NORGEN BIOTEK CORPORATION

3430 Schmon Parkway
Thorold, ON, Canada L2V 4Y6
Phone: (905) 227-8848
Fax: (905) 227-1061
Email: techsupport@norgenbiotek.com

2019-nCoV TaqMan RT-PCR Kit Dx

Product Insert

REF **DxTM67100** CE IVD PIDxTM67100-4

Intended Use

Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx is an *in vitro* diagnostic test for the qualitative detection of SARS-CoV-2 specific RNA using real-time hybridization-fluorescence detection. Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx provides SARS-CoV-2 detection based on the assays and protocols developed by the Centers for Disease Control and Prevention (CDC). The assay is designed for use with RNA isolated from nasopharyngeal swabs, oropharyngeal swabs and saliva samples collected from individuals with clinical signs/symptoms related to SARS-CoV-2 infection for *in vitro* diagnostic use.

For *In Vitro* Diagnostic Use

Product Description

Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx provides SARS-CoV-2 detection based on the assays and protocols developed by the CDC. The kit utilizes RT-PCR for the amplification of specific target sequences and target specific probes for the detection of the amplified cDNA. The Primer & Probe Mixes contain all 3 CDC developed assays in individual tubes where probes are labelled with the fluorophore FAM. All assays are premixed to the working concentrations recommended by the CDC. The Positive Control contains two nCoV nucleocapsid target gene RNA (N1 and N2) and RNase P (internal control). The kit contains a positive control to monitor for PCR inhibition, and to validate the quality of the sample and the detection result. The 2019-nCoV TaqMan RT-PCR Kit Dx comprises Master Mix for the target and PCR control detection, 3 target Primer & Probe Mixes, as well as a positive control and a negative control (nuclease-free water) to confirm the integrity of the kit reagents.

Positive results are indicative of SARS-CoV-2 RNA detection, however clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out co-infection with other viruses and therefore the agent detected may not be the definite cause of disease. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Any negative results must be combined with clinical observations, patient history, and epidemiological information.

Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx is intended for use by professional users including clinical laboratory personnel experienced and trained in molecular biology techniques including real-time PCR and *in vitro* diagnostic procedures.

Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx was developed and validated to be used with the BioRad CFX96 Touch™ Real-Time PCR Detection System.

Made in Canada
Norgen Biotek

Kit Components

Component	Product # TM67100 (50 reactions)
2019-nCoV_N1 Probe/Primer Mix Dx	80 µL
2019-nCoV_N2 Probe/Primer Mix Dx	80 µL
RNAse P Probe/Primer Mix Dx	80 µL
2019-nCoV RT-PCR Positive Control Dx - 200,000 copies/µL	50 µL
2X One-Step RT-PCR Master Mix Dx	2 x 1 mL
Nuclease-Free Water (Negative control)	1.25 mL
Product Insert	1

Storage Conditions and Product Stability

- The 2019-nCoV TaqMan RT-PCR Kit Dx is shipped on dry ice. The components of the kit should be frozen upon arrival. If one or more of the components is not frozen when the kit is received, or if any of the components have been compromised during shipment, do not use the kit and contact Norgen Biotek for assistance.
- All kit components should be stored at -20°C upon arrival.
- Repeated thawing and freezing (>3X) of the Master Mix and Positive Control should be avoided, as this may affect the performance of the assay. If the reagents are to be used only intermittently, they should be frozen in aliquots.
- All reagents can be used until the expiration date specified on their labels.

Customer-Supplied Reagents and Equipment

- Appropriate Real-Time PCR Instrument with FAM filter channel
- RNA Purification Kit
    - Performance of Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx was evaluated using Norgen's Saliva/Swab RNA Purification Kit Dx (Cat# Dx69100)
    - While the kit should be compatible with all RNA purification kits that yield high quality, inhibitor-free RNA, it is up to users to validate the use of alternate RNA purification kits
- Disposable powder-free gloves
- Benchtop microcentrifuge
- Micropipettors
- Sterile pipette tips with filters
- PCR tubes & caps (or PCR plate with appropriate plate seal)
- Vortex mixer
- PCR tube centrifuge
- PCR reaction preparation station (Recommended)

Quality Control

In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx is tested against predetermined specifications to ensure consistent product quality.

Warnings and Precautions

- Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx is intended for use by professional users including clinical laboratory personnel experienced and trained in molecular biological techniques including real-time PCR and *in vitro* diagnostic procedures.

- Follow universal precautions. All patient specimens should be considered as potentially infectious and handled accordingly.
- Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when handling specimens and kit reagents.
- Use sterile pipette tips with filters. Use proper pipetting techniques and maintain the same pipetting pattern throughout the procedure to ensure optimal and reproducible values.
- As contamination of patient specimens or reagents can produce erroneous results, it is essential to use aseptic techniques. Pipette and handle reagents carefully to avoid mixing of the samples.
- Do not use supplies and equipment across the dedicated areas of i) specimen extraction, ii) reaction set-up and iii) amplification/detection. No cross-movement should be allowed between the different areas. Personal protective equipment, such as laboratory coats and disposable gloves, should be area specific.
- Store and extract positive material (specimens, controls and amplicons) separately from all other reagents and add it to the reaction mix in a spatially separated facility.
- Dispose of unused kit reagents and human specimens according to local, provincial or federal regulations.
- Do not substitute or mix reagents from different kit lots or from other manufacturers. Do not use components of the kit that have passed their expiration date.
- As with any diagnostic test, results generated using Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx should be interpreted with regard to other clinical or laboratory findings.
- The presence of PCR inhibitors may cause false negative or invalid results.
- Potential mutations within the target regions of the SARS-CoV-2 genome covered by the primers in this kit may result in failure to detect the presence of the pathogen.
- Good laboratory practice is essential for the proper performance of this kit. Ensure that the purity of the kit and reactions is maintained at all times, and closely monitor all reagents for contamination. Do not use any reagents that appear to be contaminated.
- Ensure that appropriate specimen collection, transport, storage and processing techniques are followed for optimal performance of this test.

Assay Limitations

- Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx performance was established using nasopharyngeal swabs, oropharyngeal swabs and saliva samples. Swab samples were collected using nylon flocked synthetic swabs and were placed into Norgen's Total Nucleic Acid Preservative Tubes Dx (Cat# Dx69200) for storage until RNA isolation. Saliva samples were collected into Norgen's Saliva RNA Collection and Preservation Devices Dx (Cat# 53800) and preserved at room temperature until RNA isolation. Other specimen types and preservatives have not been validated with this kit.
- Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx performance was established using RNA that was purified with Norgen's Saliva/Swab RNA Purification Dx (Cat# Dx69100). Other RNA extraction methods have not been validated with this kit.
- The following exogenous substances were tested and determined not to interfere with the performance of the kit: nasopharyngeal swabs – blood and mucin; oropharyngeal swabs and saliva – blood, mucin sputum
- The impact of antipyretic analgesics, antitussives, expectorants, antibiotics, antivirals and corticosteroids have not been evaluated.

Instructions for Use

A. Sample Preparation

Testing for COVID-19 should be conducted in consultation with a healthcare provider, and only patients demonstrating symptomatic disease should undergo testing.

Purified RNA is the starting material for Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx. The quality of the RNA template will have a major impact on the performance of the diagnostic test. The user must ensure that the method used for RNA purification is compatible with PCR technology. We recommend the use of Norgen's Dx series of purification kits for RNA isolation, including **Norgen's Saliva / Swab RNA Purification Kit Dx (Cat# Dx69100).**

If using a different spin column-based sample preparation procedure that includes ethanol-based wash buffers, a column drying step consisting of centrifugation for 3 minutes at 20,000 x g (~14,000 RPM), using a new collection tube, is highly recommended prior to the elution of the RNA. This will help to prevent the carry-over of any ethanol into the purified RNA, as ethanol is known to be a strong inhibitor of PCR. **Ensure that any traces of ethanol from the sample preparation steps are eliminated prior to the elution of the RNA.**

B. TaqMan RT-PCR Assay Preparation

Notes:

- Before use, suitable amounts of all TaqMan RT-PCR components should be completely thawed at room temperature, mixed by gentle vortexing or by pipetting, and centrifuged briefly.
- Work quickly on ice.
- The amount of MDx TaqMan 2X RT-PCR Master Mix provided is enough for up to 50 RT-PCR reactions per each target
- For every TaqMan One-step RT-PCR run, one reaction containing 2019-nCoV Positive Control and one reaction as no template control must be included for proper interpretation of results. The recommended minimum number of RNA samples tested per TaqMan One-step RT-PCR run is 10. See the Example of Sample and Control Set-up in Table 1 below
- To avoid any contamination while preparing the TaqMan One-step RT-PCR assay, follow the order outlined in Tables 2, 3 and 4 below to prepare the Negative Control, Detection Assay and Positive Control:
    1. Prepare the RT-PCR Negative Control (Table 2)
    2. Prepare the RT-PCR 2019-nCoV Assay (Table 3)
    3. Prepare the RT-PCR Positive Control (Table 4)
- To further avoid contamination, add the components to the PCR tubes in the order shown in the tables below (i.e: 1) Nuclease-free water; 2) Primer & Probe Mix; 3) Mastermix; and 4) the Sample RNA or Positive Control).

Table 1. Example of Sample and Control Set-up

Target		1	2	3	4	5	6	7	8	9	10	11	12
2019-nCoV_N1	A	NTC	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	COVID-PC
2019-nCoV_N2	B	NTC	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	COVID-PC
RNAse P	C	NTC	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	COVID-PC
2019-nCoV_N1	D	NTC	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	COVID-PC
2019-nCoV_N2	E	NTC	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	COVID-PC
RNAse P	F	NTC	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	COVID-PC

1. For each TaqMan One-step RT-PCR set, prepare **three** no template control PCR reactions as shown in Table 2 below:

Table 2. TaqMan One-step RT-PCR Negative Control Preparation

Reagent	Vol. of Reagent Added per Reaction
Nuclease-Free Water	8.5 µL
2X One-Step RT-PCR Master Mix Dx	10 µL
2019-nCoV Primer & Probe Mix Dx*	1.5 µL
Total Volume	20 µL

* Three different reactions will be prepared using each of the 3 provided Primer & Probe target mixes: 2019-nCoV_N1 Probe/Primer Mix Dx, 2019-nCoV_N2 Probe/Primer Mix Dx, RNAse P Probe/Primer Mix Dx

2. Prepare the **three** RT-PCR reactions for sample detection as shown in Table 3 below.

Table 3. TaqMan One-step RT-PCR 2019-nCoV Assay Preparation

Reagent	Vol. of Reagent Added per Reaction
Nuclease-Free Water	3.5 µL
2X One-Step RT-PCR Master Mix Dx	10 µL
2019-nCoV Primer & Probe Mix Dx*	1.5 µL
Sample RNA**	5 µL
Total Volume	20 µL

* Three different reactions will be prepared for each sample using each of the 3 provided Primer & Probe target mixes: 2019-nCoV_N1 Probe/Primer Mix Dx, 2019-nCoV_N2 Probe/Primer Mix Dx, RNAse P Probe/Primer Mix Dx
** The recommended amount of sample RNA to be used is 5 µL. However, a volume between 1 and 5 µL of sample RNA may be used as template. Adjust the final volume of the RT-PCR reaction to 20 µL using the Nuclease-Free Water provided.

3. For each RT-PCR set, prepare **three** positive control RT-PCR as shown in Table 4 below:

Table 4. TaqMan One-step RT-PCR Positive Control Preparation

Reagent	Vol. of Reagent Added per Reaction
2X One-Step RT-PCR Master Mix Dx	10 µL
2019-nCoV Primer & Probe Mix Dx*	1.5 µL
2019-nCoV Positive Control (PosC) Dx**	5 µL
Nuclease-Free Water	3.5 µL
Total Volume	20 µL

* Three different reactions will be prepared for each sample using each of the 3 provided Primer & Probe target mixes: 2019-nCoV_N1 Probe/Primer Mix Dx, 2019-nCoV_N2 Probe/Primer Mix Dx, RNAse P Probe/Primer Mix Dx
** The positive control contains the CDC 2019-nCoV markers (N1 and N2) and RNase P gene which are compatible with the CDC 2019-nCoV specific primer/probe sets

C. 2019-nCoV TaqMan One-Step RT-PCR Assay Programming

1. Program the thermocylcer according to the program shown in Table 4 below.
2. Run one step RT-PCR.

Table 5. 2019-nCoV TaqMan One-Step RT-PCR Program

One Step RT-PCR Cycle	Step	Temperature	Duration
Cycle 1	**Step 1**	**50ºC**	**30 min**
Cycle 2	**Step 1**	**95ºC**	**3 min**
Cycle 3 (45x)	**Step 1**	**95ºC**	**3 sec**
	Step 2	**55ºC**	**30 sec**

D. 2019-nCoV TaqMan One-Step RT-PCR Assay Interpretation

- The Negative Control (NTC – No Template Control) reaction(s) must be negative and not exhibit fluorescence growth curves that cross the threshold line. If there is any amplification with the NTC the run is not valid and no interpretation of 2019-nCoV detection can be made. The assay must be repeated.
- The Positive Control (PosC) reaction(s) should produce a positive result with an expected Ct value below 20 for each target

Table 6. Example of Expected Ct Values for the Different Targets

Target	Mean Ct.*	Standard Deviation	Coefficient of variation (%)
2019-nCoV_N1	13.8	0.2	1.77
2019-nCoV_N2	15.7	0.1	0.37
RNase P	13.2	0.1	0.49

* Mean Ct. was collected from three operators testing two replicates of positive control using the BioRad CFX96 Touch™ Real-Time PCR Detection System

- If the positive control does not provide a positive result the run is not valid and no interpretation of 2019-nCoV detection can be made. The assay must be repeated.
- If the NTC and PosC are exhibiting the correct results, the results of the detection assays can be interpreted as outlined in Table 7 below

Table 7. Interpretation of Assay Results

2019 nCoV_N1	2019 nCoV_N2	RP	Expected Ct Values	Result
+	+	±	< 40.00 Ct	2019-nCoV Detected
If only one (1) of two targets is positive		±	< 40.00 Ct	Inconclusive Result
-	-	+	< 40.00 Ct	2019-nCoV Not Detected
-	-	-	N/A	Invalid Result

E. Performance Evaluation

1. Analytical Sensitivity

A. Initial Study

The analytical sensitivity of the 2019-nCoV TaqMan RT-PCR Kit Dx was initially determined by analyzing a dilution series of quantified SARS-CoV-2 RNA transcripts. Contrived nasopharyngeal swabs, oropharyngeal swabs and saliva samples were generated by spiking 5 µL of different concentrations of the 2019-nCoV RT-PCR Positive Control (200,000 copies/uL) to generate input samples of variable transcript content. Triplicate samples were tested for each concentration for all three sample types.

The limit of detection of Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx from RNA isolated from nasopharyngeal swabs, oropharyngeal swabs and saliva samples is 10 copies per PCR reaction as can be seen in Tables 8, 9 and 10 below.

Table 8. Analytical Sensitivity for Oropharyngeal Swabs

Copies/PCR reaction	N1		N2		RP	
	Average Ct Value	SDEV	Average Ct Value	SDEV	Average Ct Value	SDEV
0	N/A	N/A	N/A	N/A	30.72	1.70
1	N/A	N/A	N/A	N/A	31.45	1.31
10	33.44	0.38	34.65	0.10	36.57	0.33
100	31.26	0.18	31.78	0.05	31.14	0.33
1,000	28.26	0.24	28.13	0.32	26.98	0.62
10,000	25.69	0.29	25.19	0.72	25.01	0.91
100,000	21.60	0.21	22.00	0.49	22.08	0.15

Table 9. Analytical Sensitivity for Nasopharyngeal Swabs

Copies/PCR reaction	N1		N2		RP	
	Average Ct Value	SDEV	Average Ct Value	SDEV	Average Ct Value	SDEV
0	N/A	N/A	N/A	N/A	29.92	1.87
1	N/A	N/A	N/A	N/A	29.19	0.62
10	34.10	0.57	35.56	0.36	33.07	0.14
100	31.27	0.25	32.15	0.95	30.39	0.83
1,000	28.42	0.27	29.17	0.10	29.19	0.62
10,000	24.56	0.14	25.13	0.36	25.19	0.36
100,000	21.73	0.28	22.15	0.14	22.43	0.37

Table 10. Analytical Sensitivity for Saliva Samples

Copies/PCR reaction	N1		N2		RP	
	Average Ct Value	SDEV	Average Ct Value	SDEV	Average Ct Value	SDEV
0	N/A	N/A	N/A	N/A	30.53	0.46
1	N/A	N/A	N/A	N/A	25.09	0.87
10	34.89	0.43	34.75	0.45	31.02	0.44
100	31.92	1.20	31.93	1.11	29.57	0.94
1,000	28.44	0.32	29.70	0.38	32.49	1.27
10,000	25.27	0.35	25.68	0.46	31.93	1.11
100,000	22.56	0.21	23.41	0.25	22.96	0.34

B. Confirmatory Study

The limit of detection of the 2019-nCoV TaqMan RT-PCR Kit Dx was confirmed using 20 contrived samples of each sample type. Contrived nasopharyngeal swabs, oropharyngeal swabs and saliva samples were generated by spiking 2019-nCoV RT-PCR Positive Control corresponding to 10 copies per PCR reaction. Confirmatory results were acceptable at a 95% confidence interval. This can be achieved when obtaining a minimum of 19 positive samples out of the 20 samples spiked at the limit of detection. As seen in Tables 11, 12 and 13 the limit of detection of Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx from RNA isolated from nasopharyngeal swabs, oropharyngeal swabs and saliva samples was confirmed to be 10 copies per PCR reaction at a 95% confidence interval.

Table 11. Analytical Sensitivity Confirmation for Oropharyngeal Swabs

Concentration	N1		N2		RP	
	Detection Rate	Avg Ct Value	Detection Rage	Avg Ct Value	Detection Rate	Avg Ct Value
1 x LoD	100% (20/20)	33.22	100% (20/20)	32.96	100% (20/20)	31.21

Table 12. Analytical Sensitivity Confirmation for Nasopharyngeal Swabs

Concentration	N1		N2		RP	
	Detection Rate	Avg Ct Value	Detection Rage	Avg Ct Value	Detection Rate	Avg Ct Value
1 x LoD	100% (20/20)	31.39	100% (20/20)	33.58	100% (20/20)	25.89

Table 13. Analytical Sensitivity Confirmation for Saliva Samples

Concentration	N1		N2		RP	
	Detection Rate	Avg Ct Value	Detection Rage	Avg Ct Value	Detection Rate	Avg Ct Value
1 x LoD	100% (20/20)	32.02	100% (20/20)	33.76	100% (20/20)	28.70

2. Inclusivity/ Analytical Specificity

Inclusivity:
BLASTN analysis query alignments were performed with the SARS-CoV-2 N1 and N2 oligonucleotide primer and probe sequences with all publicly available nucleic acid sequences for 2019-nCoV in GenBank to demonstrate the predicted inclusivity of Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx. All the alignments show 100% identity to the available 2019-nCoV sequences.

Analytical Specificity (Cross-reactivity):
Cross-reactivity of Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx was evaluated using both in silico analysis and wet testing against normal and pathogenic organisms found in the respiratory tract. BLASTN analysis queries of Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx primers and probes were performed against the database of pathogens in the same genetic family and against organisms that are likely to be in the circulating area, including human sequences. The list of organisms included in the cross-reactivity matching analysis is shown in Table 14 below. Matching was performed using "Complete Genome" sequences in GenBank, using the BLASTN algorithm, with default parameters being changed to Max Target Sequence of 20000, expected threshold of 1000, word size 15, filtering "Low Complexity Regions" and "Mask For Lookup Table" turned on and automatic adjusting of parameters for short input sequences was turned off. The search was limited to sequences with a 100% query cover and percent ID from 80% to 100%.

There is cross-reactivity in the N1 and N2 primers/probes to the SARS-CoV database, however this cross-reactivity is only against one sequence, which is the bat coronavirus RaTG13 (Accession: MN996532.1), the ancestor of the SARS-CoV-2. None of the N1 primer/probes are present together in any of the organisms which are likely to be in the circulating area. Similarly, none of the N2 primer/probes are present together in any of the organisms which are likely to be in the circulating area. Only RP gene primers/probes aligned to the human sequences. Primers and probes of Norgen's 2019-nCoV TaqMan RT-PCR Kit align only to the sequence of the ancestor viral pathogen of SARS-CoV-2. They do not align to organisms that are likely to be in the circulating area or to human sequences.

Table 14: List of Organisms included in the Cross-Reactivity Matching Analysis

Group	Pathogen/Organism
Pathogens in the same genetic family	Human coronavirus 229E
	Human coronavirus OC43
	Human coronavirus HKU1
	Human coronavirus NL63
	SARS-coronavirus
	MERS-coronavirus
Organisms that are likely to be in the circulating area	Actinomycetes
	Alphacoronavirus
	bacteria
	Bordetella pertussis
	Chlamydophila pneumoniae
	Enterovirus & Rhinovirus
	Fungi
	Haemophilus influenzae
	Haemophilus parainfluenzae
	Herpes simplex virus 1
	Human adenovirus
	Human metapnemonovirus
	Human papillomavirus
	Influenza A virus
	Influenza B virus
	Leginonella
	Mollicutes
	Mycobacterium
	Mycoplasma pneumonia
	Pneumocystis jiroveci
	Pseudomonas aeruginosa
	Staphylococcus
	Streptococcus pneumoniae
	Streptococcus pyogenes

To test the analytical specificity of Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx, the 2019-nCoV RT-PCR Positive Control Dx (containing the two nCoV nucleocapsid target gene RNA (N1 and N2) and RNase P) was used to test the kits specificity against RNA purified from other related pathogens.

As can be seen in Table 15 below, only Norgen's 2019-nCoV RT-PCR Positive Control showed amplification for all genes. COVID-19 WA showed amplification of the N1 and N2 gene targets. Therefore, Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx can be used to specifically detect, confirm and discriminate COVID-19

Table 15: Pathogens Tested for SARS-CoV-2 Specificity

Sample	N1	N2	RP
2019-nCoV RT-PCR Positive Control	Positive	Positive	Positive
SARS-CoV	Not detected	Not detected	Not detected
MERS-CoV	Not detected	Not detected	Not detected
Human CoV-229E	Not detected	Not detected	Not detected
Human CoV-RPP30	Not detected	Not detected	Not detected
Human CoV-NL63	Not detected	Not detected	Not detected
Influenza A virus (H1N1)	Not detected	Not detected	Not detected
Influenza A virus (H3N2	Not detected	Not detected	Not detected
Influenza B virus	Not detected	Not detected	Not detected
Human RSV	Not detected	Not detected	Not detected

3. Precision

A. Initial Study

To generate initial precision data for the 2019-nCoV TaqMan RT-PCR Kit Dx, contrived nasopharyngeal swab samples were collected and preserved in Norgen's Total Nucleic Acid Preservative Tubes (Cat. #D69200). Nasal swabs were chosen due to the fact that they represent the most challenging matrix for isolation and testing. Collected swabs were spiked with 5 µL of one of 3 different concentrations of the 2019-nCoV RT-PCR Positive Control to generate input samples of 3 variable transcript content, which resulted in corresponding three ranges of transcript concentration in isolated RNA: High (1,000 copies/µL RNA), Mid (100 copies/µL RNA) and Low (10 copies/µL RNA). RNA was then isolated and used as a template in precision testing, using 5 replicates and performed on 3 instruments over 5 days. Data analysis was carried out by calculating the mean Ct value, standard deviation and coefficient of variation percentage.

Precision was determined as repeatability (one instrument in one day using 5 repeats of each concentration), precision between days (one instrument, 5 days using 5 repeats of each of the 3 concentrations) and precision between instruments (3 instruments, 5 days using 5 repeats of each of the 3 concentration).

3.A.1 Repeatability

Repeatability was measured by analyzing data from one instrument in one day. Data analysis showed consistent results within the same experimental session.

Table 16: Repeatability (one instrument, one day using 5 repeats of each of the 3 concentrations)

Gene	Concentration	N	Mean Value	SDEV	% CV
N1 gene	High	5	25.34	0.17	0.67
	Mid	5	28.92	0.19	0.64
	Low	5	31.27	0.15	0.48
N2 gene	High	5	26.13	0.20	0.75
	Mid	5	29.77	0.07	0.23
	Low	5	32.03	0.22	0.70
RP gene	High	5	21.42	0.17	0.80
	Mid	5	24.20	0.01	0.05
	Low	5	26.45	0.13	0.51

3.A.2 Precision Between Days

Precision between various experimental sessions was measured by analyzing data from one instrument over 5 days. Data analysis showed consistent results from day-to-day.

Table 17. Precision between days (one instrument, 5 days using 5 repeats of each of the 3 concentrations)

Gene	Concentration	N	Mean Value	SDEV	% CV
N1 gene	High	25	26.06	0.22	0.84
	Mid	25	29.65	0.27	0.92
	Low	25	32.23	0.16	0.50
N2 gene	High	25	26.86	0.20	0.74
	Mid	25	30.58	0.21	0.68
	Low	25	32.89	0.14	0.43
RP gene	High	25	21.43	0.16	0.73
	Mid	25	24.39	0.14	0.56
	Low	25	27.04	0.05	0.20

3.A.3 Precision Between Instruments

Precision between instruments was measured by analyzing data from all three instruments over 5 days. Data analysis showed consistent results from the different instruments over time.

Table 18: Precision between instruments (3 instruments, 5 days using 5 repeats of each of the 3 concentration)

Gene	Concentration	N	Mean Value	SD	% CV
N1	High	75	26.11	0.14	0.55
	Mid	75	29.94	0.41	1.38
	Low	75	32.27	0.21	0.65
N2	High	75	26.92	0.14	0.53
	Mid	75	30.78	0.34	1.10
	Low	75	33.29	0.64	1.93
RP	High	75	21.50	0.10	0.45
	Mid	75	24.22	0.32	1.33
	Low	75	26.84	0.34	1.26

B. Final Study

To generate final precision data for the 2019-nCoV TaqMan RT-PCR Kit Dx, contrived nasopharyngeal swab samples were collected and preserved in Norgen's Total Nucleic Acid Preservative Tubes (Cat. #D69200). Nasal swabs were chosen due to the fact that they represent the most challenging matrix for isolation and testing. Collected swabs were spiked with 5 µL of one of 3 different concentrations of the 2019-nCoV RT-PCR Positive Control to generate input samples of 3 variable transcript content, which resulted in corresponding three ranges of transcript concentration in isolated RNA: High (1,000 copies/µL RNA), Mid (100 copies/µL RNA) and Low (10 copies/µL RNA). RNA was then isolated and used as a template in precision testing, using 2 replicates and performed in 2 run per day over 20 days. Data analysis was carried out by calculating the mean Ct value, standard deviation and coefficient of variation percentage.

Precision was determined as repeatability (analysis of all 80 replicates), precision between days (analysis of data generated per each of the 20 days) and precision between runs (analysis of data generated per each of the 40 runs).

3.B.1 Repeatability

Repeatability was measured by analyzing data obtained from all replicates. Data analysis showed consistent results over all data points.

Table 19: Repeatability (one instrument, 80 replicates over 40 runs in 20 days)

Gene	Concentration	N	Mean value	SDEV	%CV
N1	High	80	26.21	1.00	3.81
	Mid	80	29.64	1.10	3.72
	Low	80	32.30	1.17	3.63
N2	High	80	27.15	1.62	5.96
	Mid	80	30.72	1.57	5.11
	Low	80	33.22	1.38	4.16
RP	High	80	21.65	0.43	2.01
	Mid	80	24.45	0.54	2.22
	Low	80	26.86	0.32	1.20

3.B.2 Precision Between Days

Precision between days was measured by analyzing data generated from the two sessions of each day over 20 days. Data analysis showed consistent results from day-to-day.

Table 20. Precision between days (one instrument, 20 days)

Gene	Concentration	N	Mean value	SDEV	%CV
N1	High	20	26.20	1.00	3.82
	Mid	20	29.64	1.12	3.76
	Low	20	32.30	1.08	3.34
N2	High	20	27.15	1.43	5.26
	Mid	20	30.72	1.58	5.16
	Low	20	33.22	1.40	4.20
RP	High	20	21.64	0.42	1.94
	Mid	20	24.45	0.55	2.24
	Low	20	26.86	0.31	1.17

3.B.3 Precision Between runs

Precision between runs was measured by analyzing data generated from the 40 runs. Data analysis showed consistent results from run-to-run.

Table 21: Precision between runs (one instrument, 40 runs)

Gene	Concentration	N	Mean value	SDEV	%CV
N1	High	40	26.21	1.00	3.80
	Mid	40	29.64	1.11	3.73
	Low	40	32.30	1.11	3.42
N2	High	40	27.15	1.49	5.49
	Mid	40	30.72	1.57	5.10
	Low	40	33.22	1.38	4.16
RP	High	40	21.64	0.43	1.98
	Mid	40	24.45	0.54	2.22
	Low	40	26.86	0.32	1.18

4. Accuracy

Clinical evaluation of the accuracy of the 2019-nCoV TaqMan RT-PCR Kit Dx was conducted with contrived nasopharyngeal swabs, oropharyngeal swabs and saliva samples by testing 30 positive and 30 negative samples to generate the Positive Percentage Agreement (PPA), Negative Percentage Agreement (NPA) and overall percentage agreement (OPA) as a measurement of estimated Diagnostic Accuracy. For the 30 contrived positive samples, each were spiked with 5 µL of different concentrations of the 2019-nCoV RT-PCR Positive Control to generate input

samples of variable transcript content that corresponds to a limit of detection (LoD) range from 1X to 1,000X (10 samples at 1X, 10 samples at 2X, 4 samples at 10X, 3 samples at 100X and 3 samples at 1,000X). The remaining 30 samples from each sample type were not spiked (non-reactive). RNA isolation was performed from all samples using Norgen's Saliva/Swab RNA Purification Kit (Cat. #69100) and RNA was eluted in 50 µL. Five microliters of the isolated RNA were used as a template in the Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx to detect the 3 targets of the kits (N1, N2 and RP).

As it can be seen in Table 22 below, the various SARS-CoV-2 kit targets can be detected from RNA isolated from contrived nasopharyngeal swabs, oropharyngeal swabs and saliva samples, at various detection limits using Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx with no detectable viral targets from non-reactive samples.

Table 22: Accuracy of the 2019-nCoV TaqMan RT-PCR Kit Dx

	Contrived samples					
	Nasopharyngeal		Oropharyngeal		Saliva	
	Positive	Negative	Positive	Negative	Positive	Negative
Positive	30	0	30	0	30	0
Negative	0	30	0	30	0	30
	PPA	NPA	PPA	NPA	PPA	NPA
	100	100	100	100	100	100
	Overall Percentage Agreement					
	100		100		100	

Product Use Restriction

Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx is an *in vitro* diagnostic test for the qualitative detection of SARS-CoV-2 specific RNA using real-time hybridization-fluorescence detection. Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx provides SARS-CoV-2 detection based on the assays and protocols developed by the Centers for Disease Control and Prevention (CDC). The assay is designed for use with RNA isolated from nasopharyngeal swabs, oropharyngeal swabs and saliva samples collected from individuals with clinical signs/symptoms related to SARS-CoV-2 infection for *in vitro* diagnostic use.

Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx is intended for use by professional users including clinical laboratory personnel experienced and trained in molecular biological techniques including real-time PCR and *in vitro* diagnostic procedures.

Good laboratory practice is essential for the proper performance of this kit. Ensure that the purity of the kit and reactions is maintained at all times, and closely monitor all reagents for contamination. Do not use any reagents that appear to be contaminated.

Ensure that appropriate specimen collection, transport, storage and processing techniques are followed for optimal performance of this test. The presence of PCR inhibitors may cause false negative or invalid results.

Potential mutations within the target regions of the SARS-CoV-2 genome covered by the primers in this kit may result in failure to detect the presence of the pathogen.

As with any diagnostic test, results generated using Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx should be interpreted with regard to other clinical or laboratory findings.

The respective user is liable for any and all damages resulting from application of Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx for use deviating from the intended use as specified in the user manual.

All products sold by Norgen Biotek are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately. The kit contents are for laboratory use only, and they must be stored in the laboratory and must not be used for purposes other than intended. The kit contents are unfit for consumption.

Label Legend

								
Do not reuse	Use by	Batch Code	Catalogue Number	Contains sufficient for <n> tests	Manu-facturer	In Vitro Diagnostic Medical Device	Consult instructions for use	Temper-ature limitation

Authorized Representative

Technical Support

Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

Norgen Biotek Corp.
3430 Schmon Parkway, Thorold, ON Canada L2V 4Y6
Phone: (905) 227-8848
Fax: (905) 227-1061
Toll Free in North America: 1-866-667-4362

