Department of Health & Social Care

Desktop validation report for SARS-CoV-2 Nucleic Acid Detection

Randox COVID-19 qPCR assay, CQP10449 GTIN: 05055273216929

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Contents

The product described below has been assessed by a desktop review of data by the Technologies Validation Group (TVG).

Assay description and intended purpose

1. The COVID-19 qPCR assay has been designed to provide detection of two pathogens, SARSCoV-2 (ORF1ab) and Sarbecovirus (SARS, SARS like, SARS-CoV-2) (E-gene), in a multiplex format. The multiplex primer mix in this assay allows the amplification of specific gene sequences found within the genome of the pathogen. During PCR cycling, RNA extracted and purified from the clinical sample, is reverse transcribed to complementary DNA (cDNA) which is then, along with any other extracted or purified pathogen and human DNA, denatured at high temperatures to form single strands of DNA. During the annealing step of the PCR reaction, the primers and probe anneal specifically to target sequences on the single strands of DNA after which the Taq polymerase in the master mix synthesizes new DNA strands. When the Taq polymerase meets the TaqMan® probe, the polymerase cleaves the probe due to its 5' nuclease activity, releases the fluorescent dye and produces a detectable fluorescent signal. With each cycle the signal intensity increases exponentially as more fluorescent dye is cleaved from the probe. This is proportional to the amount of PCR product produced.

2. This assay is intended for in vitro diagnostic use. The COVID-19 qPCR assay can be used for the detection of nucleic acid from 2019-Novel Coronavirus (SARS-CoV-2) and Severe Acute Respiratory Syndrome ((SARS) or SARS-like) viruses. The differentiation of SARS-CoV-2 and SARS-like corona infections from other seasonal respiratory illnesses caused by viruses can prevent rapid spread of infection through the isolation of positive cases.

3. This test is intended for use in the clinical setting as an aid in the screening and isolation of individuals that require quarantine and immediate intervention.

4. The assay is intended for use with extracted nucleic acid and as such does not have any specific biosafety requirements post sample extraction. The instructions for use (IFU) recommends that pre-extraction, clinical specimens should be handled and treated as if they are potentially infectious.

Type of sample to be used in validation

1. Stipulate the sample type (for example, whole non-extracted virus, extracted RNA, synthetic RNA, plasmid DNA containing assay target regions) and any sample

matrices (for example, saliva, plasma, nasopharyngeal or oronasal swab [dry or in VTM] etc) in which the material spiked. Is the test a stand-alone device or test or to be used in conjunction with other equipment and is biosafety containment is required?

- The assay is compatible extracted nucleic acid obtained from sample matrices including nasal and throat swabs. The type of sample used in the validations is not specified in the raw validation data report. Validation of sample types should take place at each test site.

- IFU Recommendation: 300 µl of sample to be extracted and eluted in 80 µl of elution buffer.

- The product has been validated by the manufacturer for samples extracted on the Chemagic™ 360 Instrument (Cat # 2024-0020) and the extraction Kit: Chemagic™Viral DNA/RNA 300 kit special h96 (Cat # CMG-1033). Alterative extraction methods must be validated by the user.

Equipment and Reagents

1. List all the equipment required that is not supplied by the manufacturer.

    - real-time PCR machine

    - Applied Biosystems™ QuantStudio™ 5 (0.2 mL 96 well block) or

    - Applied Biosystems™ 7500

    - microcentrifuge capable of holding 0.5 ml and 1.5 ml microcentrifuge tubes

    - 0.5 to 10 µl pipette

    - 5 to 50 µl pipette

    - 20 to 200 µl pipette

    - 200 to 1000 µl pipette

    - vortex mixer

    - ice bath or cold block

    - PCR plate centrifuge

2. List all the reagents required that are not provided by the manufacturer with shelf-life expiry dates and storage conditions. Include positive and negative control materials.

    - the COVID-19 qPCR Assay requires the use of a suitable nucleic acid extraction procedure which is not supplied in the kit. This kit is validated for use with the Perkin Elmer Chemagic™ Viral 300 DNA/RNA kit
    - special H96 on the Chemagic™ 360 instrument
    - nuclease-free water (for example, Ultrapure distilled water DNase and RNase-free (500 mL) Invitrogen Cat # 10977-015)
    - filter pipette tips (DNA, DNase & RNase-free)
    - 0.5 ml and 1.5 ml microcentrifuge tubes (DNA, DNase & RNase-free)

Performance characteristics

Analytical sensitivity and linearity of SARS CoV-2 targets

1. Dilution series: This was calculated using a validated standard dilution series and the results are tabulated below

    Randox SARS-COV-2 dilution series of Qnostics SARS-COV-2 control results

Qnostics SARS-COV-2 Copies/ml	**E gene CT**	**Orf1ab CT**
2000000	22.32445	21.87
200000	25.84332	25.52
20000	28.78787	28.52
10000	29.99409	29.63
2000	32.27035	31.93
1000	33.04457	32.96
200	35.17205	34.64
100	36.53003	36.06

2. Linearity Data from 1. was used to calculate linearity. Both gene targets demonstrate good linearity of 0.9978 and 0.999.

3. Lowest Limits of Detection (LLOD). The validation demonstrated that the limit of detection was equal or lower than that stated in the IFU (750 copies per ml). Precision and robustness Inter-assay precision: The Randox validation included inter-assay precision for 7 replicates of Qnostics control samples on ten separate runs. The average standard deviation was 0.562 and the CV was 1.77 and is within the desired specification (less than 15%)

Analytical specificity (interferences and cross-reactions)

1. Cross-reactivity to non-target samples and organisms. A range of samples either direct clinical samples or spiked samples that are known positives for other diseases, both closely related to be tested. 38 non-SARS-CoV-2 respiratory organisms were in vitro tested to demonstrate assay specificity, no cross reactivity was observed. In additional to in vitro testing, the IFU states the in-silico analysis for both assays target primer/probe regions and relevant respiratory targets in line with the recommendations of the MHRA TPP for laboratory-based SARS-CoV-2 tests.

2. TVG did not assess the assay performance for samples containing potential interfering substances

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

1. Samples selected for this validation were appropriate to the assay and according to the IFU. To avoid increasing or lowering diagnostic sensitivity or specificity. Low medium and high viral loads were distributed as summarised in the tables below.

CT value	Randox pos/Allinity pos	Sensitivity (%)
<25	104/104	100
25-30	10/10	100
30-35	12/12	100
>35	4/5	80

CT Value Range	Randox pos/Abbott M2000 pos	Sensitivity (%)
<25	25/25	100
25 to 30	n/a	n/a
30 to 35	n/a	n/a
>35	n/a	n/a

2. Diagnostic sensitivity: confirmed clinical samples from patients (positive RT-qPCR result) were included in the assay validation. In total 156 positive samples were included to align with the requirements of the MHRA TPP for laboratory-based SARS-

COV-2 tests. Clinical sensitivity (95% CI) was calculated in comparison with a CE marked reference method that itself has a stated sensitivity and specificity and a limit of detection within the specifications of the MHRA TPP. The CT values or equivalent for both the assessed and comparator assays were compared.

3. Diagnostic specificity: 267 clinical samples from patients with negative RT-qPCR result were included in total to align and exceed the requirements of the MHRA TPP for laboratory-based SARS-COV-2 tests. Clinical specificity (95% CI) was calculated in comparison to a CE marked reference method that itself had sensitivity and specificity in line with the MHRA TPP.

		Standard of Care assay		**Total**
Randox SARS-COV-2		+	-	
	+	155	0	155
	-	1	267	268
Total		156	267	423

		95%(CI)
Sensitivity	99.4	96.46 to 99.89
Specificity	100	98.58 to 100.00

Accuracy (external quality assurance)

1. Seven distributions from NEQAS (National External Quality Assurance Scheme) were reported and all distributions achieved the full available marks (all scored samples were correctly identified). The scheme distributes 2 samples in a monthly cycle. The results are allocated a maximum score of 2 points each. The current EQA performance score is 12 out of 12.

Summary and limitations of the validation report

1. A limited number of samples were tested in the CT range of less than 30

2. The validation was performed by desktop review of independent data. TVG cannot comment on the usability of the named assay in a laboratory environment

Noting the exceptions stated above, the assessed assay performs according to the acceptable criteria for laboratory-based SARS-CoV-2 assays. Additional local validation or verification is recommended as per UKAS requirements for laboratories implementing assays for clinical diagnostics and screening purposes.

TVG uses a wide range of sites in order to validate new technologies and tests. These independent sites use a range of RT-qPCR assays against different genomic regions and it is recognised that for some assay comparisons the sensitivity of RT-qPCR assay(s) may subtly differ from the true sensitivity of the test if compared to the same genomic region.

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