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COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) Dx Product Insert

REF **DxTM67200** CE IVD PIDxTM67200-9

Intended Use

Norgen's COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) Dx is an in vitro diagnostic test for the qualitative detection of SARS-CoV-2 specific RNA using a multiplexed TaqMan® fluorescence detection assay (FAM and HEX/VIC). 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 COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) 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 COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) Dx includes 2X One-Step RT-PCR Master Mix and 2 primer/probe mixes, a positive control and a negative control (nuclease-free water). The first primer/probe mix is used for first line screening and contains the E gene/RP that targets the SARS-CoV-2, SARS-CoV and bat-SARS-related CoVs Envelope gene (E gene - FAM) in addition to the human RNase P transcript (RP - HEX/VIC) as an internal control target to monitor for PCR inhibition, and to validate the quality of the sample and the detection result. The second Primer/Probe Mix is only required as a confirmatory/discriminatory step with samples showing positive amplification of the E gene. This second Primer/Probe Mix is for the RdRP gene and detects two RNA-dependent RNA Polymerase (RdRP) targets where the first RdRP target is SARS-CoV-2 specific (FAM) while the second RdRP target is to detect SARS-CoV-2, SARS-CoV and bat-SARS-related CoVs (HEX). The provided E gene/RdRP/RP Positive Control contains an *in vitro* RNA transcript for the three SARS-related target genes: E gene, RdRP gene as well as the human RP gene (internal control).

Norgen's COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) 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 # DxTM67200 (500 reactions)
E gene/RP Primer & Probe Mix Dx	850 µL
RdRP gene Primer & Probe Mix Dx *	850 µL
E/RdRP/RP Positive Control Dx†	500 µL
2X One-Step RT-PCR Master Mix Dx	12 mL
Nuclease-Free Water (Negative control)	4 x 1.25 mL
Product Insert	1

***** Confirmatory/Discriminatory assay
† Contains an *in vitro* RNA transcript for the three SARS-related target genes: E gene, RdRP gene as well as the human RP (internal control).

Storage Conditions and Product Stability

- The COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) 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 (> 3 x) 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 and HEX/VIC filter channel
- RNA Purification Kit
    - Performance of Norgen's COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) 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
- Vortex mixer
- PCR reaction preparation station

Quality Control

In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) Dx is tested against predetermined specifications to ensure consistent product quality.

Warnings and Precautions

- Norgen's COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) 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.
- 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 COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) 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 COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) 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 COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) 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 COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) 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 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.**

C. 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 2X One-Step RT-PCR Master Mix Dx provided is enough for up to 500 RT-PCR reactions per each target.
- For every TaqMan One-Step RT-PCR run, one reaction containing E gene/RdRp/RP Positive Control Dx and one reaction as a no template control (NTC) must be included for proper interpretation of results. A minimum number of 10 samples are recommended to be tested per run per assay. Table 1 and Table 2 below show an example for the samples and the controls set-up for each assay.
- For SARS-CoV-2 detection, E gene/RP Primer & Probe Mix Dx is required for initial detection. RdRP gene Primer and Probe Mix Dx is then used in a separated RT-PCR reaction as a confirmatory/discriminatory assay to validate positive samples detected by the E gene.
- The kit is used in 2 stages; the first is to perform line screening to test if the sample is positive for SARS-CoV-2, SARS-CoV or bat-SARS-related CoVs. Positive samples are then used in a second PCR (Confirmatory/discriminatory PCR) to determine if the sample is SARS-CoV-2 positive or SARS-CoV or bat-SARS-related CoVs positive.

Table 1. Samples and Controls Set-up for the E gene/RP Assay

Assay	1	2	3	4	5	6	7	8	9	10	11	12
E gene/RP	NTC	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	Positive Control

Table 2. Samples and Controls Set-up for the RdRP gene Assay* (Confirmatory/Discriminatory Step)

Assay	1	2	3	4	5	6	7	8	9	10	11	12
RdRP genes	NTC	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	Positive Control

* Only samples showing positive E gene amplification should be confirmed by the RdRP gene assay in a separated RT-PCR reaction.

- To avoid any contamination while preparing the TaqMan One-step RT-PCR assay, follow the order outlined in Tables 3, 4 and 5 below to prepare the NTC, Detection Assays and E gene/RdRP/RP Positive control:
  1. Prepare the RT-PCR NTC (Table 3)
  2. Prepare the RT-PCR E gene/RP Assay or RdRP gene Assay (Table 4)
  3. Prepare the RT-PCR E gene/RdRP/RP Positive Control (Table 5)
- 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).

1. For each TaqMan One-step RT-PCR set, prepare no template control PCR reactions as shown in Table 3 below:

Table 3. TaqMan One-Step RT-PCR NTC Preparation

Reagent	Volume of Reagent Added per Reaction
Nuclease-Free Water	8.5 µL
2X One-Step RT-PCR Master Mix Dx	10 µL
E gene/RP Primer & Probe Mix Dx*	1.5 µL
Total Volume	20 µL

* The RdRP gene Primer & Probe Mix Dx can be used instead to validate positive samples detected by the E gene.

2. Prepare the RT-PCR reactions for sample detection as shown in Table 4 below.

Table 4. TaqMan One-Step RT-PCR Target Assays Preparation

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

* The RdRP gene Primer & Probe Mix Dx can be used instead to validate positive samples detected by the E gene.
+ The recommended amount of sample RNA to be used is 5 µL. However, 1 µL - 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 in case the volume of the sample RNA used is different from the volume shown in Table 4.

3. For each RT-PCR set, prepare positive control RT-PCR as shown in Table 5 below:

Table 5. TaqMan One-Step RT-PCR E gene/RdRP/RP Positive Control Preparation

Reagent	Vol. of Reagent Added per Reaction
2X One-Step RT-PCR Master Mix Dx	10 µL
E gene/RP Primer & Probe Mix Dx*	1.5 µL
E gene/RdRP/RP Positive Control Dx +	5 µL
Nuclease-Free Water	3.5 µL
Total Volume	20 µL

* The RdRP gene Primer & Probe Mix Dx can be used instead to validate positive samples detected by the E gene.

+ The positive control contains the SARS-CoV-2 E gene, RdRP gene and RNase P RNA fragments.

D. COVID-19 TaqMan One-Step RT-PCR Assay Programming

1. Program the thermocylcer according to the program shown in Table 6 below.
2. Run one step RT-PCR.

Table 6. COVID-19 TaqMan One-Step RT-PCR Program

One Step RT-PCR Cycle	Step	Temperature	Duration
Cycle 1	**Step 1**	**50ºC**	**20 min**
Cycle 2	**Step 1**	**95ºC**	**3 min**
Cycle 3 (45x)	**Step 1**	**95ºC**	**15 sec**
	Step 2	**58ºC**	**30 sec**

E. COVID-19 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 SARS-CoV-2 detection can be made. The assay must be repeated.
- The **E gene/RdRP/RP Positive Control Dx** reaction(s) should produce a positive result with an expected Ct value (< 40.00 Ct) for each target. If the positive control does not provide a positive result the run is not valid and no interpretation of SARS-CoV-2 detection can be made. The assay must be repeated.
- **Only samples showing a positive signal for E gene should be re-tested with RdRP gene for confirmation/discrimination.**
- Table 7 below shows the targets and specificity of the primer/probes used in this assay
- If the NTC and E gene/RdRP/RP Positive Control Dx are exhibiting the correct results, the results of the detection assays can be interpreted as outlined in Tables 8 and 9 below

Table 7. Target and Specificity of Primer/Probes

Assay	Target	Specificity
Initial Screening	E gene (FAM)	SARS-CoV-2, SARS-CoV and bat-SARS-related CoVs
	RP gene (HEX)	Human transcriptome
Confirmatory / Discriminatory	RdRP Confirmatory (FAM)	SARS-CoV-2
	RdRP Discriminatory (HEX)	SARS-CoV-2, SARS-CoV and bat-SARS-related CoVs

Table 8. Interpretation of Assay Results with E gene/RP Primer & Probe Mix

E gene (FAM)	RP (HEX)	Result
+	+	Potential SARS-CoV-2 Positive
+	-	Potential SARS-CoV-2 Positive
-	+	Negative
-	-	PCR inhibited

Table 9. Interpretation of Assay Results with RdRP gene Primer & Probe Mix

RdRP Confirmatory Detection (FAM)	RdRP Discriminatory Detection (HEX)	Result
+	+	SARS-CoV-2 Positive
-	+	SARS-CoV-2, SARS-CoV and bat-SARS-related CoVs Positive
-	-	Negative
+	-	Invalid PCR

F. Performance Evaluation

1. Analytical Sensitivity

A. Initial Study

The analytical sensitivity of the COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) Dx was determined by analyzing a dilution series of quantified SARS-CoV-2 heat inactivated viral particles. Contrived nasopharyngeal swabs, oropharyngeal swabs and saliva samples were generated by spiking 5 µL of different concentrations of the viral particles to generate input samples of variable transcript content. Triplicate samples were tested for each concentration for all 3 samples.

The limit of detection of Norgen's COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) Dx from RNA isolated from nasopharyngeal swabs, oropharyngeal swabs and saliva samples is 10 copies per PCR reaction as can be seen in Tables 10, 11 and 12 below.

Table 10. Analytical Sensitivity for Oropharyngeal Swabs

Viral particles/PCR reaction	E gene		RP gene		RdRP Confirmatory		RdRP Descriminatory	
	Average Ct Value	SDEV	Average Ct Value	SDEV	Average Ct Value	SDEV	Average Ct Value	SDEV
0.00	N/A	N/A	27.85	0.13	N/A	N/A	N/A	N/A
1.00	N/A	N/A	27.80	0.09	N/A	N/A	N/A	N/A
5.00	32.34	N/A	27.95	0.12	37.26	3.04	36.90	0.39
10.00	30.60	0.41	27.79	0.13	30.78	0.42	32.58	0.21
100.00	26.75	0.17	27.68	0.17	27.19	0.06	28.44	0.03
1000.00	23.54	0.28	27.68	0.15	24.23	0.04	25.09	0.04

Table 11. Analytical Sensitivity for Nasopharyngeal Swab

Viral particles/PCR reaction	E gene		RP gene		RdRP Confirmatory		RdRP Descriminatory	
	Average Ct Value	SDEV	Average Ct Value	SDEV	Average Ct Value	SDEV	Average Ct Value	SDEV
0.00	N/A	N/A	26.22	0.12	N/A	N/A	N/A	N/A
1.00	N/A	N/A	25.94	0.08	N/A	N/A	N/A	N/A
5.00	32.54	N/A	26.03	0.06	36.42	1.19	37.42	0.78
10.00	30.63	0.13	25.92	0.06	30.98	0.13	32.46	0.20
100.00	26.98	0.39	25.89	0.08	27.13	0.05	28.42	0.03
1000.00	23.64	0.12	25.82	0.09	24.16	0.03	25.07	0.01

Table 12. Analytical Sensitivity for Saliva Samples

Viral particles/PCR reaction	E gene		RP gene		RdRP Confirmatory		RdRP Descriminatory	
	Average Ct Value	SDEV	Average Ct Value	SDEV	Average Ct Value	SDEV	Average Ct Value	SDEV
0.00	N/A	N/A	27.86	0.13	N/A	N/A	N/A	N/A
1.00	N/A	N/A	27.71	0.34	N/A	N/A	N/A	N/A
5.00	33.71	1.60	27.65	0.11	36.80	1.81	36.67	N/A
10.00	30.85	0.04	27.66	0.10	30.62	0.07	32.71	0.30
100.00	23.60	0.07	27.37	0.04	27.20	0.07	28.50	0.06
1000.00	27.03	0.17	27.58	0.11	24.20	0.07	25.15	0.12

B. Confirmatory Study

The limit of detection of the COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) 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 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 13, 14 and 15 the limit of detection of Norgen's COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) 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 13. Analytical Sensitivity Confirmation for Oropharyngeal Swabs

Concentration	E gene		RP gene		RdRP Confirmatory		RdRP Discriminatory	
	Detection Rate	Avg Ct Value	Detection Rage	Avg Ct Value	Detection Rate	Avg Ct Value	Detection Rate	Avg Ct Value
1 x LoD	100% (20/20)	30.31	100% (20/20)	28.00	100% (20/20)	31.14	100% (20/20)	32.06

Table 14. Analytical Sensitivity Confirmation for Nasopharyngeal Swabs

Concentration	E gene		RP gene		RdRP Confirmatory		RdRP Discriminatory	
	Detection Rate	Avg Ct Value	Detection Rage	Avg Ct Value	Detection Rate	Avg Ct Value	Detection Rate	Avg Ct Value
1 x LoD	100% (20/20)	30.14	100% (20/20)	27.34	100% (20/20)	31.21	100% (20/20)	32.10

Table 15. Analytical Sensitivity Confirmation for Saliva Samples

Concentration	E gene		RP gene		RdRP Confirmatory		RdRP Discriminatory	
	Detection Rate	Avg Ct Value	Detection Rage	Avg Ct Value	Detection Rate	Avg Ct Value	Detection Rate	Avg Ct Value
1 x LoD	100% (20/20)	30.19	100% (20/20)	28.44	100% (20/20)	30.43	100% (20/20)	31.62

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 E gene assay components (E forward primer, reverse primer and probe) designed for broad screening showed 100% alignments to SARS-CoV-2 sequences and the six new variants, at both 0 and 1 mismatch. In the discriminatory/confirmatory assay, RdRP, the RdRP probe 2 showed 100% alignments to SARS-CoV-2 sequences and the six new variants at both 0 and 1 mismatch. The remaining assay components (forward primer, reverse primer and RdRP probe 1) showed lower alignment to SARS-CoV-2 sequences and all variants, as they are designed to detect broad spectrum of corona viruses. RP primers and probe show 0 matching to SARS-CoV-2 sequences as they are specific to the human transcriptome. The E gene assay and RdRP probe 2 of Norgen's COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) have a 100% alignment to SARS-CoV2 variants. RdRP forward and reverse primers and Probe 1 used in broad screening efficiently align to their designated targets. This analysis can then conclude that this kit has the same efficacy of detection to the new emerging variants.

Table 16: Inclusivity analysis at 0 and 1 mismatch.

Target	Primer	At 0 mismatch		At 1 mismatch	
		Entries aligned to SARS-CoV-2 (taxid 2697049)	% homology	Entries aligned to SARS-CoV-2 (taxid 2697049)	% homology
E gene	E_Sarbeco_Forward	2810	100%	2810	100%
	E_Sarbeco_Reverse	2786	100%	2786	100%
	E_Sarbeco_Probe	2785	100%	2785	100%
RdRP gene	RdRP_Forward	0	0%	2788	95%
	RdRP_Reverse	0	0%	2868	96%
	RdRP_Probe 1	0	0%	0	0%
	RdRP_Probe 2	2782	100%	2782	100%
RP gene	Rnase-P_Forward	0	0%	0	0%
	Rnase-P_Reverse	0	0%	0	0%
	Rnase-P_Probe	0	0%	0	0%

Table 17: Variant analysis allowing for 0 mismatch:

Target	Primer	England Variant EPI_ISL_581117	Brazil Variant EPI_ISL_792680	South Africa Variant EPI_ISL_678597	Nigerian Variant EPI_ISL_1168768	California Variant EPI_ISL_1335868	Indian Variant EPI_ISL_2832106
E gene	E_Sarbeco_Forward	100%	100%	100%	100%	100%	100%
	E_Sarbeco_Reverse	100%	100%	100%	100%	100%	100%
	E_Sarbeco_Probe	100%	100%	100%	100%	100%	100%
RdRP gene	RdRP_Forward	100%	100%	100%	100%	100%	0%
	RdRP_Reverse	0%	0%	0%	0%	0%	0%
	RdRP_Probe 1	0%	0%	0%	0%	0%	0%
	RdRP_Probe 2	100%	100%	100%	100%	100%	100%
RP gene	Rnase-P_Forward	0%	0%	0%	0%	0%	0%
	Rnase-P_Reverse	0%	0%	0%	0%	0%	0%
	Rnase-P_Probe	0%	0%	0%	0%	0%	0%

Table 18: Variant analysis allowing for 1 mismatch:

Target	Primer	England Variant EPI_ISL_581117	Brazil Variant EPI_ISL_792680	South Africa Variant EPI_ISL_678597	Nigerian Variant EPI_ISL_1168768	California Variant EPI_ISL_1335868	Indian Variant EPI_ISL_2832106
E gene	E_Sarbeco_Forward	100%	100%	100%	100%	100%	100%
	E_Sarbeco_Reverse	100%	100%	100%	100%	100%	100%
	E_Sarbeco_Probe	100%	100%	100%	100%	100%	100%
RdRP gene	RdRP_Forward	100%	100%	100%	100%	100%	95%
	RdRP_Reverse	96%	96%	96%	96%	96%	96%
	RdRP_Probe 1	0%	0%	0%	0%	0%	0%
	RdRP_Probe 2	100%	100%	100%	100%	100%	100%
RP gene	Rnase-P_Forward	0%	0%	0%	0%	0%	0%
	Rnase-P_Reverse	0%	0%	0%	0%	0%	0%
	Rnase-P_Probe	0%	0%	0%	0%	0%	0%

Analytical Specificity (Cross-reactivity):

Cross-reactivity of Norgen's COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) 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 COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) 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 19 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.

The E gene primers/probe has 248 alignments to the SARS-CoV database. The RdRP forward primer, reverse primer, and probe 1 have alignments to the SARS-CoV database, as per their design. The RdRP Probe 2, which is used as the confirmatory assay, only aligns to SARS-CoV-2, however there is also one alignment to the RaTG13 (Accession: MN996532.1), the ancestor of the SARS-CoV-2, therefore there is no potential cross reactivity in RdRP probe 2 to Human viruses. The RNase-P assay does not align to anything in the related genetic family, or the circulating area, it only aligns to human sequences. Primers and probes of Norgen's COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) Dx (Cat. #DxTM67200) align specifically to sequences of the SARS-CoV-2 and can discriminate SARS-CoV-2 from SARS-CoV. These primers and probes Do not align to sequences of other pathogens in the same genetic family, or organisms that are likely to be in the circulating area. Only RP gene primer/probe will align to human sequences.

Table 19: List of Organisms included in the Cross-Reactivity Matching Analysis and their GenBank taxid

Group	Pathogen/Organism	taxid
Pathogens in the same genetic family	Human coronavirus 229E	11137
	Human coronavirus OC43	31631
	Human coronavirus HKU1	290028
	Human coronavirus NL63	277944
	SARS-coronavirus	694009
	MERS-coronavirus	1335626
Organisms that are likely to be in the circulating area	Actinomycetes: **Contains ALL Actinomycetes subspecies**	1760
	Alphacoronavirus: **Contains ALL Alphacoronavirus variants**	693996
	bacteria: **Contains entire bacterial database**	2
	Bordetella pertussis	520
	Chlamydophila pneumoniae	83558
	Enterovirus & Rhinovirus	12059
	Fungi: **Contrains entire fungal database**	4751
	Haemophilus influenzae	727
	Haemophilus parainfluenzae	729
	Herpes simplex virus 1	10298
	Human adenovirus	1907210
	Human metapnemonovirus	162145
	Human papillomavirus	10566
	Influenza A virus	11320
	Influenza B virus	11520
	Influenza C virus	11552
	Leginonella: **Includes ALL Legionella subspecies**	445
	Mollicutes: **Inclues ALL Mollicutes subspecies**	31969
	Mycobacterium: **Includes ALL Mycobacterium subspecies**	1763
	Mycoplasma pneumonia	2104
	Parechovirus	138954

	Pneumocystis jiroveci	42068
	Pseudomonas aeruginosa	287
	Staphylococcus: **Includes ALL Staphylococcous subspecies**	1279
	Streptococcus pneumoniae	1313
	Streptococcus pyogenes	1314
	Parainfluenza virus 1	11210
	Parainfluenza virus 2	11213
	Parainfluenza virus 3	11217
	Parainfluenza virus 4	11226
	Streptococcus salivarius	1304
	Human respiratory synncytial virus A	208893
	Human respiratory synncytial virus B	208895
	Mycobacterium tuberculosis	1773
	Candida albicans	5476
	Staphylococcus epidermidis	1282
	Adenoviridae: **Includes ALL Adenovirus variants**	10508

To test the analytical specificity of Norgen's COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) Dx, the E/RdRP/RP Positive Control was used in a 20 µL RT-PCR reaction to test specificity against genomic RNA of other related pathogens. Testing was done in triplicates and used pathogens spiked into both nasopharyngeal samples preserved in Norgen's Total Nucleic Acid Preservative Tubes (Cat #69200) and saliva samples collected and preserved in Norgen's Saliva RNA Collection and Preservation Devices (Cat. #RU53800). Pathogen concentrations were a minimum 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. Amplification was measured by real time RT-PCR. The E gene/RdRP/RP Positive Control reaction(s) was considered a positive result with an expected Ct value (<40 Ct) for each target.

As can be seen in Table 20 below, only Norgen's E/RdRP/RP Positive Control and COVID-19 WA showed amplification for all genes. None of the remaining pathogens showed amplification with any of the tested genes. Therefore, Norgen's COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) Dx can be used to specifically detect, confirm and discriminate COVID-19.

Table 20: Pathogens Tested for SARS-CoV-2 Specificity from nasopharyngeal and saliva samples

#	Pathogen	Material Type	Final Concentration Used	E	RdRp-1	RdRp-2	RNaseP
1	Positive Control (Norgen)	RNA transcript	4×10^6 copies/mL	Positive	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	Positive
3	Human cov-229E	Genomic RNA	1.04×10^6 genome copies/mL	Negative	Negative	Negative	Positive
4	Influenza B virus	Genomic RNA	3.16×10^6 genome copies/mL	Negative	Negative	Negative	Positive
5	Influenza A virus (H3N2)	Genomic RNA	1.56×10^8 genome copies/mL	Negative	Negative	Negative	Positive
6	Influenza A virus (H1N1)	Genomic RNA	2.76×10^7 genome copies/mL	Negative	Negative	Negative	Positive
7	Human cov-NL63	Genomic RNA	2.72×10^6 genome copies/mL	Negative	Negative	Negative	Positive
8	Human RSV	Genomic RNA	1.08×10^9 genome copies/mL	Negative	Negative	Negative	Positive
9	Betacoronavirus 1 (OC43)	Viral particle	1.0×10^5 $TCID_{50}$/mL	Negative	Negative	Negative	Positive
10	Human Coronavirus HKU1	Genomic RNA	2.16×10^6 genome copies/mL	Negative	Negative	Negative	Positive
11	Adenovirus C1 71	Genomic DNA	4.48×10^8 genome copies/mL	Negative	Negative	Negative	Positive
12	Human Metapneumovirus	Genomic RNA	1.32×10^6 genome copies/mL	Negative	Negative	Negative	Positive
13	Human Parainfluenza Virus 2	Viral particle	1.6×10^5 $TCID_{50}$/mL	Negative	Negative	Negative	Positive
14	Human Parainfluenza Virus 3	Viral particle	6.4×10^5 $TCID_{50}$/mL	Negative	Negative	Negative	Positive
15	Enterovirus D68	Viral particle	3.56×10^5 $TCID_{50}$/mL	Negative	Negative	Negative	Positive
16	*Haemophilus influenzae*	Bacterial cells	4.16×10^6 cfu/mL	Negative	Negative	Negative	Positive
17	*Legionella pneumophila*	Bacterial cells	$> 0.8 \times 10^4$ cfu/mL	Negative	Negative	Negative	Positive

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

3. Precision

A. Initial Study

To generate initial precision data for the COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) Dx, contrived nasopharyngeal swab samples were collected and preserved in Norgen's Total Nucleic Acid Preservative Tubes (Cat. #69200). 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 E/RdRP/RP 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 21: Repeatability (one instrument, one day using 5 repeats of each concentration)

Gene	Concentration	N	Mean Value	SDEV	% CV
E gene	High	5	22.36	0.06	0.25
	Mid	5	25.91	0.36	1.40
	Low	5	28.64	0.04	0.15
RP gene	High	5	21.43	0.05	0.24
	Mid	5	24.64	0.27	1.08
	Low	5	27.35	0.05	0.20
RdRP Confirmatory	High	5	24.08	0.12	0.49
	Mid	5	27.49	0.10	0.37
	Low	5	30.77	0.09	0.28
RdRP Discriminatory	High	5	21.37	0.11	0.54
	Mid	5	24.88	0.10	0.41
	Low	5	28.10	0.07	0.26

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

Gene	Concentration	N	Mean Value	SDEV	% CV
E gene	High	25	22.37	0.08	0.36
	Mid	25	25.53	0.21	0.82
	Low	25	28.48	0.14	0.50
RP gene	High	25	21.31	0.20	0.95
	Mid	25	24.33	0.21	0.88
	Low	25	27.20	0.24	0.90
RdRP Confirmatory	High	25	23.73	0.22	0.94
	Mid	25	27.14	0.22	0.81
	Low	25	30.52	0.28	0.92
RdRP Discriminatory	High	25	21.14	0.13	0.62
	Mid	25	24.54	0.16	0.67
	Low	25	28.07	0.42	1.49

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

Gene	Concentration	N	Mean Value	SDEV	% CV
E gene	High	75	22.30	0.07	0.30
	Mid	75	25.00	0.10	0.38
	Low	75	28.56	0.06	0.22
RP gene	High	75	21.38	0.05	0.23
	Mid	75	24.43	0.07	0.28
	Low	75	27.26	0.04	0.16
RdRP Confirmatory	High	75	23.90	0.12	0.50
	Mid	75	27.30	0.11	0.42
	Low	75	30.61	0.06	0.21
RdRP Discriminatory	High	75	21.28	0.10	0.46
	Mid	75	24.70	0.12	0.49
	Low	75	28.02	0.04	0.15

B. Final Study

To generate final precision data for the COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) Dx, contrived nasopharyngeal swab samples were collected and preserved in Norgen's Total Nucleic Acid Preservative Tubes (Cat. #69200). 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 E/RdRP/RP 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 24: Repeatability (one instrument, 80 replicates over 40 runs in 20 days)

Gene	Concentration	N	Mean Value	SDEV	% CV
E gene	High	80	22.42	0.45	2.00
	Mid	80	26.14	0.56	2.12
	Low	80	28.41	0.33	1.15
RP gene	High	80	21.43	0.44	2.07
	Mid	80	24.47	0.37	1.52
	Low	80	27.09	0.28	1.05
RdRP Confirmatory	High	80	21.28	0.57	2.68
	Mid	80	24.79	0.60	2.42
	Low	80	27.82	0.45	1.60
RdRP Discriminatory	High	80	23.96	0.65	2.71
	Mid	80	27.47	0.51	1.84
	Low	80	30.55	0.51	1.66

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

Gene	Concentration	N	Mean Value	SDEV	% CV
E gene	High	20	22.42	0.06	0.27
	Mid	20	26.14	0.12	0.46
	Low	20	28.41	0.07	0.24
RP gene	High	20	21.43	0.06	0.27
	Mid	20	24.47	0.12	0.48
	Low	20	27.09	0.05	0.20
RdRP Confirmatory	High	20	21.28	0.11	0.52
	Mid	20	24.79	0.10	0.42
	Low	20	27.82	0.14	0.51
RdRP Discriminatory	High	20	23.96	0.15	0.64
	Mid	20	27.47	0.14	0.51
	Low	20	30.55	0.18	0.59

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 26: Precision between runs (one instrument, 40 runs)

Gene	Concentration	N	Mean Value	SDEV	% CV
E gene	High	40	22.42	0.70	3.11
	Mid	40	26.14	0.09	0.36
	Low	40	28.41	0.06	0.22
RP gene	High	40	21.43	0.70	3.28
	Mid	40	24.47	0.09	0.38
	Low	40	27.09	0.05	0.17
RdRP Confirmatory	High	40	21.28	0.73	3.44
	Mid	40	24.79	0.08	0.33
	Low	40	27.82	0.11	0.40
RdRP Discriminatory	High	40	23.96	0.77	3.22
	Mid	40	27.47	0.12	0.43
	Low	40	30.55	0.15	0.48

4. Substance Interference Studies

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 27, 28 and 29 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 COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) Dx to detect the 4 targets of the kit (E gene, RdRP and RP).

Table 27: Assay robustness for nasopharyngeal swabs, oropharyngeal swabs and saliva samples with endogenous substances

Sample	Substance	E-gene		RdRP Confirmatory		RdRP Discriminatory		RP	
		Average Ct value	SDEV	Average Ct value	SDEV	Average Ct value	SDEV	Average Ct value	SDEV
Nasopharyngeal	water	31.14	0.08	30.50	0.07	30.23	0.06	28.87	0.13
	mucin	31.58	0.05	30.98	0.04	30.71	0.03	29.37	0.08
	blood	31.12	0.06	30.49	0.05	30.27	0.03	28.87	0.09
Oropharyngeal	water	31.11	0.09	30.40	0.03	30.25	0.05	28.91	0.10
	sputum	31.05	0.11	30.32	0.06	30.17	0.06	28.82	0.07
	blood	30.90	0.04	30.30	0.05	30.17	0.04	28.84	0.20
	mucin	31.35	0.07	30.64	0.26	30.57	0.08	29.13	0.08
Saliva	water	31.02	0.03	30.48	0.09	30.29	0.08	28.96	0.16
	sputum	30.92	0.08	30.43	0.09	30.22	0.07	28.75	0.10
	blood	30.89	0.07	30.30	0.03	30.17	0.05	28.89	0.13
	mucin	31.23	0.25	30.76	0.07	30.58	0.03	29.15	0.18

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 COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) Dx to detect the 4 targets of the kit (E gene, RdRP and RP).

Table 28: Assay robustness for nasopharyngeal swabs with exogenous substances

Condition	E-gene		RdRP Confirmatory		RdRP Descriminatory		RP	
	Average Ct value	SDEV	Average Ct value	SDEV	Average Ct value	SDEV	Average Ct value	SDEV
Mock	30.99	0.05	30.77	0.04	31.90	0.05	28.84	0.20
Chloraseptic	31.34	0.12	30.37	0.03	31.50	0.01	29.13	0.04
NasoGEL	31.32	0.00	30.35	0.07	31.48	0.12	29.21	0.05
Afrin	31.37	0.04	30.38	0.04	31.51	0.05	29.13	0.02
Sore Throat Phenol Spray	30.98	0.04	30.75	0.13	31.91	0.12	28.88	0.08
Fluticasone Propionate	30.96	0.10	30.71	0.01	31.86	0.06	28.71	0.13
POS	11.46	0.07	10.87	0.06	12.23	0.08	9.57	0.11
NTC	N/A	N/A	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	N/A	N/A	28.25	0.07

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 COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) Dx to detect the 4 targets of the kit (E gene, RdRP and RP).

Table 29: Assay robustness for saliva samples with exogenous substances

Condition	E-gene		RdRP Confirmatory		RdRP Descriminatory		RP	
	Average Ct value	SDEV	Average Ct value	SDEV	Average Ct value	SDEV	Average Ct value	SDEV
Water	31.22	0.12	31.03	0.11	30.80	0.13	29.07	0.07
Hemoglobin	31.17	0.10	30.98	0.11	30.76	0.11	28.98	0.10
IgA	31.17	0.05	30.98	0.05	30.72	0.05	29.00	0.14
BSA	31.06	0.05	30.86	0.05	30.56	0.04	28.90	0.15
Eating	31.03	0.06	30.83	0.06	30.50	0.05	28.81	0.15
Drinking	31.56	0.02	31.36	0.02	31.08	0.03	29.34	0.05
Chewing Gum	31.53	0.06	31.33	0.06	31.08	0.01	29.31	0.12
Mouthwash	31.19	0.07	31.00	0.07	30.64	0.07	28.94	0.16
Smoking	31.06	0.03	30.86	0.04	30.55	0.06	28.90	0.11

5. Clinical Study

Clinical evaluation of the accuracy of the COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) Dx was conducted with matched nasopharyngeal swabs, oropharyngeal swabs and saliva samples collected from 30 Positive Subjects and 30 Negative Subjects to generate the Positive Percentage Agreement (PPA), Negative Percentage Agreement (NPA) and overall percentage agreement (OPA) as a measurement of estimated Diagnostic Accuracy. 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 COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) Dx to detect the 4 targets of the kit (E gene, RdRP and RP).

As it can be seen in Table 30 below, the various SARS-CoV-2 kit targets can be detected from RNA isolated from nasopharyngeal swabs, oropharyngeal swabs and saliva samples using Norgen's COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) Dx with no detectable viral targets from non-reactive samples.

Table 30: Accuracy of the COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) Dx

	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 COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) Dx is an *in vitro* diagnostic test for the qualitative detection of SARS-CoV-2 specific RNA using a multiplexed TaqMan® fluorescence detection assay (FAM and HEX/VIC) based on the Charité/Berlin protocol. 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 COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) 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.

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 COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) 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 COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) 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

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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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