Department of Health & Social Care

QuantuMDx RT-PCR

SARS-CoV-2 Nucleic Acid Detection

Published: November 2020 (Version 1.0)

Contents

Assay description and intended purpose

1. 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 assay RT-PCR assay for the detection of SARS-CoV-2 genomic RNA (S, N and Orf1 genes). 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.

2. The QuantuMDx Severe Acute Respiratory Syndrome Virus coronavirus assay is intended to be used with nucleic acids that have been extracted from appropriate specimens obtained from Persons Under Investigation (PUIs) for SARS-CoV-2 infection. The assay targets S, N and Orf1. 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.

3. The assay is intended to be used with nucleic acids that have been extracted from appropriate specimens. QuantuMDx SARS-CoV-2 RT-PCR Detection Assay has been validated for use with the following nucleic acid extraction kit Qiagen QIAamp Viral RNA Mini Kit (Product number 52906). Detection has been validated on the thermocyclers by BioRad (CFX96 Dx), controlled by CFX Manager Dx software v3.1 with equivalence demonstrated for the Qiagen Rotor-Gene Q, controlled by Rotor-Gene Q Series software 2.3.4. The assay should be compatible with other equivalent real-time thermocyclers but should be validated prior to use. Testing with the QuantuMDx SARS-CoV-2 Detection Assay is intended for use by trained laboratorians who are proficient in performing molecular based tests.

Type of sample used in validation

Extracted genomic RNA from upper respiratory specimens (e.g. nasopharyngeal or oropharyngeal swabs, etc.) collected from individuals who meet criteria for SARS-CoV-2 testing.

Equipment and reagents

1. Samples require extraction prior to testing, QuantuMDx SARS-CoV-2 RT-PCR Detection Assay has been validated for use with Qiagen QIAamp Viral RNA Mini Kit (Product number 52906). Validations were performed using equivalent extraction platforms.

2. Equipment required not supplied by the manufacturer with calibration/service requirements and dates where applicable.

    - -20 °C ± 10°C Freezer
    - -80 °C ± 10°C Freezer
    - 2 to 8 °C Refrigerator
    - Microcentrifuge for 1.5mL tubes
    - Disposable, powder-free gloves (latex or nitrile)
    - 1.5 mL screw-capped microcentrifuge tubes
    - Tube racks
    - Biohazard bag for tips and tube disposal
    - Thermal cycler appropriate reaction tubes or 96-well reaction plates
    - Dedicated laboratory coats for each area
    - Extraction platform Bullet

3. Reagents required that are not provided by the manufacturer with shelf-life expiry dates and storage conditions. Include positive and negative control materials.

    - Positive and Negative control material
    - DEPC treated molecular grade water
    - 10% (v/v) freshly made from bleach solution (0.5% w/v sodium hypochlorite in water)
    - 70% ethanol (freshly made)

Performance characteristics

Analytical Sensitivity and Linearity of SARS COV-2 targets

1. Dilution series: See LoD data in table X and Graph X

2. Linearity and efficiency: See figure X R2 = 0.9915

3. Lowest Limits of Detection (LLOD): Manufacturers Lower limit of detection established at 10 copies per reaction (This is approximate to < 500 copies per ml.

Table 1. LoD as per IFU

SARS-CoV-2 (copies/reaction)	**Positivity**	**Average Ct**	**Standard Dev.**
1,000,000	3/3	19.54	0.24
100,000	3/3	22.96	0.12
10,000	3/3	26.18	0.39
1,000	3/3	29.44	0.12
100	3/3	32.44	0.32
10	3/3	36.92	0.27
1	1/3	43.81	NA
0.1	0/3	NA	NA
NTC	0/3	NA	NA

Once the presumptive LoD was determined it was verified by running 25 independent samples set at the concentration from the 10-fold serial dilution series in which 3 out of 3 samples were positive, in this case 10 copies/reaction. Table above shows the verification of the LoD for the assay when run on the Bio-Rad CFX96 Dx. Additional real-time platforms were also validated for the assay by verifying the LoD on the platform. Independent LoD analysis was carried out and measured using DPCR and is presented in Table 3 and Figure 1.

Table 2. LoD by amplification platform

Input	Copies/reaction	Average Ct	Standard deviation	Detection
SARS-CoV-2	10	33.90	1.85	25/25
Rotor-Gene Q	10	34.49	1.95	22/22
ABI 7500 Fast Dx	10	35.28	1.93	19/20
LightCycler 480 II	10	35.57	0.68	19/20
QuantStudio 7	10	35.12	2.86	19/20

Table 3. Independent LoD, Basingstoke

Triplicate Ct (x̄)	log 10 dC/ml
21.61	6.3
24.17	5.3
26.98	4.3
28.93	4
30.4	3.3

Efficiency determined by standard series dilution, R = 0.9915.

Precision and robustness

1. Intra-assay precision: 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%

2. Repeatability: LoD was verified by running 25 replicates from extraction through to detection. 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	33.90	1.85	25/25

Analytical specificity (interferences and cross-reactions)

Cross-reactivity to non-target samples/organisms. In vitro analysis for exclusivity or cross-reaction of 40 respiratory pathogens undertaken and reported by the manufacturer. No cross-reactivity observed under the tested conditions. Where quantification was possible bacteria were input at 1x106 cfu per reaction and virus at 1x105 pfu per reaction where concentration was unknown (commercially available validation panels), the highest available input volume was used. In addition, one of the independent validation sites performed analysis using Zeptometrix RVP2 panel, no cross reactivity was observed

Diagnostic sensitivity and specificity (Clinical validation with confirmed positives and negatives)

1. Low medium and high viral load samples were tested to avoid lowering Diagnostic sensitivity.

CT<25	CT 25 to 30	CT > 30
17	28	17

2. Diagnostic sensitivity: Confirmed clinical samples from patients (positive RT-qPCR result) were be used to calculate sensitivity. 454 negative samples and 379 positive samples were analysed in nine validation studies. The comparator assay details are provided in table X. CT data for the largest positive data set (Validation set 7 in table 5) is shown in table X below. All 17 sample with CTs >30 were detected of which 7 had CTs >35. The assessed assay gave CTs values that were an average of 2.3 cycles earlier than that of the comparator.

3. Diagnostic specificity: Confirmed clinical samples from patients (negative RT-qPCR result) should be used. Preferably, depending on the availability of samples, ~250 samples should be included to align with MHRA TPP. Clinical specificity (95% CI) and negative predictive value (PPV) should be calculated in comparison with a CE marked reference method that itself has sensitivity and specificity in line with the MHRA TPP. The CT values or equivalent for both the assessed and comparator assays must be included in the validation report.

Table 4. Aggregated assay performance characteristics

Comparator (Targets)	Extraction Method	Ral-Time Platform	Pos/Pos	Pos/Neg	Neg/Pos	Neg/Neg
RealStar SARS-CoV-2 RT-PCR (S, E)	MagNA Pure 96 – DNA and Viral RNA Small Kit	ABI 7500 Fast Dx	59	0	1	30
RealStar SARS-CoV-2 RT-PCR (S, E)	MagNA Pure 96 – DNA and Viral RNA Small Kit	ABI 7500 Fast Dx	24	0	1	5

Comparator (Targets)	Extraction Method	Ral-Time Platform	Pos/Pos	Pos/Neg	Neg/Pos	Neg/Neg
RealStar SARS-CoV-2 RT-PCR (S, E)	MagNA Pure 96 – DNA and Viral RNA Small Kit	ABI 7500 Fast Dx	25	0	0	5
RealStar SARS-CoV-2 RT-PCR (S, E)	MagNA Pure 96 – DNA and Viral RNA Small Kit	Bio-Rad CFX96	35	0	1	47
COVID-19 genesig Real-Time PCR Assay (RdRp)	QAIcube – QIAamp Viral RNA/DNA	Bio-Rad CFX96	26	0	0	24
COVID-19 genesig Real-Time PCR Assay (RdRp)	Maxwell RSC 48 – RSC Viral TNA Kit	Magnetic Induction Cycler	56	2	0	92
In-house	QIAamp Viral RNA Mini Kit	Bio-Rad CFX96 Deepwell	50	0	0	77
In-house	Chemagic Viral DNA/RNA 330 Kit	ABI QuantStudio Q6	38	0	1	30
COVID-19 genesig Real-Time PCR Assay (RdRp)	Maxwell RSC 48 – RSC Viral TNA Kit	Magnetic Induction Cycler	62	2	0	138
Total	Total	Total	375	4	4	448

Aggregated results

Sensitivity (95 CI)	Specificity (95 CI)
98.9% (97.1 to 99.6)	99.1% (97.6 to 99.6)

Published to GOV.UK in pdf format only.

www.gov.uk/dhsc