x LoD (75 cp/µL)	5	5	30.63	100%	5	24.00	100%
1 x LoD (50 cp/µL)	5	5	32.36	100%	5	23.79	100%
0,5 x LoD (25 cp/µL)	5	2	34.04	40%	5	24.11	100%
Negative	5	0	NA	0%	5	23.58	100%
Target Level	**Valid results**	**N-gene ORF1ab-gene**			**RNase P**		
		N positive	Mean Ct	Detection Rate	N positive	Mean Ct	Detection Rate
Qiagen Rotor-Gene Q							
2 x LoD (100 cp/µL)	5	5	29.50	100%	5	24.47	100%
1,5 x LoD (75 cp/µL)	5	5	31.28	100%	5	25.02	100%
1 x LoD (50 cp/µL)	5	5	31.99	100%	5	24.72	100%
0,5 x LoD (25 cp/µL)	5	3	34.19	60%	5	24.69	100%
Negative	5	0	NA	0%	5	24.46	100%

The tentative LoD is the same for all instruments. A Confirmative LoD study was performed with 20 replicates at the LoD Level and 20 replicates at 0.5 x LoD Level. Results are summarized below.

Table 09: Confirmatory LoD study with different instruments using the extraction SphaeraMag DNA/RNA isolation kit

Target Level	Valid results	N-gene ORF1ab-gene			RNase P		
		N positive	Mean Ct	Detection Rate	N positive	Mean Ct	Detection Rate
DNA Technologie DTPrime5							
50 cp/µL	20	20	31.43	100%	20	26.11	100%
25 cp/µL	20	12	33.94	60%	20	26.51	100%
Target Level	**Valid results**	**N-gene ORF1ab-gene**			**RNase P**		
		N positive	Mean Ct	Detection Rate	N positive	Mean Ct	Detection Rate
Thermo Fisher QS1							
50 cp/µL	20	20	32.26	100%	20	26.76	100%
25 cp/µL	20	13	33.51	65%	20	26.83	100%
Target Level	**Valid results**	**N-gene ORF1ab-gene**			**RNase P**		
		N positive	Mean Ct	Detection Rate	N positive	Mean Ct	Detection Rate
Analytik Jena qTower3G							
50 cp/µL	20	20	30.61	100%	20	26.82	100%
25 cp/µL	20	8	33.74	40%	20	26.65	100%
Target Level	**Valid results**	**N-gene ORF1ab-gene**			**RNase P**		
		N positive	Mean Ct	Detection Rate	N positive	Mean Ct	Detection Rate
Qiagen Rotor-Gene Q							
50 cp/µL	20	20	30.05	100%	20	26.46	100%
25 cp/µL	20	12	33.58	60%	20	26.53	100%

11.2) ANALYTICAL SPECIFICITY –IN VITRO ANALYSIS

A) INCLUSIVITY (IN SILICO ANALYSIS)

To demonstrate the predicted inclusivity of the PhoenixDx SARS-CoV-2 Multiplex kit, the PhoenixDx SARS-CoV-2 primer and probe sequences were aligned with all publicly available nucleic acid sequences for SARS-CoV-2 in the GenBank database as of April 16, 2020 (N2) and as of June 2, 2020 (ORF1ab). All the alignments showed 100% identity of the PhoenixDx SARS-CoV-2 oligonucleotides to the available SARS-CoV-2 sequences with the exception of 1 nucleotide mismatch within the N2 probe in 2 deposited sequences (MT263458.1 and MT263435.1), 1 nucleotide mismatch within the N2 reverse primer in 1 deposited sequence (MT159720.1), 1 nucleotide mismatch within the ORF1ab probe sequence in 1 deposited

sequence (MT325588.1) and 1 nucleotide mismatch within the ORF1ab forward primer in 1 deposited sequence (MT451726.1).

The risk of a single mismatch resulting in a significant loss in reactivity, and false negative result, is low due to the design of the primers and probes with melting temperatures ≥60°C and run conditions of the assay with annealing temperature at 60°C to tolerate one to two mismatches.

B) Cross Reactivity (Wet Testing)

In this study, the specificity of the PhoenixDx SARS-CoV-2 Multiplex Kit was evaluated by testing the organisms listed in table below. 9 reference organism and 11 clinical specimens were tested in the absence of SARS-CoV-2 RNA. The potential cross-reactive organisms were tested at concentrations between $1x10^3$ – $1x10^5$ 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 SARS-CoV-2 Multiplex Kit Real Time PCR Kit according to PhoenixDx SARS-CoV-2 Multiplex Kit Handbook. BIO-RAD CFX96-IVD Real-Time PCR Detection System was used for amplification, detection and analysis. PhoenixDx SARS-CoV-2 Multiplex Kit does not show cross-reactivity with other potential cross-reactive organisms listed in the table at the tested concentration.

Table 10: Cross Reactivity (Wet Testing)

Sample	Source	Sample ID	Replicates Detected/Total	Result
Influenza A	NIBSC	16/324	0/3	Negative
Influenza A H5	ATCC	VR-93	0/3	Negative
Influenza A H1	ATCC	VR-95	0/3	Negative
Influenza A H3	ATCC	VR-1609	0/3	Negative
Influenza A H7	ATCC	VR-1672	0/3	Negative
Influenza B	ATCC	VR-822	0/3	Negative
Parainfluenza 1	ATCC	VR-1641	0/3	Negative
Parainfluenza 2	ATCC	VR-101	0/3	Negative
Parainfluenza 3	ATCC	VR-94	0/3	Negative
Parainfluenza 4	ATCC	VR-92	0/3	Negative
RSV	ATCC	VR-93	0/3	Negative
HRV	ATCC	VR-579	0/3	Negative
HMPV	ATCC	VR-3250SD	0/3	Negative
Human coronavirus NL63	ATCC	VR-1432	0/3	Negative
Human coronavirus HKU1	ATCC	VR-154	0/3	Negative

Human coronavirus 229E	ATCC	VR-3263SD	0/3	Negative
Human coronavirus OC43	ATCC	VR-3262SD	0/3	Negative
MERS	ATCC	VR-740	0/3	Negative
Streptococcus pneumoniae	ATCC	VR-1558D	0/3	Negative
Haemophilus influenzae	ATCC	VR-3248SD	0/3	Negative
TPC			3/3	20,91
NTC			0/3	Negative

C) Cross Reactivity (In Silico Analysis)

The in-silico analysis for possible cross-reactions with all the organisms listed in Table 11 was conducted by Primer-BLAST analysis of the PhoenixDx® SARS-CoV-2 primers against individual genome sequences from the GenBank database. The specificity was checked not only for the forward-reverse primer pair, but also for forward-forward as well as reverse-reverse primer pairs.

The primer pair specificity checking parameters were as follows: 1) PCR Template was NC_045512; 2) PCR product size was 70 (Min) and 200 (Max); 3) Primer melting temperatures were 57.0 (Min), 60.0 (Opt), 63(Max) and 3 (Tm difference); 4) The program searched the primers against the selected database and determined whether a primer pair can generate a PCR product on any targets in the database based on their matches to the targets and their orientations; 5) Search mode was Automatic; 6) Database was Custom, i.e. GenBank accession number from Table 1.

BLAST analysis showed no homology with primers and probes of the PhoenixDx SARS-CoV-2 Multiplex Kit for the organisms listed in the table below.

The in-silico analysis for possible cross-reactions with all the organisms listed in Table 11 was conducted by mapping primers in PhoenixDx SARS-CoV-2 Multiplex Real Time PCR Kit individually to the sequences downloaded from NCBI database. Based on the sequence homologies with the PhoenixDx SARS-CoV-2 Multiplex primer and probe set cross reactivity with the analyzed pathogens is not expected.

Table 11: In silico Cross Reactivity – Homology with PhoenixDx SARS-CoV-2 Multiplex Primer and Probe sets

Pathogen	Strain	GenBank Accession #	% Homolgy					
			Fwd N	Rev N	Probe N	Fwd ORF1ab	Rev ORF1ab	Probe ORF1ab
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	KF530114.1	<50%	<50%	<50%	<50%	<50%	<50%

Pathogen	Strain	GenBank Accession #	% Homolgy					
			Fwd N	Rev N	Probe N	Fwd ORF1ab	Rev ORF1ab	Probe ORF1ab
	NL63/human/USA/891-4/1989, complete genome							
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	MH798556.1	<50%	<50%	<50%	<50%	<50%	<50%

Pathogen	Strain	GenBank Accession #	% Homolgy					
			Fwd N	Rev N	Probe N	Fwd ORF1ab	Rev ORF1ab	Probe ORF1ab
	and non-functional PB1-F2 protein (PB1-F2) gene, complete sequence							
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,	AE009949.1	53%	59%	<50%	<50%	50%	64%

Pathogen	Strain	GenBank Accession #	% Homolgy					
			Fwd N	Rev N	Probe N	Fwd ORF1ab	Rev ORF1ab	Probe ORF1ab
	complete genome							
Bordetella pertussis	Bordetella pertussis strain B3921, complete genome	CP011448.1	<50%	63%	<50%	<50%	<50%	52%
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:

The potential endogenous interference substances which may interfere with PCR were tested using the PhoenixDx SARS-CoV-2 Multiplex. 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 SphaeraMag DNA/RNA Isolation Kit. The extracted RNA was tested in triplicates using the PhoenixDx SARS-CoV-2 Multiplex.

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

Table 12: Testing of Potentially Interfering Substances

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

12) Clinical Data

Clinical specimens were obtained from three government laboratories that characterized the samples for SARS-CoV-2 by the use of an EUA authorized SARS-CoV-2 kit. The specimens were collected from patients with signs and symptoms of an upper respiratory infection and by qualified personnel according to the package insert of the collection device (Copan swabs in 1mL Copan UTM.

The following samples were obtained: 12 oropharyngeal and 72 nasopharyngeal swabs. Samples were extracted with the Procomcure SphaeraMag DNA/RNA Isolation Kit and tested using the Applied Biosystems ABI 7500 Fast Real time PCR Dx in a blinded manner and according to the PhoenixDx SARS-CoV-2 Multiplex Instructions for Use.

Table 13: Clinical Performance of the PhoenixDx SARS-CoV-2 Multiplex

		EUA Authorized Comparator		Total
NP swab		**Positive**	**Negative**	
PhoenixDx SARS-CoV-2 Multiplex	**Positive**	34	0	34
	Negative	0	38	38
Total (NP/VTM)		**34**	**38**	**72**

		EUA Authorized Comparator		Total
OP swabs		**Positive**	**Negative**	
PhoenixDx SARS-CoV-2 Multiplex	**Positive**	8	0	8
	Negative	0	4	4
Total (OP/VTM)		**8**	**4**	**12**

The negative percent agreement was calculated based on the result obtained from the prior testing at the government laboratories using an EUA authorized SARS-CoV-2 RT-PCR test. None of the SARS-CoV-2 negative clinical specimens gave positive test results for SARS-CoV-2. Diagnostic specificity of PhoenixDx SARS-CoV-2 Multiplex is 100 % (see performance tables below).

The positive percent agreement was calculated based on the agreement of the PhoenixDx SARS-CoV-2 Multiplex result with the positive tested samples in NP swabs and OP swaps are shown below.

Table 14: Percent Agreement for Clinical Performance Data

	N	SARS-CoV-2 (N Gene – ORF1 ab)		RNAse P	
		% positive (2-sided 95% CI)	Mean Ct	% positive (2-sided 95% CI)	Mean Ct
Nasopharyngeal Swabs					
Positive Percent Agreement (PPA)	34	100 (89.9 – 100%)	22.9	100 (89.9 – 100%)	28.9
Negative Percent Agreement (NPA)	38	100% (90.8 – 100%)	N/A	100 (90.8 – 100%)	28.4
Oropharyngeal Swabs					
Positive Percent Agreement (PPA)	8	100 (67.6 – 100%)	23.0	100 (67.6 – 100%)	27.6
Negative Percent Agreement (NPA)	8	100% (51.0 – 100%)	N/A	100 (51.0 – 100%)	28.6

13) 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)
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For questions, performance reporting, or technical support, contact Procomcure Biotech:

Procomcure Biotech GmbH
Breitwies 1
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support@procomcure.com

14) Symbol Definition (Manual & Packaging)

	Contains sufficient for <n> tests
	Catalogue Number
	Manufacturer
	Batch Code
	Temperature Limit
	Use-by Date
	Consult instructions for use
	Prescription only use

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