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

REF **DxTM67200** CE IVD PIDxTM67200-3

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) 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.

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).

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.

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 substances were tested and determined not to interfere with the performance of the kit: nasopharyngeal swabs – blood and mucin; oropharyngeal swabs and saliva – blood, mucin sputum
- The impact of antipyretic analgesics, antitussives, expectorants, antibiotics, antivirals and corticosteroids have not been evaluated.

Instructions for Use

A. Sample Preparation

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

Purified RNA is the starting material for Norgen's 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.**

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 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.

C. 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**	**50°C**	**20 min**
Cycle 2	**Step 1**	**95°C**	**3 min**
Cycle 3 (45x)	**Step 1**	**95°C**	**15 sec**
	Step 2	**58°C**	**30 sec**

D. 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

E. 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 RNA transcripts. Contrived nasopharyngeal swabs, oropharyngeal swabs and saliva samples were generated by spiking 5 µL of different concentrations of the E/RdRP/RP Positive Control (200,000 copies/uL) 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) 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

Copies/PCR reaction	E gene		RP gene		RdRP Confirmatory		RdRP Discriminatory	
	Average Ct Value	SDEV	Average Ct Value	SDEV	Average Ct Value	SDEV	Average Ct Value	SDEV
0	N/A	N/A	33.48	1.09	N/A	N/A	N/A	N/A
1	N/A	N/A	33.80	1.22	N/A	N/A	N/A	N/A
10	33.50	0.38	29.66	0.12	34.83	0.24	29.90	0.21
100	28.39	0.08	27.75	0.31	27.88	0.22	27.68	0.21
1,000	23.38	0.09	24.05	0.03	24.48	0.10	24.31	0.05
10,000	19.84	0.14	20.58	0.04	20.82	0.11	20.55	0.15
100,000	16.49	0.20	17.34	0.15	17.26	0.14	17.18	0.12

Table 11. Analytical Sensitivity for Nasopharyngeal Swabs

Copies/PCR reaction	E gene		RP gene		RdRP Confirmatory		RdRP Discriminatory	
	Average Ct Value	SDEV	Average Ct Value	SDEV	Average Ct Value	SDEV	Average Ct Value	SDEV
0	N/A	N/A	29.43	0.26	N/A	N/A	N/A	N/A
1	N/A	N/A	29.46	0.45	N/A	N/A	N/A	N/A
10	30.64	0.37	29.31	0.53	32.42	0.02	29.90	0.21
100	27.69	0.11	26.95	0.68	28.73	0.10	27.68	0.21
1,000	23.86	0.20	24.27	0.14	25.39	0.29	24.31	0.05
10,000	20.38	0.21	21.21	0.04	21.31	0.07	20.55	0.15
100,000	16.69	0.27	17.61	0.17	17.41	0.14	17.18	0.12

Table 12. Analytical Sensitivity for Saliva Samples

Copies/PCR reaction	E gene		RP gene		RdRP Confirmatory		RdRP Discriminatory	
	Average Ct Value	SDEV	Average Ct Value	SDEV	Average Ct Value	SDEV	Average Ct Value	SDEV
0	N/A	N/A	25.42	0.29	N/A	N/A	N/A	N/A
1	N/A	N/A	24.76	0.20	N/A	N/A	N/A	N/A
10	31.11	0.38	24.72	0.20	33.59	0.49	32.67	0.22
100	27.13	0.30	24.55	0.18	28.40	0.27	28.40	0.27
1,000	24.05	0.41	24.00	0.04	25.17	0.50	24.95	0.55
10,000	20.32	0.28	20.93	0.32	21.28	0.19	21.04	0.38
100,000	17.18	0.24	17.75	0.05	17.80	0.21	17.69	0.23

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 E/RdRP/RP Positive Control corresponding to 10 copies per PCR reaction. Confirmatory results were acceptable at a 95% confidence interval. This can be achieved when obtaining a minimum of 19 positive samples out of the 20 samples spiked at the limit of detection. As seen in Tables 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)	31.72	100% (20/20)	31.67	100% (20/20)	35.58	100% (20/20)	35.56

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)	31.95	100% (20/20)	25.22	100% (20/20)	33.44	100% (20/20)	33.54

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)	28.31	100% (20/20)	25.43	100% (20/20)	30.57	100% (20/20)	30.76

2. Inclusivity / Analytical Specificity

Inclusivity:
BLASTN analysis query alignments were performed with the SARS-CoV-2 E gene and RdRP oligonucleotide primer and probe sequences with all publicly available nucleic acid sequences for 2019-nCoV in GenBank to demonstrate the predicted inclusivity of Norgen's COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) Dx. All the alignments show 100% identity to the available 2019-nCoV sequences.

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 16 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 high cross-reactivity in the E gene primers/probe and the RdRP primers and probe 1 to the SARS-CoV database, however this is expected based on the design of the primers, which have a mismatch of 1 or 2 nucleotides to be able to detect the SARS RdRP gene, with RdRP probe 1 designed to allow for 4 mismatches. The RdRP probe 2 has only one alignment to the SARS family, which is the bat coronavirus RaTG13 (Accession: MN996532.1), the ancestor of the SARS-CoV-2. None of the probes and primers of the kit aligned to the other Betacoronavirus pathogens or any or the other organisms included in the analysis. Only RP gene primers/probe aligned to human sequences. Primers and probes of Norgen's COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) Dx align to sequences of the SARS-CoV, but not to sequences of pathogens in the same genetic family, or organisms that are likely to be in the circulating area. As per the kit design, only RP gene primer/probe will align to human sequences.

Table 16: List of Organisms included in the Cross-Reactivity Matching Analysis

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

To test the analytical specificity of Norgen's COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) Dx, the E/RdRP/RP Positive Control was used to test the kits specificity against RNA purified from other related pathogens. RdRP reactions were only prepared for samples required for confirmation and discriminatory detection based on the first E gene detection result. Amplification was measured by real time RT-PCR.

As can be seen in Table 17 below, only Norgen's E/RdRP/RP Positive Control showed amplification for all genes. COVID-19 WA showed amplification of the E gene and the two RdRP gene targets. SARS-CoV showed amplification only with E gene and RdRP Discriminatory. 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 17: Pathogens Tested for SARS-CoV-2 Specificity

Sample	E gene	RP gene	RdRP Confirmatory	RdRP Discriminatory
COVID-19 Positive Control	Positive	Positive	Positive	Positive
COVID-19 WA	Positive	Not detected	Positive	Positive
Human cov-RPP30	Not detected	Not detected	Not detected	Not detected
MERS-CoV	Not detected	Not detected	Not detected	Not detected
SARS-CoV	Positive	Not detected	Not detected	Positive
Human cov-229E	Not detected	Not detected	Not detected	Not detected
Influenza B virus	Not detected	Not detected	Not detected	Not detected
Influenza A virus	Not detected	Not detected	Not detected	Not detected
Influenza A virus (H1N1)	Not detected	Not detected	Not detected	Not detected
Human cov-NL63	Not detected	Not detected	Not detected	Not detected
Human RSV	Not detected	Not detected	Not detected	Not detected

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. #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 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 18: 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 19. 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 20: 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. #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 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 21: 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 22. 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 23: 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. Accuracy

Clinical evaluation of the accuracy of the COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) 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 E/RdRP/RP 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 COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) Dx to detect 3 targets of the kit (E gene, RdRP and RP).

As it can be seen in Table 24 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 COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) with no detectable viral targets from non-reactive samples.

Table 24: Accuracy of the COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) Dx

	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	

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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