Delphine PrecisQT™ COVID-19 Test Kit

One-Step RT-qPCR
DDX101: 46 Patient Tests – 100-20 µL Reaction Kit

For *in vitro* Diagnostic Use

For Prescription Use Only

Review of the validation of this test has not been completed by FDA. Review under the EUA program is pending. Distributed in accordance with the guidance on Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency, Section IV.C.2.

Instructions for Use

Delphine Diagnostics Inc.
Rutgers University EcoComplex
1200 Florence Columbus Rd, Suite 110
Bordentown, New Jersey 08505
Customer Contact:
https://delphinedx.com/contact-us/
Phone: 1-609-669-0510

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1. Intended Use

The Delphine PrecisQT™ COVID-19 Test Kit is used to assay a real-time reverse transcriptase polymerase chain reaction (RT-PCR) for the qualitative detection of SARS-CoV-2 RNA in nasopharyngeal/oropharyngeal swabs from patients suspected of having COVID-19 by a healthcare provider.

The Delphine PrecisQT™ COVID-19 Test Kit is intended for use only by qualified and trained clinical laboratory personnel who are proficient in laboratory techniques, including procedures such as RT-PCR and other complex diagnostic procedures. Testing is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meet the criteria to perform high complexity laboratory tests and procedures.

Results are for the identification of SARS-CoV-2 RNA that can be detectable in upper respiratory specimens, especially if the sample is collected from the individual during the acute phase of infection.

Positive results indicate the presence of SARS-CoV-2 RNA. Positive results do not exclude infection by other pathogens or microorganisms. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results cannot be solely used for treatment planning or other patient-related management decisions. Negative results must be combined with patient information including history, other diagnostics, and epidemiological information to access patient's status. Whenever possible, it is recommended that negative results for symptomatic patients be confirmed by testing of an alternative specimen type or a specimen obtained several days later from the same individual.

The Delphine test kit is not intended for use in a general, asymptomatic screening population. The Delphine test kit is not intended for use with pooled samples or samples obtained by home collection. The Delphine test kit is not intended for use at the Point of Care.

Review of the validation of Delphine PrecisQT™ COVID-19 Test Kit has not been completed by FDA. Review under the EUA program is pending. Distributed in accordance with the guidance on Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency, Section IV.C.2.

2. Summary and Explanation of the Test

The novel 2019 coronavirus, now referred to as SARS-CoV-2, is a beta coronavirus related to MERS-CoV and SARS-CoV and causes respiratory illness referred to as COVID-19 disease. On January 31, 2020, the US Health and Human Services Department declared a Public Health Emergency for the United States. Under this declaration the FDA identified categories of products that may be of assistance in addressing the emergency. The Delphine PrecisQT™ COVID-19 Test Kit is under review for Emergency Use Authorization in the Established Category of 2019-Novel Coronavirus Nucleic Acid Reagents.

The Delphine PrecisQT™ COVID-19 Test Kit is a real-time reverse transcription polymerase chain reaction (RT-PCR) test optimized to detect SARS-CoV-2 RNA in samples collected from patients who are suspected of COVID-19 by their health care provider or are otherwise required to be tested. The SARS-CoV-2 primer set is designed to detect the presence or absence of only SARS-CoV-2 RNA without interference from other common oral/nasal flora or infectious pathogens.

3. Principles of the Assay

The Delphine PrecisQT™ COVID-19 Test Kit can be applied to RNA extracted from nasopharyngeal or oropharyngeal samples collected on sterile swabs stored in viral transport media until tested. The RNA is extracted from patient samples using the QIAamp Viral RNA Mini kit in the QIAsymphony SP & AS Automated Total Nucleic Acid Extraction System. Extracted RNA is subjected to one-step RT-PCR using primers for the human GAPDH gene used as the internal control or primers targeting a highly-conserved region of the SARS-CoV-2 Spike protein (S) gene. Specific amplification is detected using SYBR Green binding to double-stranded products. Each assay plate run is validated through a no template control and a positive control containing synthesized SARS-CoV-2 templates spiked with synthesized human GAPDH gene. Each patient sample test is validated through limit-restricted assessment of the patient sample internal control prior to qualitative determination of a positive or negative test for SARS-CoV-2.

4. Kit Components

The Delphine PrecisQT™ COVID-19 Test Kit provides enough volume of each reagent to test 46 patient samples in a 96-well plate format including internal controls for each patient sample, a positive control, and a no template control.

Tube #	Component Name	Volume (µL)	Description
1	Master Mix	1020	SYBR Green RT-PCR mix
2	RT Enzyme Mix	21	One-Step Reverse Transcriptase mix
3	SARS-CoV-2 Primer Mix	170	Delphine patented forward and reverse primers, random hexamer primers, ROX reference dye
4	Internal Control Primer Mix	170	Human GAPDH forward and reverse primers, random hexamer primers, ROX reference dye
5	Positive Control	15	Synthetic SARS-CoV-2 and GAPDH RNA templates
6	Nuclease Free Water	200	Diluent

5. Materials Required but Not Provided

Item	Component Name	Manufacturer	Cat #
Non-Interchangeable Components			
1	QIAsymphony SP & AS Automated Total Nucleic Acid Extraction System	Qiagen	937036
2	QIAamp Viral RNA Mini kit	Qiagen	52904
3	Applied Biosystems QuantStudio 5 Dx, QuantStudio Dx	Thermo Fisher Scientific	A47326, A47327, 4480299
4	96-Well Optical Reaction Plates	Thermo Fisher Scientific	4483354
5	Optical Adhesive Cover	Thermo Fisher Scientific	4311971
Interchangeable Components			
6	Vortex		
7	Bench-top cold centrifuge		
8	Microcentrifuge		
9	Refrigerator		
10	Freezer (-20°C)		
11	P-10, P-20, P-200, P-1000 Pipettes		
12	P-10, P-20, P-200, P-1000 ART Plugged Tips		
13	10% sodium hypochlorite solution		
14	75% Ethanol		
15	Personal Protective Equipment (goggles, work clothes, hats, shoes, gloves, etc.)		
16	Microcentrifuge tube racks		
17	1.5 mL microcentrifuge tubes		

6. Storage & Handling Requirements

The Delphine PrecisQT™ COVID-19 Test Kit is shipped on dry ice and must be stored upright at -20°C upon arrival in its original packaging. If the kit's protective packaging is damaged upon receipt, contact Delphine Diagnostics Inc. using the contact information found on the cover of this document.

If all reagents are not used in a single test run, the kit may be returned to -20°C storage for use after a second thaw. If all reagents are not consumed after the second thaw they should be discarded.

An unopened Delphine PrecisQT™ COVID-19 Test Kit can be stored at -20°C for up to 6 months and should not be used past the expiration date indicated on the packaging.

7. Warnings and Precautions

- For in vitro diagnostic use. The Delphine PrecisQT™ COVID-19 Test Kit has been validated but FDA's independent review of this validation is pending.

- This test has not been FDA cleared or approved; the test is under review by FDA under an Emergency Use Authorization (EUA) request for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
- This test is only available for the duration of the declaration that circumstances exist justifying the authorization of emergency use, if granted, 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.
- Positive results are indicative of the presence of SARS-CoV-2 RNA. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.
- Before using the kit, check tubes for leakage or damage. Each component in the kit should be thawed at room temperature, thoroughly mixed, and centrifuged before use.
- Important information regarding the safe handling, transport, and disposal of this product is contained in the Safety Data Sheet available on the Delphine Diagnostics Inc. website. https://delphinedx.com.
- Cross-contamination may occur by inappropriate handling of reference materials and specimens, which will cause inaccurate results. It is recommended to use sterile disposable filter-tips to aspirate reagents and patient samples.
- All patient samples to be tested should be considered as infectious substances and processed strictly in accordance with laboratory biosafety requirements. Manipulations of potential live virus samples must be performed within a class II (or higher) biological safety cabinet. Necessary precautions must be followed when handling specimens. Personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples must be used. Sterile centrifuge tubes and filter-tips should be used. After use, the tips should be disposed into a waste bin containing a 10% sodium hypochlorite solution. After the operation, the work area surface and the instrument surface should be disinfected with a freshly prepared 10% sodium hypochlorite solution, and then cleaned with 75% ethanol or pure water. Finally, UV light must be turned on for 30 minutes to disinfect working surfaces. The guidelines in the Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2 must be followed. https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html
- The PCR instrument used for this assay should be calibrated regularly according to instrument's instructions to eliminate cross-talks between channels.
- This kit uses PCR-based technology and experiments should be conducted in three separate areas: reagent preparation area, specimen preparation area, amplification area. Protective equipment accessories (goggles, work clothes, hats, shoes, gloves, etc.) should be worn during operation and protective equipment accessories should be changed when entering

and leaving different work areas. Protective equipment accessories in each work area are not interchangeable.

8. Patient Sample Collection, Handling, and Storage

In general, patient samples should be collected, handled, and stored according to the guidelines published by the CDC:
https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html.

Patient samples should be collected by a health care worker trained in specimen collection wearing all appropriate personal protective equipment and following biosafety precautions for handling infectious materials. Patient sample types validated for testing with the Delphine PrecisQT™ COVID-19 Test Kit include nasopharyngeal and oropharyngeal swab samples stored in a sealed specimen tube with viral transport media validated for COVID-19 testing. Patient samples should be transported as shown in the table below:

Specimen type	**Collection materials**	**Transport to laboratory (48-72 hours)**	**Storage prior to testing**
Nasopharyngeal and oropharyngeal swab (Both swabs should be placed in the same tube to increase the viral load)	-Dacron or polyester flocked swabs -Viral transport medium	4°C	≤72 hours: 4°C >72 hours: ≤ -70°C

Any shipping required through public channels must be in accordance with the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation.

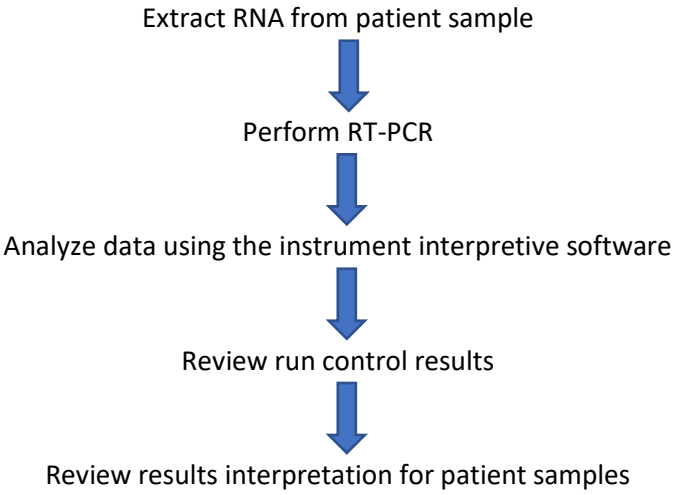
9. Assay Protocol

Sample Preparation Area. The samples are human nasopharyngeal swabs. All precautions that go with handling human and bio-hazardous material should be followed. All samples must be treated as potentially hazardous and infectious, regardless of whether or not they come from infected persons or apparently healthy, symptom-free individuals.

Aerosol Containment. COVID-19 is caused by an air-borne virus that may spread more readily through aerosol droplets; appropriate measures should be followed to protect laboratory workers.

Amplification Area Procedures should be performed in a laboratory with CDC/FDA recommended Biosafety Level (BSL), typically, Class II or higher; and samples must be handled in a biosafety cabinet/laminar flow hood. The amplification area should also be free of RNase that could degrade the sample or the extracted RNA. Samples should be on ice or cold blocks during the procedure. Pipettes, tubes, plates and other labware should be clean and free of contaminating nucleic acids and RNase free. Surfaces can also be wiped with 70% ethanol. The positive control should be handled in a separate area from samples and from PCR set up area.

Workflow



a. RNA Extraction

Specimen/sample RNA is the starting material for the RT-PCR reaction to determine COVID-19 infection. The RNA quantity and purity therefore affect the performance of the Delphine Kit and care must be taken to purify RNA according to the manufacturer's instructions.

The RNA extraction is to be performed using the QIAsymphony SP (QIAGEN) RNA Extraction system. The final elution volume of the RNA is 60 microliters (µL).

b. RT-PCR Reaction

Preparation of Primer/Enzyme Master Mixes and Loading of the 96 well Plate

1. Perform all work in a RNase free area and away from strong light.
2. Take out all the reagents from -20°C freezer for thawing at room temperature. Prepare two different types of primer master mixes for the two different target genes (target: SARS-CoV-2 and target: GAPDH)

   Note: To avoid pipetting errors and efficient dispensing, add all the reagents to the Primer/Enzyme master mix except the template.
3. In a 1.5/1 mL tube labeled COVID, add the 2X master mix, RT Enzyme Mix, COVID primer mix, and Nuclease-free water in quantities shown in the table below.
4. In a second 1.5/1 mL tube labeled IC add the 2X master mix, RT Enzyme Mix, GAPDH (IC) primer mix, and Nuclease-free water in quantities shown in the table below.

Reaction setup

Component	Full Plate (46 samples + PC + NTC+2 excess samples)*			
			Volume Added (µL)	
	Volume in each well (µL)	No of test samples (46) *calculated for 50§	COVID Mix	GAPDH Mix
Master Mix	10	X 50	500	500
RT Enzyme Mix	0.2	X 50	10	10
Primer Mix	3.1	X 50	155	155
Nuclease -free water	1.7	X 50	85	85
Total volume	15	X 50	750	750

* The 2 excess samples allow for pipetting errors and ensures there is enough mix to add into each of the 48 wells (The current table is a representative calculation for 46 samples +2 controls).
§ Scale and recalculate all component volumes proportional to the sample number and reaction volumes.

5. Mix thoroughly the Primer/Enzyme Mix to ensure homogeneity. Aliquot 15 µL of this reaction mix into each well of the Optical 96 well Reaction plate according to the following recommendations. It is important to follow good pipetting practices to avoid errors and increase test efficiency, accuracy, and precision.

Recommended RT-PCR plate setup*

	1	2	3	4	5	6	7	8	9	10	11	12
A	COVID Mix Sample 1	GAPDH Mix Sample 1	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix
B	COVID Mix Sample 2	GAPDH Mix Sample 2	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix
C	COVID Mix Sample 3	GAPDH Mix Sample 3	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix
D	COVID Mix Sample 4	GAPDH Mix Sample 4	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix
E	COVID Mix Sample 5	GAPDH Mix Sample 5	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix
F	COVID Mix Sample 6	GAPDH Mix Sample 6	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix
G	COVID Mix Sample 7	GAPDH Mix Sample 7	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix	COVID Mix NTC	GAPDH Mix NTC
H	COVID Mix Sample 8	GAPDH Mix Sample 8	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix	COVID Mix	GAPDH Mix	COVID Mix PC	GAPDH Mix PC

*Suggested plate setup for 46 patient samples and 2 controls, PC and NTC (marked in red)

6. Add patient samples, 5 µL from each patient into 2 wells (marked yellow for COVID primer and green for GAPDH primer), 5 µL of nuclease-free water into the wells labeled NTC and 5 µL of PC (supplied in the kit) to the wells labeled PC (setup illustrated in Table 2).

7. Seal the plate thoroughly with MicroAmp Optical Adhesive Film

Note: IMPORTANT! When applying the MicroAmp Optical Adhesive Film, ensure that pressure is applied across the entire plate and that there is a tight seal across every individual well. Failure to do so runs the risk of an improperly sealed well, leading to potential well-to-well contamination during vertexing and evaporation during PCR.

8. Vortex the plate at the highest setting for 10-30 seconds with medium pressure to ensure thorough mixing of all the components with the samples. Move the plate around to ensure equal contact on the vortex mixer platform.
9. Centrifuge/Spin the reaction plate for 1–2 minutes at ≥650 × *g* (≥650 RCF) to remove any air bubbles and to collect the liquid at the bottom of the reaction plate
10. Load the reaction plate immediately after the preparation/set up to avoid degradation of the RNA samples in the real-time PCR instrument and start the PCR run (RT-PCR Run Conditions provided below)
11. The QuantStudio 5 Dx Design and Analysis software used is v1.0.2; program the machine as per the following instructions

    Name: Enter a unique name
    Instrument type: **QuantStudio 5 Dx System**
    Block type: **96-Well 0.1-mL Block**
    Experiment type: **Standard Curve**
    Chemistry: **SYBR Green**
    Run Mode: **Standard**

12. Enter the total reaction volume as 20 µL and proceed with the thermal protocol RT-PCR run conditions:

Step	Cycles	Temp.	Time
Reverse transcription (RT)	1	48°C	10 minutes
Hot Start Polymerase activation	1	95°C	2 minutes
Denaturation	36	95°C	5 seconds
Anneal and Extension*		60°C	5 seconds
Melt curve*	60℃ to 95℃		

*Select SYBR/FAM channel for fluorescence data acquisition

13. In the **Plate** tab, click **Quick Setup**.
14. In the **Plate Attributes** pane, confirm that the **Passive Reference** is set to **ROX.**
15. In the plate layout pane, confirm the labelling of the control wells.
16. The template has positive (PC), and no template controls (NTC) assigned to wells for reference.

17. Move the control well assignments by copying the existing control wells and pasting them according to their location on the physical plate.
18. For all targets in the positive control well, confirm that **Task** is set to **S (Standard)**.
19. For all targets in the negative control well, confirm that **Task** is set to **N (Negative Control)**.
20. In the **Samples** table, click **Add** to define the sample names. Create a unique sample name for each well in the physical plate.
21. To assign a sample to a well, select the well in the plate layout, then select the sample from the **Samples** table. For all targets in the patient sample wells, confirm that **Task** is set to **U (Unknown).**
22. In the **Run** tab, click **Start Run**, then select your instrument from the drop-down list.
23. Enter a file name in the dialog box that prompts you to save the run file, then save the file.
24. Perform the data analysis according to the instructions provided below.

10. Interpretation of Results

a. Delphine Test Kit Controls – Positive, Negative, and Internal

All test controls must be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

For samples and controls run in the Delphine RT-PCR assay, analysis requires assessment of cycle thresholds and melt temperatures. Reportable data for the Delphine assay are cycle thresholds (Ct) and double-stranded helix melt temperatures (Tm). Cycle Threshold refers to the number of cycles that are necessary to amplify the SARS-CoV-2 RNA to reach a level that can be detected by the PCR thermocycler instrument. Generally, Ct levels are inversely proportional to the amount of target RNA in the sample. Melting Temperature refers to the temperature at which one-half of the double-stranded DNA will dissociate or 'melt' to become single-stranded. Tm values indicate the stability of the DNA duplex; and in an RT-PCR reaction, can refer to the melting temperature at which one-half of the primers and/or the primer-template DNA duplex will melt to become single-stranded. During test optimization, limits were set for Ct and Tm for controls and test samples to be considered valid and to confirm the presence or absence of SARS-CoV-2 RNA, and thus a positive or negative test result for COVID-19.

Delphine Test Kit Controls – Valid Parameters for Positive, Negative, and Internal Controls

Parameter	No Template Control		
Ct	≥ 34	< 34	< 34
Tm	Any	< 78°C or > 80°C	78°C ≤ Tm ≥ 80°C
Finding	Valid	Valid	Invalid

Parameter	Positive Control - SARS-CoV-2 primers	
Ct	≤ 35	> 35
Tm	78°C ≤ Tm ≥ 80°C	any
Finding	Valid	Invalid

Parameter	Positive Control - GAPDH primers	
Ct	≤ 30	> 30
Tm	78°C ≤ Tm ≥ 82°C	Any
Finding	Valid	Invalid

Interpretation of the NTC Result: The NTC consists of nuclease-free water and no RNA. The NTC is expected to have a Ct value ≥ 34 cycles (no amplification). In case the NTC has a Ct value < 34, the Tm should lie OUTSIDE the range of 78°C - 80°C (Tm range of the PC) for a valid result. If the NTC shows a Tm value in this range, the plate is an invalid run. The recommended plate format (provided in the Instructions for Use) calls for 2 NTC wells, one each with the SARS-CoV-2 primer mix and the GAPDH IC primer mix. If both NTC wells are invalid, the Master Mix or RT Enzyme Mix may be contaminated. If only one of the NTC wells is invalid, the primer mix used in that well may be contaminated with template RNA for that primer set. Valid NTC wells suggest the kit reagents are not contaminated and the results of the other wells can be trusted to accurately reflect interactions between the primer sets and extracted RNA or Positive Control RNA templates.

Interpretation of the PC Result: The PC test well containing the Delphine SARS-CoV primer mix must have a Ct of ≤ 35 and a Tm of 78°C - 80°C to be valid. A valid PC confirms the PCR procedure was completed successfully and the kit reagents are working properly. An invalid result in this test well suggests that one or more of the kit reagents is not performing properly or the PCR procedure did not complete successfully. Examining the IC for the PC will assist in determining the cause of the invalid finding.

Interpretation of the IC Result for the PC: The PC test well containing the GAPDH primer mix must have a Ct value ≤ 30 and a Tm within the range of 78°C - 82°C to be valid. A valid IC for the PC confirms the PCR procedure was successful and the GAPDH primer set is working properly. This allows a negative IC for a patient sample to be ascribed to the failure of the extraction procedure for the patient sample and not to a testing failure in the PCR step. An IC well with a Ct > 30 cycles is negative for GAPDH and the IC is invalid. If the IC is invalid for the PC, the IC wells for patient samples cannot be used to confirm successful RNA extraction.

b. Interpretation of Patient Sample Results

Assessment of clinical sample test results should be performed only after the No Template Control and Positive Control have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

Delphine Test Kit Findings – Examination and Interpretation of Patient Sample Result

Parameter	Patient Sample - SARS-CoV-2 primers				
Ct	≤ 35	35 < Ct ≤ 36	≤ 35	≤ 35	Undetermined
Tm	± 1°C of PC Tm	± 1°C of PC Tm	1°C < Tm < 2°C above PC Tm	> 1°C below or > 2°C above PC Tm	Any
Finding	Positive	Weakly Positive	Weakly Positive	Negative	Negative

Parameter	Patient Sample - GAPDH primers	
Ct	≤ 30	> 30
Tm	78°C ≤ Tm ≥ 82°C	Any
Finding	Valid	Invalid

Interpretation of the SARS-CoV-2 Result for the Patient Sample: When the criteria for NTC and PC are met, a patient sample with no determined Ct is a negative result for the presence of SARS-CoV-2 RNA. If the Ct value of the patient sample is ≤ 36 cycles, the melting temperature is used to interpret the result. A patient sample with a Ct ≤ 35 and Tm within 1°C of the Tm of the PC confirms the presence of the SARS-CoV-2 S gene and the patient is positive for COVID-19. A patient sample with a Ct between 35 and 36, and a Tm within 1°C of the Tm of the PC is weakly positive for SARS-CoV-2, as is a sample with Ct ≤ 35 and Tm between 1 and 2°C above the Tm of the PC. Both test results suggest the presence of SARS-CoV-2 in the patient sample, but the conclusion is not definitive. Weakly positive samples should be reextracted and rerun through the Delphine RT-PCR test to confirm the presence of SARS-CoV-2.

Interpretation of the IC Result for the Patient Sample: The patient sample test well containing the GAPDH primer mix must have a Ct value ≤ 30 and a Tm within the range of 78°C - 82°C to be valid. An invalid IC for a patient sample with a positive result for SARS-CoV-2 suggests a pipetting error in the IC test well. An invalid IC for a patient sample with a negative result for SARS-CoV-2 suggests a problem occurred in the RNA extraction procedure or in sample handling prior to testing. The sample should be reextracted and rerun through the Delphine RT-PCR test for the presence of SARS-CoV-2.

11. Assay Performance

a. Limit of Detection

The RNA extraction was accomplished using the QIAsymphony SP & AS Automated Total Nucleic Acid Extraction System as per the manufacturer's protocol. RT-PCR was carried out using the Delphine COVID-19 test kit. The Limit of Detection (LoD) study was performed with Quant Studio 5 Dx, keeping all components and parameters of the test system identical from sample preparation to detection. The preliminary LoD study was performed with commercially available ATCC SARS-CoV-2 inactivated virus spiked in a negative clinical matrix (known negative, nasopharyngeal patient sample) in 6 concentrations (2.7×10^6, 2.7×10^5, 2.7×10^4, 2.7×10^3, 2.7×10^2, 2.7×10^1 copies/mL) and subjected to individual RNA extraction in 5 replicates per concentration. Results from the preliminary LoD reading showed 5/5 consistent positive Ct values with 2.7×10^3 copies/mL. Final/confirmatory LoD study was performed with 3-fold dilutions to generate 2700 copies/mL, 900 copies/mL, 300 copies/mL, and 100 copies/mL. The confirmatory LoD was performed with 20 replicates of the 4 above-mentioned concentrations, individually extracted via QIAsymphony SP & AS Automated Total Nucleic Acid Extraction System. The final Limit of Detection was the concentration at which at least 19/20 replicates of SARS-CoV-2 showed positivity: 300 copies/mL pre-extraction sample, 20/20 positive.

b. Inclusivity

An in-silico inclusivity study was conducted with all the 48,635 different published sequences of SARS-CoV-2, according to the public database of Global Initiative on Sharing All Influenza Data (GISAID) as of December 22, 2020. The assay's forward and reverse primer targets a region of the virus spike glycoprotein (S) gene and amplifies a 124 bp sequence. The Delphine primer is designed to amplify a region in between the Receptor Binding Domain (RBD) and the Fusion Peptide (FP) corresponding to 541-788 amino acid residues respectively after translation (https://www.nature.com/articles/s41401-020-0485-4). In silico analysis confirmed that the Delphine primer set targets a stretch of the cDNA distinct from the 2 known Single nucleotide Polymorphisms (SNPs) namely, 1) Genomic coordinate: A23403G, corresponding protein effect: SD614G and 2) Genomic coordinate: C23731T, corresponding protein effect: ST723T that have been previously reported in the S gene (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7387429/). The Delphine primer target region does not target the region containing the abovementioned SNPs.

As of February 2021, several variants of SARS-CoV-2 with high infectivity rates have been traced to isolates from the UK, Brazil, and South Africa. These variants have also been isolated from different states across the US. We have performed in silico analysis with the Brazilian and S. African variant and concluded that the mutations are not interfering with the amplification process. In silico analysis and wet lab experiments have been performed with the UK variant sequences and no interference have been observed.

c. Cross-reactivity

Cross-reactivity studies were performed to demonstrate that the primer supplied in the Delphine COVID-19 test kit does not react with either related pathogens, or highly prevalent disease agents or normal/pathogenic flora that may exist in a nasopharyngeal clinical specimen. The recommended pathogens (from FDA Molecular Diagnostic Template for Commercial Manufacturers, July 28, 2020) are listed in the table below along with the results of testing each organism at high concentration

for false reporting of COVID-19 in the Delphine test kit assay. The cross-reactivity study was performed with the concentration of 10^6 CFU/mL for bacteria and 105 PFU/mL for viruses spiked in a negative clinical matrix (known negative, nasopharyngeal patient sample). All the samples were extracted using QIAsymphony SP & AS Automated Total Nucleic Acid Extraction System according to manufacturer's protocol. Amplification was performed in triplicates including a positive control and no template control using Delphine test kit.

Cross-reactivity study with High priority pathogens

Viral strains	Source Material	Concentration	Results
Cross-reactivity of High Priority Pathogens from the same Genetic Family			
Human coronavirus 229E	ATCC VR-740	10^5 PFU/mL	1/1 Negative
Human coronavirus OC43	ATCC VR-1558	10^5 PFU/mL	1/1 Negative
Cross-reactivity of High Priority Organisms likely present in a Respiratory Specimen			
Adenovirus 5 & 11	ATCC VR-5 & VR-12	10^5 PFU/mL	2/2 Negative
Human Metapneumovirus (hMPV)	ATCC VR-3250D	10^5 PFU/mL	1/1 Negative
Parainfluenza virus 2-4	ATCC VR-92, VR-93 & VR-1377	10^5 PFU/mL	3/3 Negative
Influenza A & B	ATCC VR-1738 & VR-1784	10^5 PFU/mL	2/2 Negative
Enterovirus (e.g., EV68)	ATCC VR-836	10^5 PFU/mL	1/1 Negative
Respiratory syncytial virus	ATCC VR-1540P	10^5 PFU/mL	1/1 Negative
Rhinovirus	ATCC VR-1171	10^5 PFU/mL	1/1 Negative
Chlamydia pneumoniae	ATCC VR-1356	10^6 CFU/mL	1/1 Negative
Haemophilus influenzae	ATCC 33391	10^6 CFU/mL	1/1 Negative
Legionella pneumophila	ATCC 33152	10^6 CFU/mL	1/1 Negative
Mycobacterium tuberculosis	ATCC 25177	10^6 CFU/mL	1/1 Negative
Streptococcus pyogenes	ATCC 49399	10^6 CFU/mL	1/1 Negative
Bordetella pertussis	ATCC 9797	10^6 CFU/mL	1/1 Negative
Mycoplasma pneumoniae	ATCC 15531	10^6 CFU/mL	1/1 Negative
Candida albicans	ATCC 14057	10^6 CFU/mL	1/1 Negative
Pseudomonas aeruginosa	ATCC 14053 & 10145	10^6 CFU/mL	2/2 Negative
Staphylococcus epidermis	ATCC 49134	10^6 CFU/mL	1/1 Negative
Streptococcus salivarius	ATCC 9759	10^6 CFU/mL	1/1 Negative

In silico analysis was conducted with each of the above-mentioned pathogens. The oligonucleotide primers in the Delphine test kit were queried with the whole genome sequences for each of these

pathogenic agents. Analysis found only 2 pathogens suspected to generate amplicons short enough to be detected under the PCR run conditions specified by the Delphine kit, *Parainfluenza virus 3* and *Respiratory syncytial virus*. Amplicon lengths >1000 bp are not reliably generated or detected under common RT-PCR conditions. These pathogens were evaluated for microbial interference in the Delphine assay.

d. Microbial Interference

A 'worst-case' microbial interference study was performed for the pathogens selected through in silico analysis for homology with the Delphine SARS-CoV-2 primer set and a predicted amplicon of <1000 bp. These pathogens were examined at high concentrations (10^5 PFU/mL) for interference with the proper determination of the presence or absence of low concentrations (<10^3 copies/mL) SARS-CoV-2 in test samples. Two pathogens, *Human parainfluenza virus type 3* and *Human Respiratory Syncytial Virus,* were studied with and without SARS-CoV-2 at 3x LoD (900 copies/mL) along with SARS-CoV-2 alone, a known positive patient sample and NTC, all in triplicates. The presence of the high viral load second virus shifted the Ct value for the SARS-CoV-2 read out. The samples were still reportable as positive or weakly positive meaning the interfering pathogens did not create a false negative test result under these 'worst-case' conditions.

Microbial Interference study of pathogens showing >80% homology and < 1000 bp query length

	SARS-CoV-2 SPIKE	Sample Name	S gene Mean Ct	S gene Mean Tm
1	-	*Human parainfluenza virus type 3*	Undetermined	70.0 °C
2	-	*Human Respiratory Syncytial Virus*	Undetermined	70.0 °C
3	+	*Human parainfluenza virus type 3*	35	78.8 °C
4	+	*Human Respiratory Syncytial Virus*	33	78.6 °C
5	+	Inactivated SARS-CoV-2 900 C/mL	31	78.4 °C
6	N/A	Known Positive Sample	33	78.6 °C
7	N/A	Known Negative Sample	Undetermined	69.8 °C

e. Clinical Validation

Clinical validation of the Delphine test kit was performed using nasopharyngeal samples sent to a certified clinical laboratory for COVID-19 testing. 36 positive and 36 negative samples were selected for inclusion in the study based on results using the LabGenomics LabGun COVID-19 test kit test per manufacturer's instructions. These 72 samples were subjected to RNA extraction using the QIAamp Viral RNA Mini kit. Extracted RNA was then subjected to amplification and detection with the Delphine test kit using the QuantStudio 5 Dx instrument. Positive clinical samples were pre-selected with high, medium, and low-viral loads. Both positive and negative sample tests were performed in triplicates in a blinded fashion along with appropriate controls, NTC and PC. The interpretation of the study

results was based on the Ct/melt curve interpretation tables for each test kit, as appropriate. Clinical results with the Delphine PrecisQT™ test kit yielded similar results as the LabGun COVID-19 RT-PCR Kit (detection of positive and negative samples) confirming comparable accuracy and sensitivity.

Comparison of results with comparator kit of 36 positive and 36 negative patient samples

		LabGenomics LabGun			
		Positive	Negative	Metric	Agreement
Delphine PrecisQT™	Positive	36	0	PPA*	100%
	Negative	0	36	NPA*	100%

* PPA – Positive Percent Agreement, NPA – Negative Percent Agreement

12. Limitations of the Procedure

For use as specified in this Instructions for Use only.

1. This assay is for *in vitro* diagnostic use only. Validation of this test has not been reviewed by FDA. Review under the EUA program is pending. Distributed in accordance with the guidance on Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency, Section IV.C.2.
2. Use of the Delphine PrecisQT™ COVID-19 Test Kit is limited to personnel who have been trained in the procedures of a molecular diagnostic assay and the instruments associated with the procedure.
3. Laboratories are required to report all positive results to the appropriate public health authorities.
4. The test was validated with only nasopharyngeal swab samples. Performance with other types of samples is not known.
5. A negative result is not definitive for the absence of COVID-19 infection.
6. The test result should not be used as a sole method of diagnosis of a patient's status. Additional patient testing and evaluation may be required for confirmatory diagnosis.
7. Improper sample collection, storage, transportation, contamination, or handling could result in inconclusive, false negative or false positive results.
8. Compromising the quality of the reagents by contamination, use of inhibitory substances, non-clean plates or tubes will affect kit performance adversely.
9. A positive result is not a definite indicator of presence of infectious viral particles or the sole reason for symptoms exhibited by the patient.
10. This assay cannot exclude infections or medical conditions caused by other micro-organisms or pathogens.

13. Additional Instructions for Laboratories

Should the Emergency Use Authorization request for the Delphine PrecisQT™ COVID-19 Test Kit be granted, the Delphine test Letter of Authorization will be available at the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas.

In addition to the above, the following Conditions are applicable to the Laboratories that use the Delphine PrecisQT™ COVID-19 Kit:

1. Laboratories of the Delphine Kit will use the kit as outlined in the present protocol. Any deviation from the indicated protocol, even if used with specified instruments, extraction methods, clinical samples, control materials and other accessory reagents and/or materials is not permitted.
2. Laboratories that receive or obtain the Delphine Kit will notify the relevant public health authorities of their intention to run the test prior to initiation of the testing.
3. Laboratories using the Delphine Kit will have a procedure already established to report test results to healthcare providers and relevant public health authorities as required. The report should include a general statement such as 'the test has been validated but FDA's independent review of this validation is pending'.
4. Laboratories will collect information on the performance of the Delphine Kit and report to the DMD/OHT7-OIR/OPEQ/CDRH (by email: CDRH-EUA-Reporting@fda.hhs.gov) and Delphine Diagnostics Technical Support (https://delphinedx.com/contact-us/ or phone 609-669-0510) any suspected false positive or false negative results and/or deviations from the established performance of the assay.
5. Only trained laboratory personnel proficient in RT-PCR techniques, protocols, procedures, and instrument-use that are all required for the correct usage of the Delphine Kit should use the kit. The trained personnel must have and use the approved and/or appropriate personal protective equipment (PPE) and use the kit according to the supplied labeling and in compliance with applicable safety protocols.
6. Delphine Diagnostics, its authorized distributors and laboratories using the Delphine kit, will ensure that any and all records associated with the use of this test are maintained until otherwise notified by the FDA. Such records will be made available to the FDA for inspection as necessary and upon request.

14. Bibliography

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15. Trademarks and Disclaimers

Delphine PrecisQT™ COVID-19 is a trademark owned by Delphine Diagnostics Inc. The Delphine Diagnostics Technology is protected by pending Indian and international patents. All other trademarks that appear in this Instructions for Use document are the property of their respective owners.

Delphine PrecisQT™ has been validated but FDA's independent review of this validation is pending. Use of the Delphine PrecisQT™ COVID-19 Test Kit is subject to Delphine Diagnostics' Terms and Conditions of use.

In vitro Diagnostic Medical Device Technical Assistance:

Customer Contact:
https://delphinedx.com/contact-us/
Phone: 1-609-669-0510
Delphine Diagnostics Inc.
Rutgers University EcoComplex
1200 Florence Columbus Rd, Suite 110
Bordentown, New Jersey 08505