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NoV TaqMan RT-PCR Kit Dx

Product Insert

REF DxTM41400 CE IVD PIDxTM41400-2

Intended Use

Norgen's NoV TaqMan RT-PCR Kit Dx is an *in vitro* diagnostic test for the detection and quantification of NoV specific RNA using real-time hybridization-fluorescence detection.

Background Information

Norovirus (NoV) is a single-stranded, non-enveloped RNA virus belonging to the Caliciviridae family. Norovirus is considered the major causative agent of non-bacterial gastroenteritis in the world. The main channel of transmittance is via contaminated food or water, as well as human-to-human contact. Most Noroviruses infecting humans belong to genogroup I and II (particularly genotype 4). Norovirus is very stable in the environment and is resistant to some surface disinfectants such as alcohols and detergents. Moreover, it is very difficult to culture Norovirus in vitro, making its identification by standard microbiological assays challenging.

Product Description

Norgen's NoV TaqMan RT-PCR Kit Dx is an *in vitro* diagnostic test for the detection and quantification of NoV specific RNA using real-time hybridization-fluorescence detection. The kit includes a PCR control to monitor for PCR inhibition, and to validate the quality of the sample and the detection result. The NoV TaqMan RT-PCR Kit Dx comprises Master Mix for the target and PCR control detection, Primer & Probe Mix, as well as a positive control and a negative control (nuclease-free water) to confirm the integrity of the kit reagents.

Norgen's NoV TaqMan RT-PCR Kit Dx is based on real-time PCR technology, utilizing RT-PCR for the amplification of specific target sequences and target specific probes for the detection of the amplified cDNA. The probes are labelled with fluorescent reporter and quencher dyes. Probes specific for NoV are labelled with the fluorophore FAM. The probe specific for the PCR control is labelled with the fluorophore HEX. Using probes linked to distinguishable dyes enables the parallel detection of NoV specific cDNA and the PCR control in corresponding detector channels of the real-time PCR instrument.

The supplied positive control can be used individually, or it can be diluted to generate a standard curve that can be used to determine the concentration of NoV in the sample. To generate the standard curve the following concentrations are used:

Standard Sample	Copy Number Per 5 µL	Note
A	1.00E+06	Supplied concentration
B	1.00E+05	Dilution
C	1.00E+04	Dilution
D	1.00E+03	Dilution
E	1.00E+02	Dilution
F	1.00E+01	Dilution

Norgen's NoV TaqMan RT-PCR Kit Dx was developed and validated to be used with the following real-time PCR instruments:

- Qiagen Rotor-Gene Q
- BioRad CFX96 Touch™ Real-Time PCR Detection System

Kit Components

Component	Product # DxTM41400 (24 preps)
MDx TaqMan 2X RT-PCR Master Mix Dx	550 µL
NoV Primer & Probe Mix Dx	2 x 70 µL
NoV Positive Control Dx – 200,000 copies/µL	50 µL
Nuclease-Free Water	2 x 1.25 mL
Product Insert	1

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

Storage Conditions and Product Stability

- The NoV 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, please 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.

Quality Control

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

Warnings and Precautions

- Norgen's NoV TaqMan RT-PCR Kit Dx is intended for use by professional users such as technicians and biologists experienced and trained in molecular biological techniques including 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 NoV 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 NoV 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.

Customer-Supplied Reagents and Equipment

- Appropriate Real-Time PCR Instrument with FAM and HEX filter channel
- RNA Purification Kit
    - The kit is compatible with all RNA purification kits that yield high quality, inhibitor-free RNA
    - **Recommended Purification Kit:**
        - Norgen's Stool Total RNA Purification Kit Dx- Cat# Dx49500
        - Norgen's Total RNA Purification Kit Dx - Cat# Dx17200
        - Norgen's Plasma/Serum Circulating and Exosomal RNA Purification Kit Dx (Slurry Format) - Cat# Dx42800
- Disposable powder-free gloves
- Benchtop microcentrifuge
- Micropipettors
- Sterile pipette tips with filters
- PCR tubes
- Vortex mixer
- PCR tube centrifuge
- PCR reaction preparation station (Optional)

Procedure

A. Sample Preparation

Purified RNA is the starting material for Norgen's NoV 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 Stool Total RNA Purification Kit Dx (Cat# Dx49500), Norgen's Total RNA Purification Kit Dx (Cat# Dx17200) and Norgen's Plasma/Serum Circulating and Exosomal RNA Purification Kit Dx (Slurry Format) (Cat# Dx42800).** Norgen's Dx series of purification kits have been fully validated with Norgen's TaqMan RT-PCR kits for *in vitro* diagnostic testing.

If using a different spin column based sample preparation procedure that includes ethanol-based wash buffers, a column drying step consisting of centrifugation for 10 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, mixed by gentle vortexing or by pipetting, and centrifuged briefly.
- Work quickly on ice.
- The recommended minimum number of RNA samples tested per TaqMan RT-PCR run is 8.
- The amount of MDx TaqMan 2X RT-PCR Master Mix Dx provided is enough for up to 45 PCR reactions (3 x 8 sample PCR reactions, 3 x 6 positive control serial dilution PCR reactions and 3 no template control PCR reactions).
- For every TaqMan RT-PCR run, one reaction containing NoV Positive Control (or optionally 6 reactions for the provided positive control and 5 serial dilutions for qualitative PCR) and one reaction as no template control must be included for proper interpretation of results.
- For quantitative PCR, a standard curve should be generated using the provided Positive Control and 5 dilutions from the positive control (6 PCR reactions).

i. Reaction Setup for Qualitative Analysis

1. Prepare the Reaction Master Mix by combining the PCR components in a clean tube according to the following pipetting scheme:

PCR component	1 reaction	*11 reactions
Nuclease-Free Water	3 µL	33 µL
MDx TaqMan 2X RT-PCR Master Mix	10 µL	110 µL
NoV Primer & Probe Mix	2 µL	22 µL
Total Volume	**15 µL**	**165 µL**

* The volume of the Reaction Master Mix depends on the number of samples to be prepared.

2. Mix well by pipetting or gentle vortexing. Spin briefly to collect any liquid in the tube cap.
3. Pipette 15 µL of the Reaction Master Mix into the appropriate PCR tubes.

4. Add 5 µL of the Negative Control, Sample or Positive Control, and pipette to mix. A minimum of one positive control and one negative control wells should be used.
5. Cap the tubes with the appropriate optical lid and spin briefly at 3000 RPM for a few seconds.

Note: To avoid any contamination while adding the templates, ensure that they are added in the follow the order:

1. Negative Control
2. Test Sample
3. Positive Control

ii Reaction Setup for Quantitative Analysis

1. Prepare the Reaction Master Mix by combining the PCR components in a clean tube according to the following pipetting scheme:

PCR component	1 reaction	*16 reactions
Nuclease-Free Water	3 µL	48 µL
MDx TaqMan 2X RT-PCR Master Mix	10 µL	160 µL
NoV Primer & Probe Mix	2 µL	32 µL
Total Volume	**15 µL**	**240 µL**

* The volume of the Reaction Master Mix depends on the number of samples to be prepared.

2. Mix well by pipetting or gentle vortexing. Spin briefly to collect any liquid in the tube cap.
3. Pipette 15 µL of the Reaction Master Mix into the appropriate PCR tubes.
4. Add 5 µL of the Negative Control or Sample, and pipette to mix. A minimum of one negative control well should be used.
5. Cap the tubes with the appropriate optical lid and spin briefly at 3000 RPM for a few seconds.
6. Prepare a serial dilution of the Positive Control Dx (200,000 copies/µL), according to the following steps:
   a. Prepare 5 clean tubes containing 45 µL of Nuclease-Free water.
   b. Use 5 µL of the Positive Control Standard supplied with the kit (200,000 copies/µL) to prepare 5 serial dilutions as outlined in Figure 1.
   c. Mix well by vortexing or pipetting after each transfer and before moving to the next transfer.
   d. Add 5 µL of the original supplied concentration as well as the 5 prepared serial dilutions to wells containing the Reaction Master Mix. This will result in 5 concentrations: 1 x 10^6, 1 x 10^5, 1 x 10^4, 1 x 10^3, 1 x 10^2 and 1 x 10^1 copies/reaction.
   e. Cap the tubes with the appropriate optical lid and spin briefly at 3000 RPM for a few seconds.

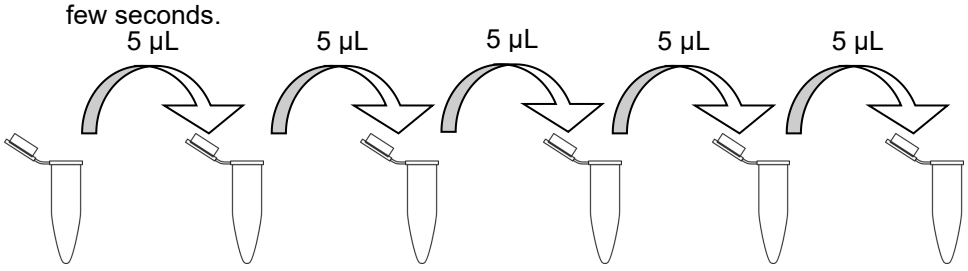

Figure 1. Serial Dilution Preparation

C. NoV TaqMan RT-PCR Assay Programming

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

Table 1. NoV TaqMan 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 1 (40x)	Step 1	94°C	15 sec
	Step 2	60°C	30 sec
	Step 3	72°C	45 sec
Cycle 4	Step 1	72°C	5 min
Cycle 5	Step 1	4°C	∞

D. NoV TaqMan RT-PCR Assay Interpretation

i. Qualitative Analysis

Table 2. Interpretation of Assay Results.

FAM (Target detection)	HEX (PCR validation)	Result
+	+	Positive
-	+	Negative
-	-	PCR inhibited

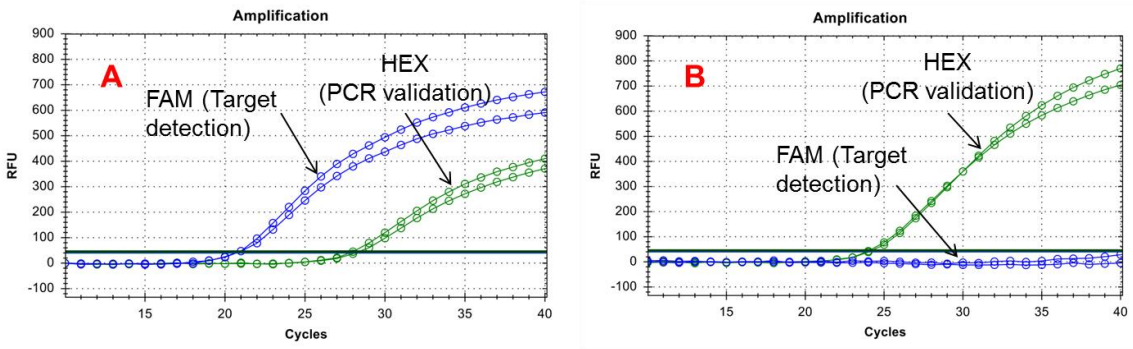

Figure 2. Example of TaqMan RT-PCR positive (A) and negative (B) results. Both PCR signals above the baseline from FAM and HEX channel indicate the successful PCR.

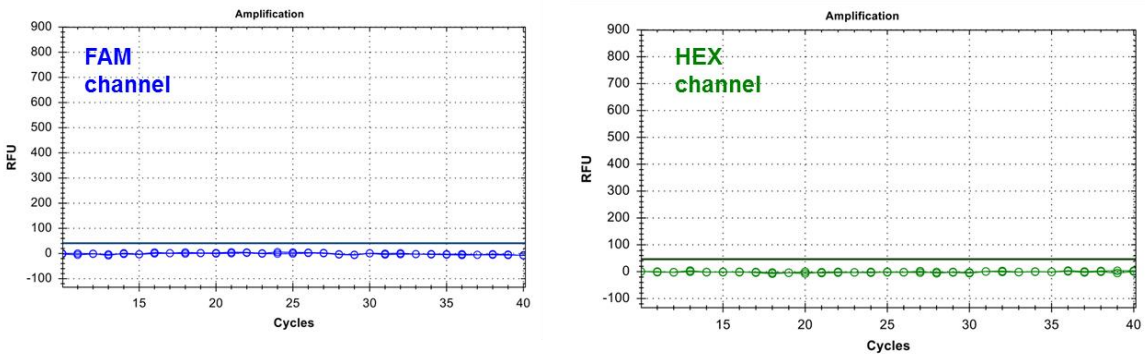

Figure 3. Example of TaqMan RT-PCR inhibition result. No signal from both FAM and HEX channel was detected. It is suggested to repeat the sample preparation using recommended kit for RNA purification.

ii. Quantitative Analysis

1. Use the Ct values obtained from the amplification of the Positive Control and the 5 serial dilutions of the Positive Control to generate a standard curve to illustrate the linear relation between log starting Positive Control copy number (Log Copy Number; X axis) and the obtained Ct value of each concentration (Y axis).
2. Add the linear trend line to the graph and obtain the Correlation Coefficient (R2), slope (m) and intercept (b).
3. Use the slope to calculate the PCR efficiency by the following equation:

   PCR efficiency (%) = ((10^(-1/slope))-1)*100

4. For a valid quantitative diagnostic run the following control parameters of the standard curve must be achieved:

Control Parameter	Value
R^2	> 0.98
Slope	-2.92 to -3.92
PCR Efficiency	80% to 120%
PCR Control Ct	26 to 30.5

5. Use the Ct value of the test sample together with the slope (m) and intercept (b) to calculate the initial test sample concentration from the following equation:

   Initial concentration = $10^{(Ct-b)/m}$

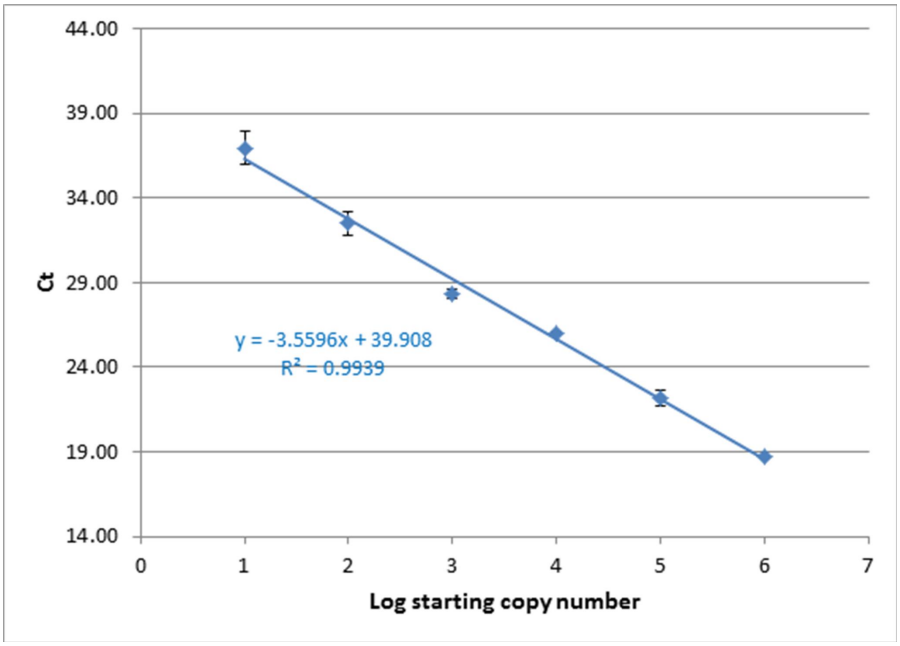

Figure 4. Standard curve between log starting copy number of NoV Positive Control Standard and Ct value.

E. Performance Evaluation

i. Analytical Sensitivity

The analytical sensitivity of the NoV TaqMan RT-PCR Kit Dx was determined by analyzing a dilution series of quantified NoV RNA.

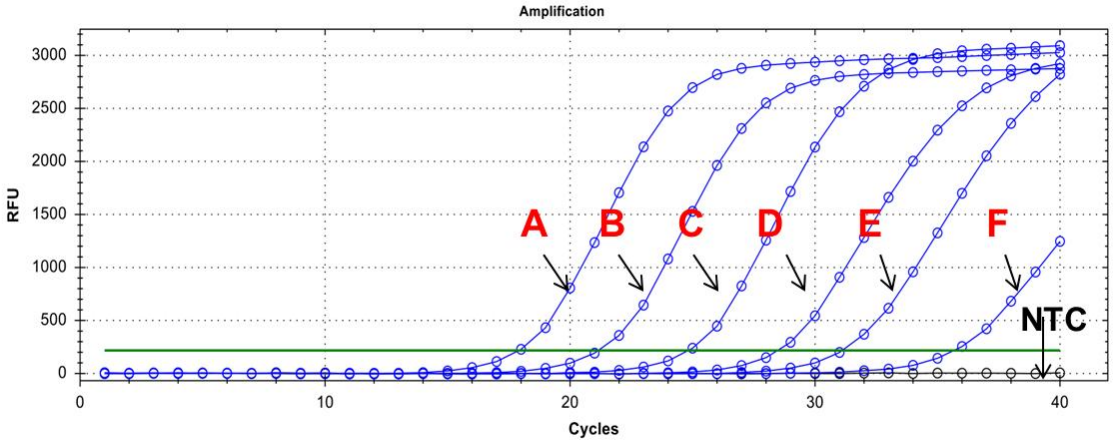

Figure 5. Analytical Sensitivity of the NoV TaqMan RT-PCR Kit Dx. A serial dilution (A to F) of NoV RNA was amplified using NoV TaqMan RT-PCR Kit Dx.

Table 3. RT-PCR Results used to determine the Analytical Sensitivity of the NoV TaqMan RT-PCR Kit Dx

Standard Sample	NoV Copy Number Per PCR Reaction (20 µL)	NoV Detection
A	1.00E+06	Positive
B	1.00E+05	Positive
C	1.00E+04	Positive
D	1.00E+03	Positive
E	1.00E+02	Positive
F	1.00E+01	Positive
NTC	0	Negative

Therefore, the limit of detection (LoD) for NoV using the NoV TaqMan RT-PCR Kit Dx is 10 copies.

ii. Analytical Specificity

The analytical specificity of the NoV TaqMan RT-PCR Kit Dx is ensured by the selection of the NoV-specific primers, as well as the selection of stringent reaction conditions. The primers were checked for possible homologies in published sequences by sequence comparison analysis.

Furthermore, the specificity of the NoV-specific primers were evaluated by testing a panel of RNA extracted from related pathogens.

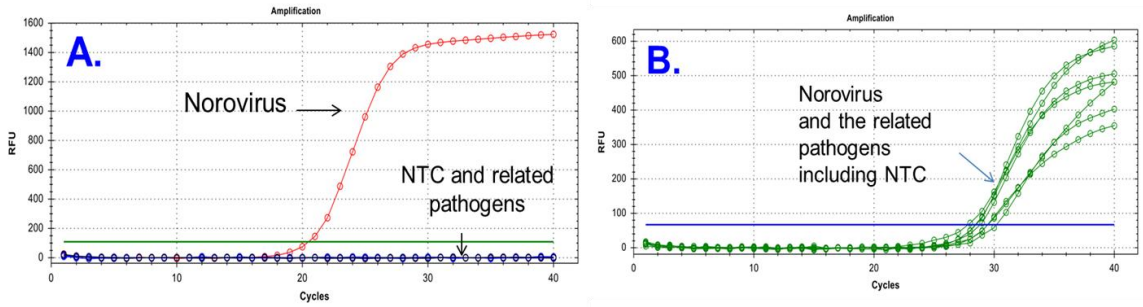

Figure 6. Analytical Specificity of the NoV TaqMan RT-PCR Kit Dx. RNA from related pathogens (see Table 2) was amplified using Norgen's NoV TaqMan RT-PCR Kit Dx. A: detection of NoV, B: PCR internal control.

Table 4. Organisms tested to demonstrate the analytical specificity of the NoV TaqMan RT-PCR Kit Dx

Pathogen	NoV Detection	Internal Control
NoV	Positive	Positive
West Nile Virus	Negative	Positive
RSV Virus	Negative	Positive
REO Virus	Negative	Positive
HIV	Negative	Positive
Campylobacter jejuni	Negative	Positive

The NoV TaqMan RT-PCR Kit Dx did not cross-react with any of the specified organisms.

iii. Precision

Precision data of the NoV TaqMan RT-PCR Kit Dx was determined as intra-assay variability (variability within one experiment), inter-assay variability (variability between different experiments) and inter-lot variability (variability between different production lots). Variability data are expressed as a percentage of accurate NoV detection. The data is based on three different operators performing two experiments each with the same diluted standard (6 dilutions) sample. Each of the 2 experiments carried out by each operator used different production lots. Therefore, 14 replicates per sample were analyzed for intra-assay, inter-assay and inter-lot variability. The precision data was found to be 100% in all cases (intra-assay variability, inter-assay variability, and inter-lot variability).

Table 5. Precision Data for the NoV TaqMan RT-PCR Kit Dx.

NoV Precision	R^2	Slope	Efficiency
Intra-Assay Variability	100% PASS	100% PASS	100% PASS
Inter-Assay Variability	100% PASS	100% PASS	100% PASS
Inter-Lot Variability	100% PASS	100% PASS	100% PASS

iv. Reproducibility

Specificity, sensitivity and precision of the NoV TaqMan RT-PCR Kit Dx were evaluated by carrying out an Internal Split-Sample Proficiency Testing Procedure, in which 2 different NoV samples were split between 3 operators and analyzed in 3 replicates over 2 different tests.

Product Use Restriction

Norgen's NoV TaqMan RT-PCR Kit Dx is an *in vitro* diagnostic test for the detection and quantification of NoV specific RNA using real-time hybridization-fluorescence detection.

Norgen's NoV TaqMan RT-PCR Kit Dx is intended for use by professional users such as technicians and biologists experienced and trained in molecular biological techniques including 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 NoV 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 NoV 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 NoV 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.

Technical Support

Contact our Technical Support Team between the hours of 9:00 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 support@norgenbiotek.com.

Authorized Representative

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