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2019-nCoV TaqMan RT-PCR Kit Dx

Product Insert

REF **DxTM67120** CE IVD PIDxTM67120-9

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. 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.

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.

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.

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.

Kit Components

Component	Product # DxTM67120 (500 reactions)
2019-nCoV_N1 Probe/Primer Mix Dx	850 µL
2019-nCoV_N2 Probe/Primer Mix Dx	850 µL
RNAse P Probe/Primer Mix Dx	850 µL
2019-nCoV RT-PCR Positive Control Dx - 200,000 copies/µL	500 µL
2X One-Step RT-PCR Master Mix Dx	20 x 1 mL
Nuclease-Free Water (Negative control)	10 x 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
- RN 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 and endogenous substances were tested and determined not to interfere with the performance of the kit:
    - Nasopharyngeal swabs: blood, mucin, Chloraseptic, NasoGEL, Afrin, Sore Throat phenol spray and Fluticasone Propionate.
    - Oropharyngeal swabs: blood, mucin and sputum.

  - Saliva: blood, mucin sputum, amylase, hemoglobin, IgA, protein, eating, drinking, chewing gum, rinsing with mouse wash and smoking.
- The impact of antipyretic analgesics, antitussives, expectorants, antibiotics, antivirals and corticosteroids have not been evaluated.
- The performance of this device has not been assessed in a population vaccinated against COVID-19

Instructions for Use

A. Sample Stability and Handling Information

Nasopharyngeal swabs and oropharyngeal swabs should be collected into Norgen's Total Nucleic Acid Preservation Tubes Dx (Cat# Dx69200). Saliva samples should be collected into Norgen's Saliva RNA Preservation Devices Dx (Cat# 53800). The RNA (including viral RNA) in the preserved samples is stable for up to 2 months at ambient temperature (20-27°C) without any detectable RNA degradation.

For information on how to safely collect samples please refer to the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19); https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html.

B. 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

D. 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**	**50°C**	**30 min**
Cycle 2	**Step 1**	**95°C**	**3 min**
Cycle 3 (45x)	**Step 1**	**95°C**	**3 sec**
	Step 2	**55°C**	**30 sec**

E. 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

F. 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 heat inactivated SARS-CoV-2 particles. Contrived nasopharyngeal swabs, oropharyngeal swabs and saliva samples were generated by spiking 5 µL of different concentrations of the heat inactivated SARS-CoV-2 viral particles to generate input samples of variable viral particle count. 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

SARS-CoV-2 Viral Particles	N1		N2		RP	
	Average Ct Value	SDEV	Average Ct Value	SDEV	Average Ct Value	SDEV
0	N/A	N/A	N/A	N/A	30.49	0.03
1	N/A	N/A	N/A	N/A	30.51	0.05
5	38.95	0.69	38.54	1.83	31.23	0.05
10	34.86	0.09	35.62	0.12	30.03	0.08
100	31.02	0.02	30.98	0.03	30.02	0.10
1000	27.42	0.03	27.20	0.02	31.49	0.04
Positive Ctrl	20.66	0.15	18.00	0.22	14.56	0.79

Table 9. Analytical Sensitivity for Nasopharyngeal Swabs

SARS-CoV-2 Viral Particles	N1		N2		RP	
	Average Ct Value	SDEV	Average Ct Value	SDEV	Average Ct Value	SDEV
0	N/A	N/A	N/A	N/A	27.85	0.04
1	N/A	N/A	N/A	N/A	27.89	0.03
5	38.68	1.82	38.95	0.69	27.88	0.12
10	33.15	0.09	34.86	0.09	27.68	0.06
100	29.27	0.05	31.02	0.02	27.94	0.09
1000	25.76	0.04	27.42	0.03	27.89	0.13
Positive Ctrl	16.80	0.49	20.66	0.15	18.63	0.35

Table 10. Analytical Sensitivity for Saliva Samples

SARS-CoV-2 Viral Particles	N1		N2		RP	
	Average Ct Value	SDEV	Average Ct Value	SDEV	Average Ct Value	SDEV
0	N/A	N/A	N/A	N/A	25.53	0.06
1	N/A	N/A	N/A	N/A	28.57	0.14
5	37.79	2.97	38.06	1.12	26.86	0.02
10	35.47	0.13	35.70	0.15	26.85	0.10
100	32.59	0.06	33.10	0.06	26.96	0.15
1000	27.83	0.04	28.39	0.11	26.36	0.07
Positive Ctrl	16.19	0.44	17.16	0.46	15.92	0.15

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 heat-inactivated SARS-CoV-2 viral particles 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 (10 viral copies)	100% (20/20)	33.83	100% (20/20)	34.63	100% (20/20)	29.39

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 (10 viral copies)	100% (20/20)	32.42	100% (20/20)	33.75	100% (20/20)	26.80

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 (10 viral copies)	100% (20/20)	35.31	100% (20/20)	34.07	100% (20/20)	25.55

2. Inclusivity/ Analytical Specificity

Inclusivity:

Primers and probes were input into BLASTN offered through NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) updated on July 1, 2021. Primers were each aligned to the database of every SARS_CoV2 (taxid:2697049) "Complete Genome" sequence in GenBank, using the megablast algorithm, with default parameters being changed to Max Target Sequence of 5000, expected threshold of 1000, word size 16, filtering "Low Complexity Regions" and "Mask

For Lookup Table" turned on and automatic adjusting of parameters for short input sequences was turned off. Every other parameter was left at blast default. To allow for no mismatching, the search was limited to those with a query cover of 100% and percent ID of 100%. To account for 1 mismatch, the search was limited to those with a query cover of 100% and percent ID of up to 99.99%. For the variant analysis sequences were aligned using Clustal Omega using the ClustalW algorithm, allowing for 0 and 1 mismatch. The sequences of the England Variant, lineage B.1.1.7 (GISAID: EPI_ISL_581117), Brazil Variant, lineage B.1.1.248 (GISAID: EPI_ISL_792680), South Africa, lineage B.1.351 (GISAID: EPI_ISL_678597) the Nigerian Variant, lineage B.1.525 (GISAID: EPI_ISL_1168768) California Variant, lineage B.1.429 (GISAID: EPI_ISL_1335868) and the Indian Variant, lineage B.1.617.2 (GISAID: EPI_ISL_2832106). The designated specific primers and probes of Norgen's 2019-nCoV TaqMan RT-PCR Kit have a 100% alignment to SARS-CoV-2 sequences. Primers and probes of the human RP gene (internal control) show 0 matching to SARS-CoV-2 sequences. The number of entries aligning to SARS-CoV-2 did not change between 0 and 1 mismatch for the N1, however that number has increased for the N2 at 1 mismatch. As the assay result interpretation is dependent on a matching signal (either positive or negative) from both the N1 and N2, therefore any potential alignment of the N2 primers due to a single mismatch will not affect the kit's ability to accurately call for positive or negative result. The variant analysis illustrates that the designed primers and probes can target the new variants as of July 1, 2021 of SARS-CoV-2. The alignment scores for the 2019-nCoV TaqMan RT-PCR Kit to the variants can be seen in Table 15 and Table 16, which shows that the alignment of primers & probes is identical between variants as well as the initial SARS-CoV-2 Wuhan strain. This analysis can then conclude that all three kits have the same efficacy of detection to the new emerging variants.

Table 14: Inclusivity analysis at 0 and 1 mismatch.

Target	Primer	At 0 mismatch		At 1 mismatch	
		Entries aligning to SARS-CoV-2 (taxid 2697049)	% homology	Entries aligning to SARS-CoV-2 (taxid 2697049)	% homology
N1	2019-nCoV_N1-F	2731	100%	2731	100%
	2019-nCoV_N1-R	2730	100%	2730	100%
	2019-nCoV_N1-P	2731	100%	2731	100%
N2	2019-nCoV_N2-F	2750	100%	3536	100%
	2019-nCoV_N2-R	2753	100%	3533	100%
	2019-nCoV_N2-P	2745	100%	3527	100%
RP	RNAse-P_F	0	0%	0	0%
	RNAse-P_R	0	0%	0	0%
	RNAse-P_P	0	0%	0	0%

Table 15: Variant analysis allowing for 0 mismatch:

Target	Primer	England Variant EPI_ISL _581117	Brazil Variant EPI_ISL_7 92680	South Africa Variant EPI_ISL_6785 97	Nigerian Variant EPI_ISL_1 168768	California Variant EPI_ISL_1 335868	Indian Variant EPI_ISL_ 2832106
N1	2019-nCoV_N1-F	100%	100%	100%	100%	100%	100%
	2019-nCoV_N1-R	100%	100%	100%	100%	100%	100%
	2019-nCoV_N1-P	100%	100%	100%	100%	100%	100%
N2	2019-nCoV_N2-F	100%	100%	100%	100%	100%	100%
	2019-nCoV_N2-R	100%	100%	100%	100%	100%	100%
	2019-nCoV_N2-P	100%	100%	100%	100%	100%	100%
RP	RNAse-P_F	0%	0%	0%	0%	0%	0%
	RNAse-P_R	0%	0%	0%	0%	0%	0%
	RNAse-P_P	0%	0%	0%	0%	0%	0%

Table 16: Variant analysis allowing for 1 mismatch:

Target	Primer	England Variant EPI_ISL _581117	Brazil Variant EPI_ISL_7 92680	South Africa Variant EPI_ISL_6785 97	Nigerian Variant EPI_ISL_1 168768	California Variant EPI_ISL_1 335868	Indian Variant EPI_ISL_ 2832106
N1	2019-nCoV_N1-F	100%	100%	100%	100%	100%	100%
	2019-nCoV_N1-R	100%	100%	100%	100%	100%	100%
	2019-nCoV_N1-P	100%	100%	100%	100%	100%	100%
N2	2019-nCoV_N2-F	100%	100%	100%	100%	100%	100%
	2019-nCoV_N2-R	100%	100%	100%	100%	100%	100%
	2019-nCoV_N2-P	100%	100%	100%	100%	100%	100%
RP	RNAse-P_F	0%	0%	0%	0%	0%	0%
	RNAse-P_R	0%	0%	0%	0%	0%	0%
	RNAse-P_P	0%	0%	0%	0%	0%	0%

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 17 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 17: 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: **Contains ALL Actinomycetes subspecies**
	Alphacoronavirus: **Contains ALL Alphacoronavirus variants**
	bacteria: **Contains entire bacterial database**
	Bordetella pertussis
	Chlamydophila pneumoniae
	Enterovirus & Rhinovirus
	Fungi: **Contrains entire fungal database**
	Haemophilus influenzae
	Haemophilus parainfluenzae
	Herpes simplex virus 1
	Human adenovirus
	Human metapnemonovirus
	Human papillomavirus
	Influenza A virus
	Influenza B virus
	Influenza C virus
	Leginonella: **Includes ALL Legionella subspecies**
	Mollicutes: **Includes ALL Mollicutes subspecies**
	Mycobacterium: **Includes ALL Mycobacterium subspecies**
	Mycoplasma pneumonia
	Parechovirus
	Pneumocystis jiroveci
	Pseudomonas aeruginosa
	Staphylococcus: **Includes ALL Staphylococcous subspecies**
	Streptococcus pneumoniae

	Streptococcus pyogenes
	Parainfluenza virus 1
	Parainfluenza virus 2
	Parainfluenza virus 3
	Parainfluenza virus 4
	Streptococcus salivarius
	Human respiratory synncytial virus A
	Human respiratory synncytial virus B
	Mycobacterium tuberculosis
	Candida albicans
	Staphylococcus epidermidis
	Adenoviridae: **Includes ALL Adenovirus variants**

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 other related pathogens. The test was performed on both nasopharyngeal and saliva samples at minimum pathogen concentrations of 10^6 CFU/mL for bacteria and 10^5 PFU/mL for viruses. For pathogens that did not have high concentration to achieve 10^6 CFU/mL (for bacteria) or 10^5 PFU/mL (or $TCID_{50}$, for viruses), a maximum volume of 200 µL was used to spike 800 µL of collected preserved material (swab or saliva). In some cases, genomic RNA or DNA was used instead of the whole organism, and the concentration used is shown in the tables below.

As can be seen in Tables 18 and 19 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 18: Pathogens Tested for SARS-CoV-2 Specificity from nasopharyngeal samples

#	Pathogen	Material Type	Final Concentration Used	N1	N2	RNaseP
1	Positive Control (Norgen)	RNA transcript	4 x 10^6 copies/mL	Positive	Positive	Positive
2	SARS-CoV-2 (COVID-19 WA)	Heat inactivated Viral particle	1.68 x 10^6 genome copies/mL (2.58 x 10^7 $TCID_{50}$/mL based on concentration before heat inactivation)	Positive	Positive	Positive
3	Human cov-229E	Genomic RNA	1.04 x 10^6 genome copies/mL	Negative	Negative	Positive
4	Influenza B virus	Genomic RNA	3.16 x 10^6 genome copies/mL	Negative	Negative	Positive
5	Influenza A virus (H3N2)	Genomic RNA	1.56 x 10^8 genome copies/mL	Negative	Negative	Positive
6	Influenza A virus (H1N1)	Genomic RNA	2.76 x 10^7 genome copies/mL	Negative	Negative	Positive

7	Human cov-NL63	Genomic RNA	2.72×10^6 genome copies/mL	Negative	Negative	Positive
8	Human RSV	Genomic RNA	1.08×10^9 genome copies/mL	Negative	Negative	Positive
9	Betacoronavirus 1 (OC43)	Viral particle	1.0×10^5 $TCID_{50}$/mL	Negative	Negative	Positive
10	Human Coronavirus HKU1	Genomic RNA	2.16×10^6 genome copies/mL	Negative	Negative	Positive
11	Adenovirus C1 71	Genomic DNA	4.48×10^8 genome copies/mL	Negative	Negative	Positive
12	Human Metapneumovirus	Genomic RNA	1.32×10^6 genome copies/mL	Negative	Negative	Positive
13	Human Parainfluenza Virus 2	Viral particle	1.6×10^5 $TCID_{50}$/mL	Negative	Negative	Positive
14	Human Parainfluenza Virus 3	Viral particle	6.4×10^5 $TCID_{50}$/mL	Negative	Negative	Positive
15	Enterovirus D68	Viral particle	3.56×10^5 $TCID_{50}$/mL	Negative	Negative	Positive
16	*Haemophilus influenzae*	Bacterial cells	4.16×10^6 cfu/mL	Negative	Negative	Positive
17	*Legionella pneumophila*	Bacterial cells	$> 0.8 \times 10^4$ cfu/mL	Negative	Negative	Positive
18	*Streptococcus pneumoniae*	Bacterial cells	1.12×10^6 cfu/mL	Negative	Negative	Positive
19	Human coronavirus 229E	Viral particle	1.0×10^5 $TCID_{50}$/mL	Negative	Negative	Positive
20	Human adenovirus 5	Viral particle	1.0×10^7 NIU/mL	Negative	Negative	Positive
21	Influenza A (H1N1)	Viral particle	1.0×10^5 PFU/mL	Negative	Negative	Positive
22	Influenza A (H3N2)	Viral particle	1.0×10^5 $TCID_{50}$/mL	Negative	Negative	Positive
23	Influenza B (Victoria)	Viral particle	2.0×10^6 $TCID_{50}$/mL	Negative	Negative	Positive
24	Influenza B (Yamagata)	Viral particle	1.39×10^6 $TCID_{50}$/mL	Negative	Negative	Positive
25	Human respiratory syncytial virus	Viral particle	1.75×10^4 PFU/mL	Negative	Negative	Positive
26	*Chlamydophila pneumoniae*	Bacterial cells	1.0×10^6 IFU/mL	Negative	Negative	Positive
27	*Bordetella pertussis*	Bacterial cells	$> 0.25 \times 10^4$ CFU/mL	Negative	Negative	Positive
28	*Candida albicans*	Yeast cells	1.0×10^6 CFU/mL	Negative	Negative	Positive
29	*Pseudomonas aeruginosa*	Bacterial cells	1.0×10^6 CFU/mL	Negative	Negative	Positive
30	*Staphylococcus epidermidis*	Bacterial cells	1.14×10^6 CFU/mL	Negative	Negative	Positive
31	Human parechovirus 2	Viral particle	2.78×10^6 $TCID_{50}$/mL	Negative	Negative	Positive
32	Corynebacterium diphtheria	Bacterial cells	1.0×10^6 CFU/mL	Negative	Negative	Positive

33	Legionella longbeachae	Bacterial cells	1.0×10^6 CFU/mL	Negative	Negative	Positive
34	*Leptospira interrogans*	Bacterial cells	Not available	Negative	Negative	Positive
35	*Moraxella (Branhamella) catarrhalis*	Bacterial cells	> 0.25×10^4 CFU/mL	Negative	Negative	Positive
36	*Neisseria meningitidis*	Bacterial cells	> 1.0×10^4 CFU/mL	Negative	Negative	Positive
37	*Staphylococcus aureus*	Bacterial cells	1.67×10^7 CFU/mL	Negative	Negative	Positive

Table 19: Pathogens Tested for SARS-CoV-2 Specificity from saliva samples

#	Pathogen	Material Type	Final Concentration Used	N1	N2	RNaseP
1	Positive Control (Norgen)	RNA transcript	4×10^6 copies/mL	Positive	Positive	Positive
2	SARS-CoV-2 (COVID-19 WA)	Heat inactivated Viral particle	1.68×10^6 genome copies/mL (2.58×10^7 $TCID_{50}$/mL based on concentration before heat inactivation)	Positive	Positive	Positive
3	Human cov-229E	Genomic RNA	1.04×10^6 genome copies/mL	Negative	Negative	Positive
4	Influenza B virus	Genomic RNA	3.16×10^6 genome copies/mL	Negative	Negative	Positive
5	Influenza A virus (H3N2)	Genomic RNA	1.56×10^8 genome copies/mL	Negative	Negative	Positive
6	Influenza A virus (H1N1)	Genomic RNA	2.76×10^7 genome copies/mL	Negative	Negative	Positive
7	Human cov-NL63	Genomic RNA	2.72×10^6 genome copies/mL	Negative	Negative	Positive
8	Human RSV	Genomic RNA	1.08×10^9 genome copies/mL	Negative	Negative	Positive
9	Betacoronavirus 1 (OC43)	Viral particle	1.0×10^5 $TCID_{50}$/mL	Negative	Negative	Positive
10	Human Coronavirus HKU1	Genomic RNA	2.16×10^6 genome copies/mL	Negative	Negative	Positive
11	Adenovirus C1 71	Genomic DNA	4.48×10^8 genome copies/mL	Negative	Negative	Positive
12	Human Metapneumovirus	Genomic RNA	1.32×10^6 genome copies/mL	Negative	Negative	Positive
13	Human Parainfluenza Virus 2	Viral particle	1.6×10^5 $TCID_{50}$/mL	Negative	Negative	Positive
14	Human Parainfluenza Virus 3	Viral particle	6.4×10^5 $TCID_{50}$/mL	Negative	Negative	Positive
15	Enterovirus D68	Viral particle	3.56×10^5 $TCID_{50}$/mL	Negative	Negative	Positive
16	*Haemophilus influenzae*	Bacterial cells	4.16×10^6 cfu/mL	Negative	Negative	Positive

17	*Legionella pneumophila*	Bacterial cells	> 0.8 x 10^4 cfu/mL	Negative	Negative	Positive
18	*Streptococcus pneumoniae*	Bacterial cells	1.12 x 10^6 cfu/mL	Negative	Negative	Positive
19	Human coronavirus 229E	Viral particle	1.0 x 10^5 $TCID_{50}$/mL	Negative	Negative	Positive
20	Human adenovirus 5	Viral particle	1.0 x 10^7 NIU/mL	Negative	Negative	Positive
21	Influenza A (H1N1)	Viral particle	1.0 x 10^5 PFU/mL	Negative	Negative	Positive
22	Influenza A (H3N2)	Viral particle	1.0 x 10^5 $TCID_{50}$/mL	Negative	Negative	Positive
23	Influenza B (Victoria)	Viral particle	2.0 x 10^6 $TCID_{50}$/mL	Negative	Negative	Positive
24	Influenza B (Yamagata)	Viral particle	1.39 x 10^6 $TCID_{50}$/mL	Negative	Negative	Positive
25	Human respiratory syncytial virus	Viral particle	1.75 x 10^4 PFU/mL	Negative	Negative	Positive
26	*Chlamydophila pneumoniae*	Bacterial cells	1.0 x 10^6 IFU/mL	Negative	Negative	Positive
27	*Bordetella pertussis*	Bacterial cells	> 0.25 x 10^4 CFU/mL	Negative	Negative	Positive
28	*Candida albicans*	Yeast cells	1.0 x 10^6 CFU/mL	Negative	Negative	Positive
29	*Pseudomonas aeruginosa*	Bacterial cells	1.0 x 10^6 CFU/mL	Negative	Negative	Positive
30	*Staphylococcus epidermidis*	Bacterial cells	1.14 x 10^6 CFU/mL	Negative	Negative	Positive
31	Human parechovirus 2	Viral particle	2.78 x 10^6 $TCID_{50}$/mL	Negative	Negative	Positive
32	Corynebacterium diphtheria	Bacterial cells	1.0 x 10^6 CFU/mL	Negative	Negative	Positive
33	Legionella longbeachae	Bacterial cells	1.0 x 10^6 CFU/mL	Negative	Negative	Positive
34	*Leptospira interrogans*	Bacterial cells	Not available	Negative	Negative	Positive
35	*Moraxella (Branhamella) catarrhalis*	Bacterial cells	> 0.25 x 10^4 CFU/mL	Negative	Negative	Positive
36	*Neisseria meningitidis*	Bacterial cells	> 1.0 x 10^4 CFU/mL	Negative	Negative	Positive
37	*Staphylococcus aureus*	Bacterial cells	1.67 x 10^7 CFU/mL	Negative	Negative	Positive

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 20: 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 21. 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 22: 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 23: 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 24. 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 25: 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. Robustness

Robustness studies were performed to determine the effect of the following substances on the performance of the kit:

A- Endogenous substances for all samples: sputum, blood and mucin.

B- Exogenous substances for Nasopharyngeal swabs: Chloraseptic, NasoGEL, Afrin, Sore Throat phenol spray and Fluticasone Propionate.

C- Exogenous substances for Saliva: amylase, hemoglobin, IgA, protein, eating, drinking, chewing gum, rinsing with mouse wash and smoking.

As it can be seen in Tables 26, 27 and 28 below the presence of endogenous and exogenous substances did not affect the detection of SARS-CoV-2 targets of the kit at 3X the limit of detection, from nasopharyngeal swabs, oropharyngeal swabs or saliva samples.

A- Endogenous Substances for All Samples

Biological samples of each Nasopharyngeal swabs (n=3), Oropharyngeal swabs (n=4) and Saliva (n=4) were collected from healthy donors. Swabs were preserved in Norgen's Total Nucleic Acid Preservative Tubes (Cat #69200) while saliva samples were collected and preserved in Norgen's Saliva RNA Collection and Preservation Devices (Cat. #RU53800). Nasopharyngeal swab tubes were spiked with blood and mucin. Oropharyngeal swab tubes and preserved saliva samples were spiked with blood, mucin and sputum. Water was used to generate control conditions from all specimens. Spiking of all conditions was done at 10% (v/v) final concentration. Tubes within each group were used for RNA isolation in triplicates after spiking with the heat inactivated SARS-CoV-2 to generate input samples that correspond to a limit of detection (LoD) of 3X. Isolated RNA was used as a template in Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx to detect the 3 targets of the kits (N1, N2 and RP).

Table 26: Assay robustness for nasopharyngeal swabs, oropharyngeal swabs and saliva samples with endogenous substances

Sample	Substance	N1		N2		RP	
		Average Ct value	SDEV	Average Ct value	SDEV	Average Ct value	SDEV
Nasopharyngeal	water	31.21	0.05	31.18	0.07	24.95	0.03
	mucin	36.90	0.30	35.48	0.26	27.93	0.38
	blood	32.55	0.03	32.37	0.13	24.07	0.03
Oropharyngeal	water	31.20	0.06	31.30	0.04	28.05	0.05
	sputum	31.05	0.02	31.29	0.18	26.02	0.07
	blood	32.07	0.06	32.29	0.11	24.27	0.09
	mucin	33.63	0.27	33.39	0.35	29.46	0.14
Saliva	water	32.52	0.10	32.77	0.14	22.36	0.08
	sputum	33.50	0.18	33.90	0.08	22.20	0.13
	blood	33.68	0.25	34.43	0.08	22.71	0.24
	mucin	37.68	0.61	35.77	0.48	23.50	0.21

B- Exogenous Substances for Nasopharyngeal Swabs

Nasopharyngeal swabs (n=7) were collected from healthy donors and preserved in Norgen's Total Nucleic Acid Preservative Tubes (Cat #69200). Exogenous substances were used to spike the collected tubes at the specified concentration in the following table:

Substance	Concentration
Mock	None
Chloraseptic	1.5 mg/mL
NasoGEL	5% (v/v)
Afrin	15% (v/v)
Sore Throat Phenol Spray	15% (v/v)
Fluticasone Propionate	5% (v/v)

Tubes within each group were used for RNA isolation in triplicates after spiking with the heat inactivated SARS-CoV-2 to generate input samples that correspond to a limit of detection (LoD) of 3X. The isolated RNA was used as a template in Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx to detect the 3 targets of the kits (N1, N2 and RP).

Table 27: Assay robustness for nasopharyngeal swabs with exogenous substances

Condition	N1		N2		RP	
	Average Ct value	SDEV	Average Ct value	SDEV	Average Ct value	SDEV
Mock	33.54	0.24	34.56	0.06	27.50	0.09
Chloraseptic	34.42	0.10	34.78	0.05	28.93	0.02
NasoGEL	34.78	0.05	34.74	0.08	28.62	0.14
Afrin	36.71	0.25	36.64	0.15	30.71	0.09
Sore Throat	32.79	0.15	33.19	0.09	28.41	0.06
Fluticasone Propionate	33.14	0.09	33.75	0.17	28.93	0.09
POS	15.34	0.07	17.38	0.01	15.20	0.04
NTC	N/A	N/A	N/A	N/A	N/A	N/A
Non-Spiked (no viral particles)	N/A	N/A	N/A	N/A	28.42	0.01

C- Exogenous Substances for Saliva Samples

Saliva samples (n=9) were collected from healthy donors and preserved in Norgen's Saliva RNA Collection and Preservation Devices (Cat. #RU53800). Exogenous substances/conditions were considered before collection (eating, drinking, chewing gum, rinsing with mouth wash or smoking) or used to spike the collected tubes at specified concentration (hemoglobin, IgA and protein), as per the following table:

Exogenous substance/condition	Concentration
Water	15% (v/v)
Hemoglobin	15% (v/v), 22.5 ug/uL
IgA	15% (v/v), 150 ug/mL
Protein (BSA)	15% (v/v), 7.5 ug/uL
Eating	Immediately before collection
Drinking	Immediately before collection
Chewing gum	Immediately before collection
Rinsing with mouthwash	Immediately before collection
Smoking	Immediately before collection

Tubes within each group were used for RNA isolation in triplicates after spiking with the heat inactivated SARS-CoV-2 to generate input samples that correspond to a limit of detection (LoD) of 3X. The isolated RNA was used as a template in Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx to detect the 3 targets of the kits (N1, N2 and RP).

Table 28: Assay robustness for saliva samples with exogenous substances

Condition	N1		N2		RP	
	Average Ct value	SDEV	Average Ct value	SDEV	Average Ct value	SDEV
Water	33.04	0.02	34.22	0.08	25.90	0.03
Hemoglobin	33.78	0.07	35.44	0.35	26.45	0.07
IgA	32.36	0.24	34.75	0.43	26.13	0.13
BSA	32.56	0.09	34.46	0.41	25.96	0.11
Eating	33.10	0.05	34.32	0.22	25.82	0.04
Drinking	34.40	0.37	36.28	0.26	25.24	0.08
Chewing Gum	31.47	0.05	32.46	0.11	27.58	0.05
Mouthwash	32.33	0.03	33.25	0.08	27.08	0.03
Smoking	32.78	0.17	33.91	0.09	26.69	0.06

5. 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 29 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 29: Accuracy of the 2019-nCoV TaqMan RT-PCR Kit Dx using Contrived Samples

	Contrived samples					
	Nasophareyngeal		Orophareyngeal		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	

6. Clinical Study

Final clinical evaluation of the 2019-nCoV TaqMan RT-PCR Kit Dx was conducted using 30 SARS-CoV-2 Positive Subjects and 30 SARS-CoV-2 Negative Subjects. From each donor, 4 matched samples were collected for a total of 240 samples. Each matched sample set consists of 1 nasopharyngeal swab and 1 oropharyngeal swab collected into Norgen's Total Nucleic Acid Preservative Tubes (Cat. #69200). The third matched sample is saliva collected into Norgen's Saliva RNA Collection and Preservation Devices (Cat. #53800). The fourth and final sample is a second nasopharyngeal swab collected into a CE marked and FDA approved sample collection container in order to verify the status of the samples. Swab sampling order and location were randomized.

RNA was isolated from the CE marked / FDA approved collection containers using the associated approved RNA isolation kit from the workflow. The isolated RNA was validated with a CE marked and FDA approved qRT-PCR SARS-CoV-2 detection kit. SARS-CoV-2 detection was conducted on the 60 nasopharyngeal samples to validate the samples as being true positive or true negative. Donors who were verified to be true negative did not exhibit any symptoms related to SARS-CoV-2 infection at the time of clinical sample (saliva, nasopharyngeal swab, oropharyngeal swab) collection. Donors who verified as true positive had self-reported symptoms related to SARS-CoV-2 infection.

RNA from the remaining 90 matched Positive samples (nasopharyngeal, oropharyngeal and saliva samples, collected on Norgen's preservatives) and 90 matched Negative samples (nasopharyngeal, oropharyngeal and saliva samples, collected on Norgen's preservatives) were then isolated utilizing Norgen's Saliva/Swab Total RNA Purification Kit (Cat. #69100). An input of 250 µL of each of the 180 samples was used and RNA was eluted in 50 µL. Five microliters of the isolated RNA were used as a template in Norgen's 2019-nCoV TaqMan RT-PCR Kit (Cat. #TM67100).

The resulting data was used to generate the Positive Percentage Agreement (PPA), Negative Percentage Agreement (NPA) and overall percentage agreement (OPA) as a measurement of estimated Diagnostic Accuracy. As it can be seen in the table below, the various SARS-CoV-2 kit targets can be detected from RNA isolated from clinical nasopharyngeal swabs, oropharyngeal swabs and saliva samples, using Norgen's 2019-nCoV TaqMan RT-PCR Kit Dx with no detectable viral targets from non-reactive samples.

Table 30: Accuracy of the 2019-nCoV TaqMan RT-PCR Kit Dx using Clinical Samples

	Clinical 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
	PPA 95% CI	NPA 95% CI	PPA 95% CI	NPA 95% CI	PPA 95% CI	NPA 95% CI
	88.6 – 100%	88.6 – 100%	88.6 – 100%	88.6 – 100%	88.6 – 100%	88.6 – 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. 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

		LOT	REF	Σ		IVD	i	
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.

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