White Paper

Title: SARS-CoV-2 RT-PCR Detection Assay – Design, Analytical and Multi-centre Evaluation of Clinical Performance

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Abstract: To address the testing needs in response to the SARS-CoV-2 pandemic QuantuMDx has designed, developed and validated a RT-PCR test. While similar in many ways to other assays that have been commercialized, the QuantuMDx assay has been validated for a broad range of extraction methods, real-time instrumentation and most importantly for detection direct from nasopharyngeal and saliva specimens. These differentiators make the QuantuMDx assay highly flexible and easy-to-use allowing it to be utilized in almost all clinical diagnostic settings. Understanding the analytical and clinical performance of a molecular assay targeting a new analyte is critical in gauging how well the assay will perform in sites of intended use. Upon completion of the development of the SARS-CoV-2 RT-PCR detection Assay, QuantuMDx undertook with partners, extensive clinical performance testing. Clinical performance testing was done as blinded, independent studies carried out by a number of sites. In all cases the external sites chose all samples which would be tested and QuantuMDx was not privy to the results until the study was completed and analysed by the site. In a number of cases the studies were conducted to support the equivalent use of extraction methods and platforms as well as additional real-time instruments. Overall clinical performance was outstanding with a positive percent agreement (PPA) or 99.4% and a negative percent agreement of 98.7%. Based upon the analytical and clinical performance data the QuantuMDx SARS-CoV-2 RT-PCR Detection Assay provides a highly sensitive, specific and easy to use solution for centralized laboratory testing.

Introduction: On December 31, 2019, an outbreak of a pneumonia of unknown aetiology 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. If disease progresses in severity the individual can develop pneumonia, severe acute respiratory syndrome, kidney failure and death.

Nucleic acid amplification tests (NAATs) are the primary means for the detection of infections caused by the SARS-CoV-2 virus. Current data indicates that viral genomic RNA can be detected in upper- and lower-respiratory tract specimens prior to an individual being symptomatic [1-4].

In response the SARS-CoV-2 pandemic, QuantuMDx quickly designed and developed a molecular assay. The design of an assay for the detection of SARS-CoV-2 genomic RNA is critical due

to the potential for genomic loci to be shared across coronavirus species. Herewith we describe the analytical and clinical performance characteristics of the QuantuMDx SARS-CoV-2 RT-PCR Detection Assay.

Methods and Materials:

Design. A vetted database was generated of sequences from NCBI and GISAID (10,151 genomic sequences of greater than 25,000 bases). A systematic and holistic approach was then undertaken for the selection of a minimum of three unique loci within the SARS-CoV-2 genome which had no cross-reactivity to any other known coronaviruses. The analysis was performed locally and utilized open source software. In addition, a set of selection criteria were put into place. The analysis generated multiple solutions for multiple loci within the SARS-CoV-2 genome. Based upon set criteria, a subset if the solutions were selected for *in vitro* assessment. The selected solutions were then down selected based upon pre-set performance criteria.

QuantuMDx SARS-CoV-2 RT-PCR Detection Assay utilizes 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-labelled with a reporter dye attached to the 5'-end and a quencher attached to the 3'-end. In this process, the oligonucleotides anneal 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. The assay utilizes FAM for the detection of the three SARS-CoV-2 loci, Orf1ab, N gene and the S gene. HEX is utilized for the detection of the RNAse P gene, which serves as a specimen and whole process control.

Positive Control. The QuantuMDx SARS-CoV-2 RT-PCR Detection Kit does not provide a positive control. However, QuantuMDx has validated Positive Control materials. The Positive Control is comprised of Twist Synthetic SARS-CoV-2 RNA Control-2 (Twist Biosciences) and QPCR Human Reference Toral RNA (Agilent) at fixed concentrations in RNAse free molecular grade water. For the purposes of all of the studies herein the Positive Control was fixed at 500 copies of SARS-CoV-2 and 25 ng of human RNA per reaction.

Analytical Performance. Performance studies were undertaken following the design, development and transfer of manufacturing to a contract manufacturing organization (CMO). All studies were undertaken with manufactured and quality control released product.

(i) **Limit of Detection (LoD).** The LoD was determined as the lowest concentration of SARS-CoV-2 target that could be detected by the assay with a greater than 95% positivity rate (i.e. 19 out of 20). A presumptive LoD was determined by a 10-fold dilution of a contrived sample containing known concentrations of SARS-CoV-2 targeted loci and a fixed concentration of human RNA. Triplicates of independent samples were run for each of the 8 concentrations of SARS-CoV-2 target. Once the presumptive LoD was determined it was verified by running a minimum of 20 independently processed samples at the presumptive LoD. This was done for all real-time platforms that have been validated for use with the assay. Verification of LoD was deemed to be a minimum of 19 out 20 independent samples being positive.

(ii) **Inclusivity (*in silico*).** Inclusivity testing was performed on the locked primer and probe combinations and with downloaded and vetted whole genome sequences from NCBI and GISAID (NCBI = 1,022 and GISAID = 9,126). Analysis was performed for the QuantuMDx primers and probes which are in the N and S gene and the Orf1ab. Each primer and probe set were analyzed for inclusivity versus the vetted database of sequences. Part of the analysis was mismatching with a cut-off of no more than 1 mismatch under a primer and probe and with the mismatch present at no greater than 1% of all sequences within the database.

(iii) **Exclusivity (*in silico*).** The locked primers and probes were also analyzed for exclusivity or cross-reactivity to a predefined list of organisms. The list was an expanded list from the FDA of potential commensal and infectious organisms which could be found in upper respiratory specimens. Due to a limit in computational power, for all non-viral organisms a reference genome was utilized for the analysis, for most viral organisms when available a maximum of 100 unique genome sequences were utilized and in certain cases with analyzed viruses a fixed number of genomes were utilized. A cut-off of greater than or equal to 80% homology was recorded as a potential positive binding event. If this did occur additional analysis was performed to determine if a true real-time amplification reaction could occur with a concomitant hydrolysis of a probe.

(iv) **Exclusivity/Cross-reactivity (wet testing).** *In vitro* or wet analysis was also carried out for a limited subset of the potential organisms (n=29) which could cause cross-reactivity. In all cases independent samples in a background of 25ng of human total RNA were run in triplicate. Where quantification was possible bacteria were run at $1x10^6$ cfu/reaction and viruses at $1x10^5$ pfu/reaction. In cases were quantification was unknown (commercially available panels) the highest available

input volume was used. The samples were processed with the validated QIAamp Viral RNA Mini Kit (Qiagen) and run on the CFX96 Dx (Bio-Rad). Analysis looked at all potential cross-reactivity for both cross-reactivity in the FAM (SARS_CoV-2) and HEX (RNAse P) channels.

(v) **Interfering Organisms.** The same 29 organisms were also run as independent samples with 20 copies/reaction of the Twist Biosciences SARS-CoV-2 RNA control material and 25ng human total RNA. Each organism was run in triplicate. Where quantification was possible bacteria were run at $1x10^6$ cfu/reaction and viruses at $1x10^5$ pfu/reaction. In the cases where quantification was unknown (commercially available panels) the highest available input volume was used. The samples were processed with the validated QIAamp Viral RNA Mini Kit (Qiagen) and run on the CFX96 Dx (Bio-Rad). Analysis looked at all potential forms of inhibition of the reactions, including Ct shifts, amplification efficiencies as well as amplification endpoints.

(vi) **Interfering Substances.** A selected list of potential interfering substances was determined based upon the potential for the substance to be present in upper respiratory specimens. Twelve potential interfering substances were determined, of both endogenous and exogenous origin. Each substance was tested at three concentrations, in triplicate with and without the addition of SARS-CoV-2 target (20 copies/reaction SARS-CoV-2 (Twist Bioscience) and human total RNA at 2.5 ng/reaction). The samples were processed with the validated QIAamp Viral RNA Mini Kit (Qiagen) and run on the CFX96 Dx (Bio-Rad). Analysis looked at all potential forms of inhibition of the reactions, including Ct shifts, amplification efficiencies as well as amplification endpoints.

(vii) **Acceptance and Validity Criteria.** All testing included a set of criteria for validity and acceptance of the data sets generated. These included the running of positive and negative controls as well as Ct acceptance ranges.

(viii) **Analysis.** All analysis criteria were prescribed prior to experimentation. Analysis differed dependent upon the experimentation.

External Clinical Performance Evaluations. Sites which performed clinical performance evaluations were provided with positive control materials and manufactured QuantuMDx SARS-CoV-2 RT-PCR Detection Kits. The external sites in consultation with QuantuMDx decided on the extraction method and real-time platform which would be utilized for the study. Analysis was initially performed by the site and then in combination with QuantuMDx. Studies were performed on discarded/residual samples and results were not utilized for any clinical decision making. In all cases performance was assessed by

comparison of the QuantuMDx assay versus the site's standard of care method. All analysis was carried out using two-by-two tables (e.g. positive and negative percentage agreement and sensitivity and specificity). Confidence intervals were determined by the Wilson method [Statistics with Confidence (2nd Ed.) DC Altman, et. al. (pub: British Medical Journal Books, UK, 2000) p.46-7].

Results:

Analytical Performance

Limit of Detection. A presumptive LoD was initially determined utilizing the Bio-Rad CFX96 Dx by utilizing a ten-fold serial dilution series (Table 1). For all LoD testing the following validity and acceptance criteria had to be met in order to consider the run valid and the data acceptable; (i) Positive control must provide positive signal for SARS-CoV-2 (Ct ≤ 40) and RNAse P (Ct ≤ 36) , (ii) Negative control negative for all signals (FAM and HEX) and (iii) RNAse P signal (HEX) for all samples (Ct ≤ 36). In addition, acceptance of the verification data was based upon 95% positivity of 19 out 20 independent samples.

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

Table 1. Determination of the assays presumptive LoD.

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 X shows the verification of the LoD for the assay when run on the Bio-Rad CFX96 Dx.

Input	Copies/reaction	Average Ct	Standard Deviation	Detection
SARS-CoV-2	10	33.90	1.85	25/25

Table 2. Verification of the LoD for the QuantuMDx SARS-CoV-2 RT-PCR Detection Assay on the Bio-Rad CFX96 Dx.

Additional real-time platforms were also validated for the assay by verifying the LoD on the platform. Table 3 provides the verification data for four additional real-time platforms.

Platform	Copies/reaction	Average Ct	Stand. Dev.	Detection
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. Verification of the LoD for an expanded number of real-time platforms.

These data are consistent with the LoD of the QuantuMDx SARS-CoV-2 RT-PCR Detection Assay being 10 copies/reaction for all tested real-time platforms.

Inclusivity (*in silico*). Analysis was carried out to determine if the proposed primer and probe solutions would amplify and detect all known sequences of SARS-CoV-2. Table X provides the output of the analysis (15 April, 2020). Cut-offs were set that such that (i) a primer and probe could only have one mismatch cross its entire length and (ii) that mismatch can only occur in less than 1% of all known sequences of SARS-CoV-2.

QuantuMDx Name	Mismatch rates of single base
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%

Table 4. *In silico* analysis of primers and probes utilized in the QuantuMDx SARS-CoV-2 RT-PCR Detection Assay versus all known full-length sequences of the virus (15 April, 2020).

Based upon the *in silico* analysis there is no evidence that the assay could not detect all of the current full length SARS-CoV-2 genome data (15 April, 2020). In addition, in no case was an individual sequence identified which had mismatches in primers or probes for all three targeted loci.

Exclusivity (*in silico*). 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 Beta-coronavirus	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

Organism	Type	Count	Sequence Type
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
Human gamma herpesvirus 4 (Epstein 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

Organism	Type	Count	Sequence Type
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

Table 5. List of all organisms utilized for in silico analysis of cross-reactivity and the number of unique sequences utilized for the analysis.

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 defined above, indicating by *in silico* analysis there is almost no chance of off target signal generation.

Exclusivity (wet testing). Wet testing was carried out as described for a subset of the organisms utilized for *in silico* analysis. Twenty-nine organisms were tested. 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.

Organism	SARS-CoV-2 Signal	RNAse P Signal (HEX)		
		Average	St. Dv.	%CV
Enterobacter cloacae	0/3	28.10	0.05	0.18

Escherichia coli	0/3	28.14	0.09	0.32
Klebsiella pneumoniae	0/3	27.98	0.10	0.36
Streptococcus agalactiae	0/3	28.27	0.22	0.78
Legionella pneumophilia	0/3	28.19	0.13	0.46
Streptococcus pyrogenes	0/3	28.07	0.18	0.64
Mycobacterium tuberculosis	0/3	27.76	0.12	0.43
Mycobacterium bovis subsp Bovis	0/3	27.95	0.13	0.47
Neisseria meningitides	0/3	28.53	0.43	1.51
Candida albicans	0/3	28.32	0.05	0.18
Proteus vulgaris	0/3	28.19	0.25	0.89
Pseudomonas aeruginosa	0/3	28.40	0.29	1.02
Staphylococcus aureus	0/3	28.27	0.24	0.85
Staphylococcus epidermidis	0/3	28.33	0.17	0.60
Bordetella pertussis	0/3	28.15	0.15	0.53
Adenovirus Group A - G, 7, 8	0/3	28.41	0.28	0.99
Enterovirus A-L	0/3	28.24	0.24	0.85
Human coronavirus HKU1	0/3	28.29	0.08	0.28
Human coronavirus OC43	0/3	28.32	0.23	0.81
Human metapneumonvirus	0/3	28.32	0.17	0.60
Influenza A	0/3	28.58	0.29	1.01
Influenza B	0/3	26.97	0.23	0.85
MERS coronavirus	0/3	26.82	0.41	1.53
Parainfluenza 1	0/3	26.64	0.10	0.38
Respiratory syncytial virus A	0/3	28.11	0.25	0.89
Respiratory syncytial virus B	0/3	27.87	0.34	1.22
Rotavirus	0/3	26.67	0.32	1.20
SARS coronavirus	0/3	26.90	0.26	0.97
Human immunodeficiency virus HIV-1	0/3	26.95	0.30	1.11

Table 6. Results for wet testing of cross-reactivity

Across all wet testing versus potential commensal and infectious organisms no cross-reactivity was observed**.**

Interfering Organisms. The same twenty-nine organisms were also utilized for wet testing to determine if any commensal or potential co-infecting organism might interfere in the detection of SARS-CoV-2. 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.

Organism	SARS-CoV-2 Signal	SARS-CoV-2 Signal (FAM)		
		Average	St. Dev.	%CV
Enterobacter cloacae	3/3	33.36	0.65	1.95
Escherichia coli	3/3	33.67	1.03	3.06
Klebsiella pneumoniae	3/3	34.87	1.4	4.01
Streptococcus agalactiae	3/3	33.86	0.96	2.84
Legionella pneumophilia	3/3	33.37	0.49	1.47
Streptococcus pyrogenes	3/3	34.36	1.13	3.29
Mycobacterium tuberculosis	3/3	32.58	0.14	0.43

Mycobacterium bovis subsp Bovis	3/3	33.51	0.95	2.83
Neisseria meningitides	3/3	34.63	1.65	4.76
Candida albicans	3/3	33.78	0.52	1.54
Proteus vulgaris	3/3	33.72	0.12	0.36
Pseudomonas aeruginosa	3/3	34.05	1.28	3.76
Staphylococcus aureus	3/3	32.98	0.23	0.70
Staphylococcus epidermidis	3/3	34.01	0.79	2.32
Bordetella pertussis	3/3	33.53	0.5	1.49
Adenovirus Group A - G, 7, 8	3/3	33.6	1.04	3.10
Enterovirus A-L	3/3	34.00	0.58	1.71
Human coronavirus HKU1	3/3	33.34	0.13	0.39
Human coronavirus OC43	3/3	33.27	0.44	1.32
Human metapneumonvirus	3/3	33.72	0.89	2.64
Influenza A	3/3	33.01	0.48	1.45
Influenza B	3/3	35.66	0.04	0.11
MERS coronavirus	3/3	34.88	0.46	1.32
Parainfluenza 1	3/3	34.72	0.36	1.04
Respiratory syncytial virus A	3/3	35.9	1.64	4.57
Respiratory syncytial virus B	3/3	34.8	0.62	1.78
Rotavirus	3/3	34.97	0.59	1.69
SARS coronavirus	3/3	34.25	1.94	5.66
Human immunodeficiency virus HIV-1	3/3	35.23	0.51	1.45
SARS-CoV-2 (No interfering organism)	3/3	34.09	0.74	2.17

Table 7. Results for interference testing

Across all wet testing versus potential commensal and infectious organisms no interference was observed**.**

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	Detection (SARS-CoV-2 present)	Detection (No target present)
Mucin – bovine submaxillary gland, type I-S*	5 mg/mL	3/3	0/3
Blood (Human)	5% v/v	3/3	0/3
Phenylephrine hydrochloride, Max strength Cold and Flu Relief	0.2 mg/mL	3/3	0/3
Oxymetazoline hydrochloride, Blocked nose relief	30% v/v	3/3	0/3
Beclomethasone dipropionate	0.037% v/v	3/3	0/3
NasalGuard Cold and Flu block	0.125 drops in 2mL	3/3	0/3
Galphimia glauca, cardiospermum and Luffa operculata, Rhinital	1 tablet in 2mL	3/3	0/3
Benzocaine	2.5 mg/mL	3/3	0/3
Menthol	0.084% v/v	3/3	0/3
Zanamivir, antiviral drug	5 mg/mL	3/3	0/3
Mupirocin, antibiotic	5 mg/mL	3/3	0/3

Tobramycin, antibiotic	1.2 mg/mL	3/3	0/3

Table 8. Summary of interfering substances testing

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. These data show that under the conditions tested these twelve potential interfering substances had no effect upon the performance of the assay for the detection of SARS-CoV-2

Clinical Performance

Clinical performance was independently evaluated at five sites in the United Kingdom across eight different studies. One of the goals for these studies was to extend the label claims to increase the number of validated extraction methods and real-time platforms on which the assay can be utilized. While clinical studies were discussed with the clinical testing sites, the clinical testing sites had full control over all specimen selection and protocols. QuantuMDx did provide in some cases guidance on the raw data analysis.

Comparator (Targets)	Extraction Method	Real-time Platform	Results (QMDx/comparator)				PPA (95% CI)	NPA (95% CI)
			Pos/Pos	Pos/Neg	Neg/Pos	Neg/Neg		
RealStar SARS-CoV-2 RT-PCR (S, E)	MagNA Pure 96 – DNA and Viral RNA Small Volume Kit	ABI 7500 Fast Dx	59	0	1	30	100% (95.0 – 100%)	96.7% (86.9 – 96.7%)
RealStar SARS-CoV-2 RT-PCR (S, E)	MagNA Pure 96 – DNA and Viral RNA Small Volume Kit	ABI 7500 Fast Dx	24	0	1	5	100% (90.3 – 100%)	83.3% (44.5 – 83.3%)
RealStar SARS-CoV-2 RT-PCR (S, E)	MagNA Pure 96 – DNA and Viral RNA Small Volume Kit	ABI 7500 Fast Dx	25	0	0	5	100% (91.1 – 100%)	100% (55.7 – 100%)
RealStar SARS-CoV-2 RT-PCR (S, E)	MagNA Pure 96 – DNA and Viral RNA Small Volume Kit	Bio-Rad CFX96	35	0	1	47	100% (91.6 – 100%)	97.9% (91.8 – 97.9)
COVID-19 genesig Real-Time PCR Assay (RdRp)	QAIcube – QIAamp Viral RNA/DNA 96 Kit	Bio-Rad CFX96	26	0	0	24	100% (89.19 – 100%)	97.9% (89.1 – 97.9)
COVID-19 genesig Real-Time PCR Assay (RdRp)	Maxwell RSC 48 – RSC Viral TNA Extraction Kit	Magnetic Induction Cycler	56	2	0	92	96.6% (93.4 – 98.4%)	96.1% (94.3 – 97.1)
In house	QIAamp Viral RNA Mini Kit	Bio-Rad CFX96 Deepwell	50	0	0	77	100% (94.5 – 100%)	100% (96.4 – 100%)
In house	Chemagic Viral DNA/RNA 330 Kit	ABI QuantStudio Q6	38	0	1	30	100% (92.4 – 100%)	96.8% (87.4 – 96.8%)
		Aggregated results	**313**	**2**	**4**	**310**	**99.4% (98.1 – 99.9%)**	**98.7% (97.4 – 99.2%)**

Table 9. Overview of clinical performance data

Discussion: Extensive testing has been conducted on the QuantuMDx SARS-CoV-2 RT-PCR Detection Assay to understand both analytical and clinical performance specifications for a number of extraction methods, real-time PCR instruments. The assay performed consistently across multiple sites and users as well as across multiple combinations of extraction methods and real-time instruments. The utilization of three unique loci from the SARS-CoV-2 provides built-in redundancy. In addition, because the E gene is not utilized a presumptive positive result due to cross-over with other coronaviruses as the E gene is conserved (pan Sarbecovirus) will not occur.

In conclusion, the QuantuMDx SARS-CoV-2 RT-PCR Detection assay is highly sensitive and specific for the detection of the SARS-CoV-2 virus. The assay is simple to use as the Master Mix is provided as a single reagent containing all required components for the specific detection of three loci within the SARS-CoV-2 genomic RNA.

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