QuantuMDx SARS-CoV-2 RT-PCR Detection Assay

Instructions for Use

Version 6. September 2020

Product Code: Q22003

For *in vitro* Diagnostic Use Only

CE IVD

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1. INTENDED USE

The QuantuMDx Severe Acute Respiratory Syndrome Virus coronavirus-2 (SARS-CoV-2) real-time reverse transcription polymerase chain reaction (RT-PCR) Detection Assay is a qualitative RT-PCR assay for the detection of SARS-CoV-2 genomic RNA from upper respiratory specimens (e.g. nasopharyngeal or oropharyngeal swabs, etc.) collected from individuals who meet criteria for SARS-CoV-2 testing.

Results are for the presumptive detection and identification of SARS-CoV-2 targeted genomic RNA sequences. SARS-CoV-2 genomic RNA is detectable in upper respiratory specimens during the acute phase of the infection and for a time after symptoms have abated. Positive results are indicative of active infection with SARS-CoV-2 but do not rule out co-infections with other viruses or bacteria. Clinical correlation with patient history and other diagnostic information is necessary to determine an individual's infection status. The detection of SARS-CoV-2 genomic RNA may not indicate the definitive cause of disease.

Negative results do not preclude SARS-CoV-2 infection and should not be used as a sole basis for treatment or other care management decisions. Negative results must be combined with clinical observations, the individual's history and epidemiological data and information.

Testing with the QuantuMDx SARS-CoV-2 Detection Assay is intended for use by trained laboratorians who are proficient in performing molecular based tests.

2. SUMMARY AND EXPLANATION

On December 31, 2019, an outbreak of a pneumonia of unknown etiology was reported to the WHO, the focal point of the outbreak was the city of Wuhan, Hubei Province, China. Authorities in China identified a novel coronavirus (2019-nCoV) as the causative organism. The causative agent SARS-CoV-2 is a member of a large family of viruses that can cause illness in humans ranging from the common cold to severe diseases. SARS-CoV-2 is a novel strain of coronavirus which has not been previously identified from human isolates.

Common signs of infections are primarily respiratory in nature and include:

- Fever
- Cough
- Shortness of breath
- Breathing difficulties

If disease progresses in severity the individual can develop pneumonia, severe acute respiratory syndrome, kidney failure and death.

3. PRINCIPLES OF THE PROCEDURE

The QuantuMDx SARS-CoV-2 RT-PCR Detection Assay is a qualitative *in vitro* diagnostic assay consisting of reagents for real-time RT-PCR amplification, and detection of genomic RNA from SARS-CoV-2 virus, and a Specimen Process Control (SPC) from clinical samples. The assay is intended to be used with nucleic acids that have been extracted from appropriate specimens obtained from individuals suspected and consistent with being infected with SARS-CoV-2.

The assay is composed of two principal steps:

- extraction and enrichment of nucleic acids from an appropriate specimen obtained from an individual, and
- reverse transcription and PCR amplification (RT-PCR) using oligonucleotide primers and fluorogenic DNA hybridization probes for the specific detection of amplified target sequences.

This kit utilizes RNase P as a SPC.

NOTE: This kit does not include a Positive Control (PC) or Negative Control (NC).

The assay run time from PCR setup to result is less than 75 minutes (excluding nucleic acid extraction time).

An overview of the procedure is as follows:

- An appropriate specimen from a symptomatic individual is obtained using appropriate materials and by approved techniques.
- Perform extraction and enrichment of nucleic acids from the specimen.
- Re-hydrated the lyophilized Master Mix with the appropriate volume of DEPC treated molecular grade water.
- Aliquot the appropriate volume of the Master Mix into as many PCR reaction tubes as required.
- Add the NC to the appropriate reaction tube, followed by the specimen nucleic acid to appropriate

reaction tubes and finally the PC to the appropriate reaction tube.

Utilize a validated real-time thermocycler to perform reverse transcription of the SARS-CoV-2 genomic RNA to synthesize complementary DNA (cDNA) and subsequently amplify the cDNA into specific amplicons of DNA by PCR. The primers are complementary to highly conserved regions of the virus. The probes are dual-labeled with a reporter dye attached to the 5'-end and a quencher attached to the 3'-end. In this process, the probe anneals specifically to the template followed by primer extension and amplification. The QMDx SARS-CoV-2 Detect assay is based on TaqMan chemistry, which utilizes the 5'-3' exonuclease activity of the Taq polymerase to cleave the probe thus separating the reporter dye from the quencher. This generates an increase in fluorescent signal upon excitation from a light source. With each cycle, additional reporter dye molecules are cleaved from their respective probes, further increasing fluorescent signal. The amount of fluorescence at any given cycle is dependent on the amount of amplification products present at that time and is monitored by the real-time instrument during each PCR cycle.

4. ASSAY MATERIALS PROVIDED

QMDx SARS-CoV-2 RT-PCR Detection Assay Components:

Master Mix – 1 vial sufficient for 100 reactions. Store at 2 – 8 °C

Component	Label	Cap Colour	Resuspension volume per vial	Reactions per vial
Master Mix	SARS-CoV-2 RT-PCR Detection Assay	White	1.56 mL	100 x 15µL reaction

The SARS-CoV-2 RT-PCR Detection Assay contains the primer and probes required for amplification of the SARS-CoV-2 and RNase P amplicons as detailed below.

Analyte	Gene Targeted	Probe Fluorophore	Absorbance Peak	Emission Peak	Equivalent Dye
SARS-CoV-2	Orf1	FAM	495nm	520nm	FAM
SARS-CoV-2	N	FAM	495nm	520nm	FAM
SARS-CoV-2	S	FAM	495nm	520nm	FAM
Human Cells	RNase P	HEX	535nm	556nm	CAL Fluor Orange 560

5. STORAGE AND HANDLING CONDITIONS

Store the kit at 2 to 8 °C.
The rehydrated Master Mix have been validated for up to three (3) freeze and thaw cycles.
Rehydrated Master Mix can be stored at 2 to 8 °C for up to 48 hours.
Do not used reagents after their labeled expiration date.

6. ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

This kit utilizes RNase P as a SPC.

NOTE: This kit does not include a Positive (PC) or Negative Control (NC).

The Negative Control should be DEPC treated molecular grade water. The following materials have been validated as adequate in performance to act as a PC:

Manufacturer	Product Name	Product Code
Twist Biosciences	Twist Synthetic SARS-CoV-2 RNA Control 2 (MN908947.3)	102024
Agilent	Quantitative PCR Human Reference Total RNA	750500

7. EQUIPMENT AND DISPOSABLES REQUIRED

The following equipment and disposables are required:

- Biological safety cabinet
- RNA Extraction Kit - see Section 8.
- Real-time thermal cycler – calibrated for the detection of fluorescent dyes: FAM and HEX
- Vortex Mixer
- Pipettes for delivery of volumes between 1-10mL, 10-100mL, and 100-1000µL
- Microcentrifuge for 1.5mL tubes
- -20 °C ± 10°C Freezer
- -80 °C ± 10°C Freezer
- 2 to 8 °C Refrigerator
- Disposable, powder-free gloves (latex or nitrile)
- 1.5 mL screw-capped microcentrifuge tubes
- Tube racks
- Biohazard bag for tips and tube disposal
- 10% (v/v) freshly made from household bleach solution (0.5% w/v sodium hypochlorite in water)
- 70% ethanol (freshly made)
- Thermal cycler appropriate reaction tubes or 96-well reaction plates
- DEPC treated molecular grade water
- Dedicated laboratory coats for each area

8. RNA EXTRACTION KITS

QuantuMDx SARS-CoV-2 RT-PCR Detection Assay has been validated for use with the following nucleic acid extraction kits:

Manufacturer	Kit	Product Number
Qiagen	QIAamp© Viral RNA Mini Kit (automated QIAcube)	52906
Promega	Maxwell® RSC Viral TNA Purification Kit (automated Maxwell RSC 48)	AS1330
PerkinElmer	chemagic™ Prime Viral DNA/RNA	CMG-1433
Roche	MagNA Pure 96 DNA and Viral RNA SV Kit (automated MagNA Pure 96)	06543588001

9. REAL-TIME THERMALCYCLER INSTRUMENTS

QuantuMDx SARS-CoV-2 RT-PCR Detection Assay has been validated for use with the following real-time thermocycling instruments and associated software:

Manufacturer	Model	Controller Software (version)
Qiagen	Rotor-Gene Q	v2.3.4
Bio-Rad	CFX96™ Dx	v3.1
Bio-Rad	CFX96™ Deepwell	v3.1
Thermo Fisher	ABI™ 7500 Fast Dx	v1.4.0
Roche	LightCycler® 480 II	v1.5.1
Thermo Fisher	QuantStudio™ 7 384 well	v1.3

All instruments should be installed, calibrated for the fluorophores FAM and HEX and maintained according to the manufacturer's instructions and recommendations.

10. FACILITY AND TRAINING REQUIREMENTS

Testing for the presence of SARS-CoV-2 RNA should be performed in an appropriately equipped and maintained laboratory. Staff should be trained in the relevant technical and safety procedures.
Please refer to the Center for Disease Control and Prevention (CDC) guidelines:
https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html

11. PRECAUTIONS AND HANDLING REQUIREMENTS

Warnings and Precautions

- As with all testing and testing procedures, good laboratory practices are essential to ensure proper performance of the assay. General care should be taken to ensure there is no contamination of reagents.
- For in vitro diagnostic use
- Positive results are indicative of the presence of SARS-CoV-2 within the specimen and the PUI being positive for SARS-CoV-2 infection.
- Negative results do not preclude the presence of SARS-CoV-2 within the specimen
- Proper personal protective equipment including lab coats, gowns, gloves, eye protection and a biological safety cabinet are recommended for manipulation of clinical specimens. All specimen processing should be performed in accordance with national, local and institutional biological safety recommendations.
- All specimens and samples should be handled as infectious, using good laboratory procedures and Universal Precaution. Only personnel proficient in handling infectious materials and the use of the QuantuMDx SARS-CoV-2 RT-PCR Detection Assay should perform this procedure.
- If spillage should occur, immediately disinfect with a freshly prepared solution of 10% household bleach in distilled or deionized water or follow appropriate procedures as outlined by the site or facility.
- Safety Data Sheets (SDS) are available upon request and on the QuantuMDx website
- Closely follow procedures and guidelines provided to ensure that the assay is performed correctly. Any deviation from the procedure and guidelines may affect optimal performance of the assay.
- False Positive results may occur if carryover is not adequately controlled during specimen and sample handling and processing.

Reagent Handling

- Wear gloves when handling specimens and reagents.
- Protect the SARS-CoV-2 RT-PCR Detection Assay from light at all times.
- Wash hands thoroughly after handling specimens, samples, and kit reagents and after removing one's gloves.
- The use of sterile-aerosol barrier nuclease-free pipette tips is recommended and good practice when handling specimens, samples, and reagents for the detection of RNA.
- Handle all reagents, controls, specimens, and samples according to good laboratory practice in order to prevent carryover and/or contamination of specimens, samples or controls.
- Before use, visually inspect the vial to ensure that there were or are no signs of breakage or leakage. If there are signs of breakage or leakage DO NOT use that material for testing and immediately contact QuantuMDx Customer Service.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents, specimens and samples are handled.
- Decontaminate and dispose of all potentially infectious materials in accordance with institutional, local and relevant national regulations.
- Dispose of all cleaning materials as biological waste.
- Amplification technologies such as RT-PCR are sensitive to accidental introduction of product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product. Measures to reduce the risk of contamination in the laboratory include physically separating the activities involved in performing RT-PCR in compliance with good laboratory practices and establishing a unidirectional workflow.
- Avoid microbial and nuclease contamination of the RT-PCR MM when removing aliquots. Use sterile, disposable pipettes and aerosol barrier pipette tips.
- Change aerosol barrier pipette tips between all liquid transfers.
- During preparation of samples, compliance with good laboratory techniques is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with nucleic acids.
- Work area and instrument platforms must be considered potential sources of contamination. Change

gloves after contact with potential contaminants (specimens, eluates, and/or amplified product) before handling unopened reagents, controls, calibrators, or specimens.
- Bring all reagents to room temperature, then place on ice prior to use.
- RNA and extracted sample nucleic acids should be maintained on a cold block during preparation and use, to ensure stability.
- Add reagents to the bottom of the reaction tube or reaction well without touching the pipette tip to the rim or side of the tube or well.
- Reaction plates or reaction tubes should be setup such that they are maintained at 2 to 8 °C.
- Do not use assay components beyond their recommended storage dates.
- Set up each run separately.
- Dispose of unused kit reagents and human specimens according to all relevant local and national regulations.

12. PREVENTION OF NUCLEIC ACID CONTAMINATION

The possibility of nucleic acid contamination is minimized when:
- PCR amplification, and oligonucleotide hybridization occur in a properly sealed 96-well reaction plate or reaction tube.
- Detection is carried out automatically without the need to open the 96-well reaction plate or tubes.
- Once the reaction has been carried out the 96-well reaction plate or tubes should never be opened and should be disposed of properly.
- Aerosol barrier pipette tips are used for all pipetting. The pipette tips are discarded after use.
- Unidirectional workflow practices are followed, such that reaction well aliquoted RT-PCR MM is brought to the target preparation location for mixing, then the complete reaction plate to the instrument location.
- Work surfaces and equipment are regularly cleaned with the appropriate solutions.

13. SAMPLE COLLECTION, HANDLING AND STORAGE

Collecting the Specimen: Refer to the manufacturer's package insert or facility's procedures.
Storing Specimens: Store specimens refrigerated (2 to 8 °C) for up to 24 hours prior to processing. Store any leftover specimens at -80 °C ± 15 °C.
Storing Purified Nucleic Acids Samples: It is recommended to aliquot and store enriched nucleic acids at -80 °C ± 15 °C.
Notes: The following can affect the results obtained:
- Inadequate or inappropriate collection of specimens
- Incorrectly stored specimens and samples
- Incorrectly transported specimens
- Use of non-validated specimen matrices
- Inadequate specimen volume

14. SAMPLE PREPARATION

The QuantuMDx SARS-CoV-2 RT-PCR Detection Assay does not include reagents for extracting and enriching of nucleic acids from specimens. The user is responsible for following all procedures recommended by the manufacturer of the sample preparation kit. This assay has been validated against the kits named within this document ONLY.

15. REAGENT PREPARATION

Batch Size: The reagents included with a single vial of the QuantuMDx SARS-CoV-2 Detection RT-PCR Assay Kit are sufficient for 100 tests. In each testing batch, at least one replicate of a Negative/ No Template Control (NTC) should be included in addition to at least one replicate of a Positive Control (PC).

Master Mix: Once rehydrated, the Master Mix should be stored on ice, while dispensing.

SARS-CoV-2 RT-PCR Detection Assay Controls
Specimen Process Control (SPC) – the human RNase P gene is utilized by the assay to provide guidance as to

the performance of specimen acquisition, performance of the extraction/enrichment process, the performance of the assay chemistry and real-time instrumentation. The SPC should be present in all correctly collected human specimens. The SPC has been designed not to compete with the detection of SARS-CoV-2 target loci. The SPC signal should be present in all negative samples and most SARS-CoV-2 positive samples. However, in the presence of high concentrations of SARS-CoV-2 genomic RNA the RNase P signal may not be detected. The reactivity of the SPC is part of the validity and acceptance criteria for every batch run of the assay.

Negative Control (NC) – the NC should be included in every batch run of the assay. The NC is part of the validity and acceptance criteria for each batch run of the assay. The NC is needed to determine if carry-over contamination has occurred in the preparation of test reactions. For the SARS-CoV-2 Detect assay the NC should be 5µL of DEPC treated molecular grade water.

Positive Control (PC) – at least 1 PC reaction should be included in every batch run of the assay. The PC is utilized for validity and acceptance criteria for both the assay chemistry and the real-time instrumentation. It is recommended that the user make up their positive control to provide a Ct of between 24-30 cycles on the FAM channel and a Ct of 24-30 cycles on the HEX channel. 5µL of PC material is utilized instead of extracted material from a PUI specimen. Expected results are a positive signal in the FAM and HEX channels. The PC material has been validated for use with the assay and should be made as instructed below.

Material	Stock Concentrations	µL of Stock per 50 µL of Working Reagent	Working concentration	Concentration per reaction
Twist synthetic SARS-CoV-2 RNA Control 2	1000 copies/µL*	5 µL	100 copies/µL	500 copies
QPCR Human Reference Total RNA	100 ng/µL**	2.5 µL	5 ng/µL	25 ng
RNase Free Molecular grade water	N/A	42.5 µL	N/A	N/A

Formulation of the SARS-CoV-2 Q-Detect Positive Control. * Twist Synthetic SARS-CoV-2 RNA Control 2 is supplied at 1000000 copies/µL, a 1 in 1000 dilution is required to produce a 1000 copies/µL stock. ** QPCR Human Reference Total RNA is supplied at 1000 ng/µL, a 1 in 10 dilution is required to produce a 100 ng/µL stock.

16. ASSAY PROCEDURE

Prepare the Positive Control (PC) and Negative Control (NC) as detailed in the controls section. Place on ice. Prepare the Reaction Mix on ice as follows:

- To an individual tube of lyophilized Master Mix, add 1560 µL of DEPC treated molecular grade water (not provided). Ensure that the lyophilized Master Mix has been fully resuspended and vortex briefly (2 – 5 seconds) to mix.
- Aliquot 15 µL of Master Mix per PCR reaction tubes or wells.
- Add 5µL of the NC to the appropriate reaction tube of well.
- Add 5µL of each extracted nucleic acid specimens to individual reaction tubes or wells.
- Add 5µL of the PC to the appropriate reaction tube or well.
- Cap the tubes.

17. EQUIPMENT PREPARATION

QuantuMDx utilizes the following protocol when utilizing validated instrumentation. QuantuMDx recommends calibration of instrument(s) for the fluorophores FAM and HEX prior to the initial utilization of the assay. Follow the manufacturer's instructions. Refer to manufacturer's documentation for detailed

operating instructions for thermocyclers.

- Switch on the instrument and open the associated software.
- Utilize the thermal protocol in the following table by loading a run file or adjusting the run profile settings ensuring acquisition is set for FAM and HEX fluorophores.
- Load reactions into the instrument, ensuring no air pockets remain at the base of the reaction tubes or wells
- Save the file when prompted.
- Run the protocol.
- Data is collected automatically by the integrated software.

Reaction Step	Action	Temperature	Time	Cycles
Stage 1	Hold	50°C	10 min	1
Stage 2	Hold	95°C	2 mins	1
Stage 3	Cycle	95°C	5 seconds	45
	Cycle (acquiring)	63°C	20 seconds	

18. QUALITY CONTROL

Quality control requirements should be performed in conformance with local and national regulations or accreditation requirements and your laboratory's standard quality control procedures. Quality control procedures are intended to monitor reagent and assay performance.

Control Type	Used to Monitor
Specimen Process (SPC)	Sample extraction efficiency PCR inhibition Process error
Positive (PC)	Substantial reverse transcriptase and polymerase failure Substantial primer and probe failure
Negative (NC)	Reagent contamination Environmental contamination

The PC should be diluted and tested prior to running samples with each new kit lot to ensure proper functioning of all reagents and kit components.

Never run the PC through nucleic acid extraction and enrichment.

Always include at a minimum of one NC and one PC in each batch run performed.

Failure of the controls (PC or NC) invalidates the run. Results should not be reported as repeat testing should be done starting from purified nucleic acid, using a new aliquot of positive control. If repeat results are still invalid, results should not be reported, and testing should be repeated from the original specimen or a new specimen should be collected and tested.

Failure of an SPC for a sample invalidates the sample result when the sample is negative for the presence of SARS-CoV-2. In most cases the SPC signal should be present for a SARS-CoV-2 positive sample, however, the presence of high concentrations of SARS-CoV-2 genomic RNA the SPC signal may not present. This sample result is still valid and acceptable

In the case of higher than expected numbers of test failures and/or suspected inaccurate test results, please contact QuantuMDx Customer Service.

19. INTERPRETATION OF RESULTS

All assay controls must be examined prior to interpretation of test sample results. If the controls are not valid and acceptable, the run is invalid and must be repeated.

Amplification plots should be examined for every positive sample. If the amplification plot shows an exponential increase, the amplification curve is valid. All positive samples should be analyzed for the presence of probe drift if corrections are not applied via the instrument controller software. In instances where background fluorescence appears high, the initial PCR cycles can be omitted from analysis.

If a sample is rerun and remains invalid with non-amplification of the SPC, this suggests the presence of PCR inhibitors in the sample. An additional sample should be obtained and submitted for testing if clinically warranted. The results should be reported as 'Indeterminate due to Inhibition'.

SARS-CoV-2 (FAM)	RNase P (HEX)	Interpretation
+	+/-	Positive for the presence of SARS-Cov-2 genomic RNA
-	+	Negative for the presence of SARS-CoV-2 genomic RNA
-	-	Invalid; determine route cause and take appropriate action

All instances of SARS-CoV-2 amplification of Ct ≤ 40 indicate a SARS-CoV-2 positive result. Manual inspection of curves for positive amplification is advised.

20. LIMITATIONS

Training and familiarity with testing procedures and interpretation of results is necessary prior to performing the assay.

Performance of the QuantuMDx SARS-CoV-2 RT-PCR Detection Assay has only been established in upper respiratory swab specimens. Specimen types may yield inaccurate results.

The detection of viral nucleic acid is dependent upon proper specimen collection, handling, transportation, storage, and preparation, including extraction. Failure to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of false negative values resulting from improperly collected, transported, or handled specimens.

There is a risk of false negative results due to the presence of sequence variants in viral targets of the assay, procedural errors, amplification inhibitors in specimens, or inadequate numbers of organisms present in a clinical sample for amplification.

This assay cannot rule out diseases caused by other bacterial or viral pathogens.

The prevalence of infection will affect the test's predictive value.

Once reactions are established in tubes/plates, thermal cycling should proceed immediately.

A trained health professional should interpret test results in conjunction with the PUI's medical history, clinical signs and symptoms, and the results of other tests.

Analyte targets (viral nucleic acids) may persist *in vivo*, independent of virus viability. Detection of the analyte does not imply that the corresponding virus is infectious or is the causative agent for clinical symptoms.

A sample yielding negative results can contain pathogens other than SARS-CoV-2.

21. PERFORMANCE CHARACTERISTICS

Analytical sensitivity

The Limit of Detection (LoD) or analytical sensitivity was determined as the lowest concentration of SARS-CoV-2 target, that could be detected by the QuantuMDx SARS-CoV-2 RT-PCR Detection Assay with a ≥95% positivity rate. All samples underwent extraction as well as amplification and detection on the CFX96 Dx.

SARS-CoV-2 Copies	Replicates	Average Ct	Standard Dev.	Human RNA Concentration	Average Ct	Standard Dev.
1,000,000	3/3	19.54	0.24	25ng	25.45	0.79
100,000	3/3	22.96	0.12	25ng	26.45	0.23
10,000	3/3	26.18	0.39	25ng	28.03	0.47
1,000	3/3	29.44	0.12	25ng	28.24	0.53
100	3/3	32.44	0.32	25ng	28.62	0.88
10	3/3	36.92	0.27	25ng	28.91	0.12
1	1/3	43.81	NA	25ng	29.04	0.34
0.1	0/3	NA	NA	25ng	28.78	0.03
NTC	0/3	NA	NA	25ng	25.45	0.79

Presumptive LoD determined by triplicate amplification of SARS-CoV-2 RNA in multiplex with a fixed concentration of human RNA.

LoD was verified by running 25 replicates from extraction through to detection on Bio-Rad CFX96™ Dx. The data is presented in the table below and demonstrates detection of 25/25 samples.

Input	Copies/Reaction	Average Ct	Standard Dev.	Total Detected
SARS-CoV-2	10	35.20	0.43	25/25

Verification of presumptive LoD determined by 25 replicates of SARS-CoV-2 RNA at 10 copy input in multiplex with a fixed concentration of human RNA.

Equivalence Studies. Studies were performed to determine the equivalent performance for LoD for a number of real-time platforms. Extracted replicates were run and analyzed for 10 copies/reaction.

Platform	Copies/Reaction	Average Ct	Standard Dev.	Total Detected
Rotor-Gene Q	10	34.52	1.01	22/22
ABI 7500 Fast Dx	10	35.28	1.93	19/20
LightCycler 480 II	10	35.57	0.68	19/20
Quant Studio 7 384 well	10	35.12	2.86	20/20

22. REPRODUCIBLITY

The SARS-CoV-2 assay was evaluated at 10x LoD for day-to-day, tech-to-tech, instrument-to-instrument, site-to-site and lot-to-lot for reproducibility and repeatability under the conditions tested. Across all evaluations, reproducibility was 100%.

23. INCLUSIVITY (REACTIVITY)

Inclusivity testing was performed after selection of a short list of primers and probes specific for the S and N genes and Orf1. Sequences for the SARS-CoV-2 virus were downloaded from the NCBI and GISAID. Sequences were filtered to remove duplicate downloads, then filtered on size (≥ 25,000 bases in length) to ensure that only full or close full-length sequences were used for inclusivity testing. The downloading and vetting of the SARS-CoV-2 sequence data occurred on 15 Apr 2020 with a resulting 10,151 sequences (1,022 = NCBI and 9,126 = GISAID) utilized for inclusivity analysis. Each primer and probe solution were then analyzed versus each SARS-CoV-2 sequence one at a time. Each primer and probe solution were analyzed to ensure that they complemented the SARS-Cov-2 sequence at the desired position. Mismatching was then checked to ensure that there was no more than 1 mismatch at greater than 1% within the SARS-CoV-2 database (10,151 sequences). The following table provides the output of the analysis.

QuantuMDx Name	Mismatch rates of single base 15-April-2020
Orf1_Span20_FPrimer	(27) = 0.27%
Orf1_Span20_Probe1	(23) = 0.23%
Orf1_Span20_RPrimer	(0) = 0.0%
sGene_Span3_Fprimer	(11) = 0.11%
sGene_Span3_Probe	(1) = 0.01%
sGene_Span3_Rprimer	(0) = 0.0%
nGene_Span1_Fprimer	(29) = 0.29%
nGene_Span1_Probe	(6) = 0.06%
nGene_Span1_Rprimer	(0) = 0.0%

Based upon the *in silico* analysis the selected primers and probes will detect all known sequences (as of the date of analysis) of SARS-CoV-2.

24. EXCLUSIVITY (CROSS-REACTIVITY)

The primers and probes identified for the assay underwent additional analysis for exclusivity or cross-reaction analysis. The table below provides the lists of organisms utilized for the analysis. Within each table the number of unique whole genome sequences utilized for the analysis is presented.

Organism	Type	Count	Sequence Type
Acinetobacter baumannii	Prokaryote	1	Reference Genome
Adenovirus (e.g. C1 Ad. 71)	Virus	100 (Limited)	Any
Aspergillus fumigatus	Eukaryote	1	Reference Genome
Bacillus anthracis	Prokaryote	1	Reference Genome
Bat Betacoronavirus	Virus	100 (Limited)	Any
Bocavirus	Virus	74	Any
Bordetella pertussis	Prokaryote	1	Reference Genome
Candida albicans	Eukaryote	1	Reference Genome
Candida glabrata	Eukaryote	1	Reference Genome
Chlamydia pneumoniae	Prokaryote	5	Complete Genomes
Chlamydia psittaci	Prokaryote	1	Reference Genome
Coronaviridae	Virus	100 (Limited)	Any
Coronavirinae	Virus	4	Any
Corynebacterium diphtheriae	Prokaryote	1	Reference Genome
Corynebacterium sp.	Prokaryote	1	Reference Genome
Coxiella burnetii	Prokaryote	1	Reference Genome
Cryptococcus neoformans	Eukaryote	1	Reference Genome
Cytomegalovirus	Virus	1	Any
Enterobacter cloacae	Prokaryote	1	Reference Genome
Enterovirus (e.g. EV68)	Virus	17	Complete Genomes
Enterovirus A	Virus	1	Reference Genome
Enterovirus B	Virus	1	Reference Genome
Enterovirus C	Virus	1	Reference Genome
Enterovirus D	Virus	1	Reference Genome
Enterovirus E	Virus	1	Reference Genome
Enterovirus F	Virus	1	Reference Genome
Enterovirus G	Virus	1	Reference Genome
Enterovirus H	Virus	1	Reference Genome
Enterovirus I	Virus	1	Reference Genome
Enterovirus J	Virus	1	Reference Genome
Enterovirus K	Virus	1	Reference Genome
Enterovirus L	Virus	1	Reference Genome
Escherichia coli	Prokaryote	1	Reference Genome
Haemophilus influenzae	Prokaryote	1	Reference Genome
Haemophilus parainfluenzae	Prokaryote	1	Reference Genome
Human	Eukaryote	1	Reference Genome
Human coronavirus 229E	Virus	100 (Limited)	Any
Human coronavirus HKU1	Virus	100 (Limited)	Any
Human coronavirus NL63	Virus	100 (Limited)	Any
Human coronavirus OC43	Virus	100 (Limited)	Any

Organism	Type	Count	Sequence Type
Human gammaherpesvirus 4 (Epstien Barr Virus)	Virus	1	Reference Genome
Human herpesvirus 1	Virus	1	Reference Genome
Human herpesvirus 2	Virus	1	Reference Genome
Human Metapneumovirus (hMPV)	Virus	100 (Limited)	Any
Influenza A & B	Virus	100 (Limited)	Complete Genomes
Klebsiella pneumoniae	Prokaryote	1	Reference Genome
Legionella pneumophila	Prokaryote	1	Reference Genome
Lymphocytic choriomeningitis virus – Segment L	Virus	1	Reference Genome
Lymphocytic choriomeningitis virus - Segment S	Virus	1	Reference Genome
Measles	Virus	1	Reference Genome
MERS-coronavirus	Virus	100 (Limited)	Any
Mycobacterium tuberculosis	Prokaryote	1	Reference Genome
Mycoplasma hominis	Prokaryote	1	Reference Genome
Mycoplasma hyorhinis	Prokaryote	1	Reference Genome
Mycoplasma pneumoniae	Prokaryote	1	Reference Genome
Mycoplasma synoviae	Prokaryote	1	Reference Genome
Neisseria sicca	Prokaryote	1	Reference Genome
Parainfluenza virus 1-4	Virus	29	Any
Pneumocystis jirovecii (PJP)	Eukaryote	1	Reference Genome
Proteus vulgaris	Prokaryote	1	Reference Genome
Pseudomonas aeruginosa	Prokaryote	1	Reference Genome
Respiratory syncytial virus	Virus	1	Complete Genome
Rhinovirus	Virus	4	Complete Genomes
SARS-coronavirus	Virus	100 (Limited)	Any
Staphylococcus aureus	Prokaryote	1	Reference Genome
Staphylococcus epidermis	Prokaryote	1	Reference Genome
Staphylococcus salivarius	Prokaryote	1	Reference Genome
Streptococcus gallolyticus (firmicutes)	Prokaryote	1	Reference Genome
Streptococcus pneumoniae	Prokaryote	1	Reference Genome
Streptococcus pyogenes	Prokaryote	1	Reference Genome

All sequences were downloaded from the NCBI database on 15 Apr 2020. For small genome organisms (virus) a limit of 100 unique whole genome sequences were utilized for analysis. Whereas for larger genome organisms only a single reference sequence was used for analysis. This was done to ensure that analysis occurred within a reasonable time frame. Upon analysis completion, no primers or probes met the criteria of greater than 80% homology, indicating by *in silico* analysis there is minimal chance of off target signal generation.

In vitro analysis for exclusivity or cross-reaction was also undertaken. The table below provides the lists of organisms utilized for the analysis with no cross-reactivity observed under the tested conditions. Where quantification was possible bacteria were input at $1x10^6$ cfu per reaction and virus at $1x10^5$ pfu per reaction where concentration was unknown (commercially available validation panels), the highest available input volume was used.

RNA standard/sample	Repeats Positive
Enterobacter cloacae	0/3
Escherichia coli	0/3
Klebsiella pneumoniae	0/3
Streptococcus agalactiae	0/3
Legionella pneumophilia	0/3
Streptococcus pyogenes	0/3
Mycobacterium tuberculosis	0/3
Mycobacterium bovis subsp Bovis	0/3
Neisseria meningitides	0/3
Candida albicans	0/3
Proteus vulgaris	0/3
Pseudomonas aeruginosa	0/3
Staphylococcus aureus	0/3
Staphylococcus epidermidis	0/3
Bordetella pertussis	0/3
Adenovirus Group A - G, 3, 7, 8	0/3
Coronavirus NL63	0/3
Coronavirus 229E	0/3
Coronavirus OC43	0/3
Coronavirus HKU-1	0/3
Enterovirus A-L	0/3
Human coronavirus HKU1	0/3
Human coronavirus OC43	0/3
Human metapneumonvirus	0/3
Influenza A (H1N1)	0/3
Influenza A (H3)	0/3
Influenza A 2009 (H1N1pdm)	0/3
Influenza B	0/3
MERS coronavirus	0/3
Parainfluenza 1	0/3
Parainfluenza 1 (Type 1)	0/3
Parainfluenza 1 (Type 2)	0/3
Parainfluenza 1 (Type 3)	0/3
Parainfluenza 1 (Type 4)	0/3
Respiratory syncytial virus A	0/3
Respiratory syncytial virus B	0/3
Rhinovirus 1A	0/3
Rotavirus	0/3
SARS coronavirus	0/3
Human immunodeficiency virus HIV-1	0/3

25. INTERFERING SUBSTANCES

Potential interfering substances that could be present in an upper respiratory specimen were evaluated. The substances listed include both endogenous as well as exogenous substances. None of the tested substances under the conditions tested showed an ability to interfere with the detection of SARS-CoV-2.

Substance	Concentration Tested	Repeats/Positive
Mucin – bovine submaxillary gland, type I-S*	5 mg/ml *	3/3
Blood (Human)	5% v/v	3/3
Phenylephine hydrochloride, Max strength Cold and Flu Relief	0.2 mg/ml	3/3
Oxymetazoline hydrochloride, Blocked nose relief	30% v/v	3/3
Beclomethasone dipropionate	0.037% v/v	3/3
NasalGuard Cold and Flu block	0.125 drops in 2ml	3/3
Galphimia glauca, cardiospermum and Luffa operculata, Rhinital	2 tablets in 2ml	3/3
Benzocaine	2.5 mg/ml	3/3
Menthol	0.084% v/v	3/3
Zanamivir, antiviral drug	5 mg/ml	3/3
Mupirocin, antibiotic	10 mg/ml	3/3
Tobramycin, antibiotic	1.2 mg/ml	3/3

** Interference demonstrated as a % v/v of mucin directly spiked into SARS-CoV-2 RT-PCR v2 Detection Assay. Interference with RNA extraction system should be validated by the user.*

The performance of this assay has not been established in patients receiving intranasal administered influenza vaccine. The performance of this assay has not been established in immunocompromised individuals.

26. CLINICAL PERFORMANCE

		Comparator Assay	
		Positive	Negative
QuantuMDx SARS-CoV-2	Positive	201	0
	Negative	2	293

PPA (clinical sensitivity): 99.0% (95% CI: 96.5% – 99.9%)
NPA (clinical specificity): 100.0% (95% CI: 98.8% – 100.0%)
99.6% agreement across all samples

Five external clinical evaluations were undertaken at five different sites using four different RNA extraction kits and four different thermal cycler platforms. All platforms used have been validated for use with the QMDx SARS-CoV-2 RT-PCR Detection Assay product as described in section 21.

27. DISPOSAL

Dispose of hazardous or biologically contaminated materials accordance with local laws and according to the practices of your institution.

28. REFERENCES

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Clinical and Laboratory Standards Institute (CLSI). Statistical Quality Control for Quantitative Measurements: Principles and Definitions Approved Guideline – Second Edition. CLSI Document C24-A2; Wayne, PA.

29. SYMBOLS

Symbol	Interpretation	Symbol	Interpretation
	Consult instructions		Indicates the kit contents sufficient for <n> tests
	Used for both warnings and cautions. A warning indicates the risk of personal injury or loss of life if the operating procedures and practices are not correctly followed. A caution indicates the possibility of loss of data or damage to, or destruction of equipment if operating procedures and practices are not strictly observed		Biohazard: Follow proper infection control guidelines for handling all specimens and samples. Properly dispose of all contaminated waste according to federal, state, and local requirements
	Indicates the product's temperature limits		Indicates Positive Control material
	Indicates Use by Date		Indicates Negative Control Material
	Indicates the product batch code		*In Vitro* Diagnostic medical device
	Indicates the name and location of the product manufacturer		Do not use if package is damaged
	Indicates the product's catalogue number		Device meets the essential requirements of the IVDD 98/79/EC
	Fragile		

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