



Technical Validation Report for Roche Cobas SARS CoV-2

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Assay description

1. What is the principle and method of the assay (description of the assay according to the manufacturer's Instructions for Use (IFU)?

As stipulated in the IFU 09179909001-07EN, Doc Rev 7:

Cobas SARS CoV-2 for use on the Cobas 6800/8800 platform is a real-time RT-PCR for the detection of SARS CoV-2 RNA by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time RT-PCR and in vitro diagnostic procedures. Cobas SARS CoV-2 is based on fully automated sample preparation followed by PCR amplification and detection. The Cobas 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the Cobas 6800/8800 software, which assigns test results for all tests. Results can be reviewed directly on the system screen and printed as a report.

Nucleic acid from patient samples and internal control RNA samples (RNA IC) are simultaneously extracted. Nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris and potential PCR inhibitors, are removed with subsequent wash steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature. External controls (positive and negative) are processed in the same way with each Cobas SARS-CoV-2 run.

Selective amplification of target nucleic acid from the sample is achieved by the use of target-specific forward and reverse primers for ORF1 a/b non-structural region that is unique to SARS-CoV-2. Additionally, a conserved region in the structural protein envelope E-gene were chosen for pan-Sarbecovirus detection. The pan-Sarbecovirus detection sets will also detect SARS-CoV-2 virus.

Selective amplification of RNA Internal Control is achieved by the use of non-competitive sequence specific forward and reverse primers which have no homology with the coronavirus genome. A thermostable DNA polymerase enzyme is used for amplification.

2. What is the use for which the device is intended according to the data supplied by the manufacturer on the label, in the IFU, in promotional or sales materials or statements, or as specified by the manufacturer in the performance evaluation?

As stipulated in the IFU 09179909001-07EN, Doc Rev 7:

Cobas SARS CoV-2 is intended for the qualitative detection of nucleic acids from SARS CoV-2 in healthcare provider-instructed self-collected anterior nasal swabs (collected on site) and healthcare provider-collected nasal, nasopharyngeal and oropharyngeal swabs collected in Copan Universal Transport Medium System (UTM-RT), BD™ Universal Viral Transport System (UVT), cobas PCR Media, or 0.9% physiological saline from any individuals including those suspected of COVID-19 by their healthcare provider and/or those without symptoms or any other reason to suspect COVID-19.

3. Is the test a stand-alone device/test or to be used in conjunction with other equipment and is biosafety containment is required?

The test is designed to be used with the Cobas 6800 or 8800 Systems. All human samples should be considered as infectious and handled according to Good Laboratory procedures. From the recommended viral transport media, only Cobas PCR Media has known inactivation activity of ≥ 3.9 TCID₅₀/mL viral titre as assessed by PHE

([https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/898541/HCM-CoV2-004-](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/898541/HCM-CoV2-004-v3_Roche_Cobas_PCR_Media_TCF.pdf)

[v3_Roche_Cobas_PCR_Media_TCF.pdf](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/898541/HCM-CoV2-004-v3_Roche_Cobas_PCR_Media_TCF.pdf)). This media is also supplied in the tube required to be used in the Cobas System, and so there are no pipetting steps once the sample is taken. For all other samples, there is a pipetting step that is required to pipette sample from the primary tube to a Cobas Omni secondary tube for processing. If the transport media used to transport and store the sample does not inactivate the sample, this should be performed in a biosafety cabinet.

Type of sample to be used in validation

1. Stipulate the sample type (e.g. whole non-extracted virus, extracted RNA, synthetic RNA, plasmid DNA containing assay target regions) and any sample matrices (e.g. saliva, plasma, nasopharyngeal/oronasal swab [dry or in VTM] etc) in which the material is to be spiked.

Whole non-extracted virus from nasopharyngeal, oropharyngeal or nasal swabs in virus transport medium. The swab types and transport media recommended in the IFU are Copan Universal Transport Medium System (UTM-RT), BD™ Universal Viral Transport System (UVT), cobas PCR Media, or 0.9% physiological saline.

2. Stipulate if the material is required to be extracted i.e. volume received, volume extracted, volume eluted, elution buffer to be used in the assay.

The Cobas 6800 and 8800 platforms both provide sample extraction as part of the automated workflow.

3. If possible, stipulate if any interfering substances such as preservatives are likely to be present. Shelf life and number of freeze thaw events should also be stated, if known. Where dry swabs are to be used, samples will need to be collected prospectively; two swabs per participant, one for a new test and one to be tested using reference method; or one swab for a new test collected within 24 hours of a positive reference method swab.

Equipment and reagents

1. List all the equipment required that are not supplied by the manufacturer with calibration/service requirements and dates where applicable.

All equipment and instrumentation are supplied.

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.

Product components

- Cobas SARS CoV-2 positive control kit
- Cobas buffer negative control kit
- Cobas Omni MGP reagent
- Cobas Omni specimen diluent
- Cobas Omni lysis reagent
- Cobas Omni wash reagent
- Cobas Omni processing plate
- Cobas Omni amplification plate
- Cobas Omni liquid waste container
- Cobas Omni pipette tips
- Cobas Omni secondary tubes

Performance characteristics

Sensitivity and Linearity

1. Dilution series: This should be calculated using a validated standard dilution series. If this is not possible (as standard material is not available), use 5 clinical positive replicates, with a 5 log₁₀ dilution, plus 5 negatives, plus the use of inhibition controls. If feasible, repeat over several days, different users/machines (feasibility may be limited due to availability of positive material). Where dry swabs are to be used, known amounts of standard material should be added to the swab, and then tested as per IFU. Reference to be made on the reporting of results and for example CT values.
 - From the IFU 09179909001-07EN, Doc Rev 7, details of a limited dilution series were provided.

To determine the LOD, a cultured virus of an isolate from a US patient (USA/WA-1/2020, catalog number NR-52281, lot number 70033175, 2.8E+05 TCID₅₀/mL) was serially diluted in simulated clinical matrix. A total of 7 concentration levels, with 3-fold serial dilution between the levels, were tested with a total of 21 replicates per concentration, with an additional 10 replicates of a blank sample (i.e, simulated clinical matrix).

As shown in Table 1, the concentration level with observed hit rates greater than or equal to 95% were 0.009 and 0.003 TCID₅₀/mL for SARS-CoV-2 (Target 1) and pan-Sarbecovirus (Target 2), respectively.

Table 1. Concentration level with observed hit rates greater than or equal to 95%

Strain	Concentration (TCID ₅₀ /mL)	Total valid results	Hit Rate (%)^		Mean Ct*	
			Target 1	Target 2	Target 1	Target 2
USA-WA1/2020§ (stock concentration 2.8E+05 TCID ₅₀ /mL)	0.084	21	100	100	31.0	33.0
	0.028	21	100	100	31.8	34.1
	0.009	21	100	100	32.7	35.2
	0.003	21	38.1	100	33.5	36.4
	0.001	21	0	52.4	n/a	37.9

Strain	Concentration (TCID ₅₀ /mL)	Total valid results	Hit Rate (%) [^]		Mean Ct [*]	
			Target 1	Target 2	Target 1	Target 2
	0.0003	21	0	14.3	n/a	37.2
	0.0001	21	0	9.5	n/a	38.5
	0.0 (blank)	10	0	0	n/a	n/a

2. § Reagent was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH: SARS Related Coronavirus 2, Isolate USA-WA1/2020, NR-52281

[^] All replicates where Target 1 was positive were also positive for Target 2.

^{*} Calculations only include positive results.

From Lab 1:

The Limit of detection of the assay was assessed by testing the Qnostics SARS-CoV-2 Analytical Q Panel in triplicate. The results are shown in Table 2 below:

Table 2. Qnostics Panel Limit of Detection

Qnostics Panel	Target 1 Number Positive (Av Ct Value)	Target 2 Number Positive (Av Ct Value)
S01 1000000 copies/ml	3 of 3 (24.0)	3 of 3 (24.4)
S02 100000 copies/ml	3 of 3 (27.2)	3 of 3 (27.7)
S03 10000 copies/ml	3 of 3 (30.2)	3 of 3 (30.9)
S04 5000 copies/ml	3 of 3 (31.2)	3 of 3 (32.1)
S05 1000 copies/ml	3 of 3 (33.0)	3 of 3 (34.1)
S06 500 copies/ml	3 of 3 (33.7)	3 of 3 (34.9)
S07 100 copies/ml	3 of 3 (34.9)	3 of 3 (36.5)
S08 50 copies/ml	3 of 3 (35.6)	3 of 3 (37.1)
S09 Negative	0 of 3 (0)	0 of 3 (0)

3. The 50 copies/ml control was detected 3 out of 3 times, with both targets.

- From Lab 2

A dilution series of extracted viral RNA was run through the Cobas with 10% Triton-x in a 3:2 sample:lysis ratio. Each diluted sample was tested in triplicate. The Cobas assay remained positive in all three replicates to a 10⁻⁹ dilution. The data is presented in Table 3 below.

Table 3. Dilution Series of Extracted Viral RNA

Template RNA Dilution	Target 1	Target 2
10 ⁻³	n.t.	n.t.
10 ⁻⁴	22.06 22.22 22.37	22.04 22.15 22.12
10 ⁻⁵	25.47 25.61 25.32	25.36 25.48 25.1
10 ⁻⁶	28.7 28.8 28.63	28.68 28.75 28.71
10 ⁻⁷	31.67 31.64 31.59	31.82 31.84 31.96
10 ⁻⁸	33.84 33.93 33.97	34.82 34.55 34.99
10 ⁻⁹	35.16 36.09 35.92	37.53 37.65 37.84

4. For local verification, a dilution series using vero culture SARS CoV-2 virus (taken from p3 supernatant of SARS-CoV-2 vero cultured virus: HCM/R/053) was generated, with 10-fold dilutions of stock virus made in 2% FBS and MEM (see Table 4). The extracted viral RNA was run through the Cobas with 10% Triton-x in a 3:2 sample:lysis ratio. Each sample was run in triplicate. It is highly probably that the cultured virus had more inactive virus genomes than virus still able to infect vero cells in which the virus was titrated to give the PFU/mL, hence

the otherwise improbable positive for 10^{-2} virus PFU/mL. Once a greater knowledge of the virus biology is known, questions such as Vero cell infective dose (multiplicity of infection) can be answered and cleaner stock solutions will be possible.

Table 4. Dilution Series of Vero-Cultured SARS CoV-2 Virus in 2% FBS-MEM

Sample (PFU/mL)	Target 1	Target 2	IC
10^4	18.1 17.94 19.95	18.64 18.23 18.41	33.15 32.89 32.92
10^3	21.36 21.36 21.31	21.58 21.57 22.01	32.9 33 33.06
10^2	24.56 24.34 24.48	24.9 24.67 24.93	32.87 32.79 33.19
10^1	27.7 27.56 27.47	28.3 27.97 27.86	32.9 33.24 22.04
10^0	30.76 30.97 30.5	31.37 31.68 31.12	33.56 33.57 33.27
10^{-1}	33.12 33.25 33.39	34.42 34.57 34.35	33.42 33.2 33.69
10^{-2}	35.73 35.53 35.77	37.33 36.72 36.83	33.17 33.55 33.23
NTC virus	ND ND ND	ND ND ND	33.22 33.39 33.12

5. Linearity and efficiency: Ideally for linearity, the use of a standardised reference panel should be used to ensure effective benchmarking and assure that dilutions are accurate. If an alternative route for establishing linearity is undertaken, the method will need to be documented For LAMP assays the linearity will need to be standardised via dilutions in appropriate matrixes using untreated virus. Plot the data from 4.1.1 and calculate linearity and efficiency. Compare the data with that supplied by the manufacturer, if applicable.

- From Lab 2

The RNA and virus dilution series in tables 3 and 4 above were used to assess the linearity and efficiency of the Cobas SARS CoV-2 assay.

The linearity values for both assay targets fall within an R2 value of between 1-0.98.

The efficiency values for target 1 are high at 117.8 and 131.93% for virus and RNA respectively, this is less likely to be due to excess RNA or the presence of inhibitors in the high concentrations of sample (ThermoFisher, 2016), but more probably due to Roche deciding to sacrifice quantitative accuracy for qualitative assay sensitivity at lower concentrations of virus in the swab samples. The efficiency values for target 2 are at the more acceptable levels of 108.78 and 108.88% (calculated using the ThermoFisher efficiency calculator). If this assay was expected to report quantitative results then additional optimisation would be recommended for target 1, however this assay is qualitative and relative Ct values should not be reported to the end recipient.

6. Lowest Limits of Detection (LLOD): Where a validated standard dilution series was used LLOD should be calculated, using data from 4.1.2, in copies/ml (to align with the relevant MHRA TPP). Where clinical positive material is used, copies/ml cannot be calculated; median CT value or dPCR should be given for the lowest dilution detected from the samples used in 4.1.1.

- From the IFU 09179909001-07EN, Doc Rev 7:

As shown in Table 5, the Probit predicted 95% hit rates were 0.007 and 0.004 TCID50/mL for SARS-CoV-2 (Target 1) and pan-Sarbecovirus (Target 2), respectively.

Table 5. Probit predicted 95% hit rates using USA-WA1/2020 strain

Strain	Probit Predicted 95% Hit Rate [TCID50/mL]	
	Target 1	Target 2
USA-WA1/2020 (stock concentration 2.8E+05 TCID50/mL)	0.007 (95% CI 0.005 – 0.036)	0.004 (95% CI 0.002-0.009)

7. The analytical sensitivity of the assay was tested with AccuPlex SARS-CoV-2 (Lot #105324), a quantitated reference material – recombinant Sindbis virus particle containing target sequences from the SARS-CoV-2 genome. The

concentration level in a dilution series with observed hit rates greater than or equal to 95% was 46 copies/mL for both Target 1 and Target 2. Probit model 95% LoD estimates based on these data were 25 copies/mL (95% CI: 17 – 58 copies/mL) for Target 1 and 32 copies/ml (95% CI: 21 – 73 copies/mL) for Target 2.

- From Lab 2

Using the Accuplex SARS CoV-2 reference material, it is possible to agree with Roche that the limit of detection is approximately 50 copies/mL with Ct~35.5 and ~38 for target 1 and 2 respectively (see Table 6).

Table 6. A dilution in 2% FBS-MEM of SeraCare Accuplex reference material supplied at 5000 copies/mL processed with the 3:2 ratio in 5% Triton-X-100 lysis buffer.

Accuplex (copies/mL)	Target 1	Target 2	IC
5000	31.02	32.03	33.31
	31.29	32.13	33.79
	31.14	31.95	33.34
500	33.45	35.12	33.72
	33.59	35.8	33.62
	33.54	35.03	33.74
50	35.65	37.32	33.33
	35.58	38.09	33.23
	35.41	38.69	33.35
5	Negative	42.31	33.3
	Negative	Negative	33.72
	36.26	Negative	33.29

Precision and robustness

- Intra-assay precision: Use the data for 5 replicate values from a single day from 4.1.1 to calculate Standard Deviation & Coefficient of Variation measurement, with the values for the latter to be <10%. To include the use of inhibition controls
 - From the IFU 09179909001-07EN, Doc Rev 7
- Equivalence between nasopharyngeal swab (NPS) and oropharyngeal swab (OPS) sample types was evaluated using cultured virus (USA-WA1/2020 strain) spiked into paired negative samples (individual samples, not pooled) to prepare contrived low positive (approximately 1.5x Target 1 LoD) and moderate positive (approximately 4x Target 1 LoD) samples for each sample type. A total of 21 low positive paired samples, 11 moderate positive paired samples, and 11 negative paired samples were tested.

As shown in Table 7 below, all low positive and moderate positive paired samples were positive in both sample matrices. All negative paired samples were negative in both sample types. The observed Ct values for contrived positive samples were comparable in both sample types.

Table 7. Comparison of nasopharyngeal and oropharyngeal swab types

Sample type	Sample concentration	N	Hit Rate (%)^		Mean Ct*	
			% positive	Mean Ct (95% CI)	% positive	Mean Ct (95% CI)
NPS	~1.5 x LOD (Target 1)	21	100	31.9 (31.7-32.0)	100	33.6 (33.5-33.7)
OPS			100	32.2 (31.8-32.6)	100	33.7 (33.4-34.1)
NPS	~4.0 x LOD (Target 1)	11	100	30.9 (30.3-31.5)	100	32.2 (31.6-32.9)
OPS			100	31.5 (31.2-31.9)	100	32.7 (32.4-33.0)
NPS	Negative	11	0	n/a	0	n/a
OPS			0	n/a	0	n/a

- From Lab 2

8 replicates of viral RNA diluted to 10^{-8} were passed through the assay on one Cobas 6800 instrument (s/n 1040) and 12 replicates of viral RNA diluted to 10^{-8} were passed through the assay on another Cobas 6800 instrument (s/n 1694). The results are shown in tables 8 and 9 below. The level of variation is low and well within the acceptable variation for a qualitative diagnostic assay, none of the results varying as much as 1 Ct, supporting the data provided by Roche.

Table 8. Intra-assay variation as assessed by 8 replicates with a 10^{-8} dilution on Cobas 1040

	Target 1	Target 2
Mean	34.19	35.12
SD	0.25	0.42
% CV	0.73	1.21

Table 9: Intra-assay variation as assessed by 12 replicates with a 10^{-8} dilution on Cobas 1694

	Target 1	Target 2	IC
Mean	33.91	34.86	33.22
SD	0.16	0.25	0.16
% CV	0.48	0.71	0.47
Min	33.69	34.54	32.91
Max	34.22	35.28	33.39
Difference	0.53	0.74	0.48

Cultured virus was spiked directly onto 6 swabs, these swabs were then placed directly into 0.85% saline. The same cultured virus was added directly into three tubes with only 0.85% saline (no swab). The 9 tubes were then stored for 48 hrs at 4°C, the spiked saline solution was then tested on the SARS-CoV-2 assay. Table 10 shows the results.

Table 10. Intra-assay variation as assessed by virus spiked swabs (n=6) in 0.85 % saline or (n=3) 0.85% saline, stored for 48 hrs at 4°C, then extracted and assessed, OBL: 3:2 ratio and 5% triton-x100.

	Target 1	Target 2	IC
Saline (no swab)	33.93	35.21	34.43
	33.41	34.5	34.34
	33.83	34.78	34.81
Inoculated swab stored in saline	33.47	34.67	34.29
	33.39	34.66	34.68
	33.51	35.05	34.91
	33.39	34.84	34.56
	33.43	35.28	34.25
	33.06	34.34	34.96
No virus	Neg	Neg	34.6
	Neg	Neg	34.33
	neg	neg	34.22
Mean	33.49	34.80	34.58
SD	0.26	0.30	0.27
% CV	0.76	0.86	0.79
Min	33.06	34.34	34.25
Max	33.93	35.21	34.96
Difference Ct	0.87	0.87	0.71

The level of variation is low for these limited datasets and within the acceptable limits for a diagnostic assay and is supportive of the data provided by Roche.

- Inter-assay precision: Use the data for 5 replicate values from multiple days from 4.1.1 for Standard Deviation & Coefficient of Variation with the values for the latter to be <15%.

- From Lab 2

Samples were added to lysis buffer and then tested after 4 hours and 72 hours for 2 different dilutions of virus, 10^{-1} and 10^{-2} . The level of variation between the samples were minimal. The results are presented in Table 11.

Table 11. Inter-assay precision assessed using the 10⁻¹ and 10⁻² dilution of SARS-CoV-2 virus run 4hrs and 72hrs after addition to the lysis buffer.

Virus	Time	Target 1	Mean	SD	%CV	Target 2	Mean	SD	%CV	IC
10 ⁻¹	4 hrs	33.12 33.25 33.39	33.34	0.16	0.47	34.42 34.57 34.35	34.47	0.08	0.23	33.42 33.2 33.69
	72 hrs	33.28 33.56 33.45				34.47 34.53 34.47				33.38 33.23 33.3
10 ⁻²	4 hrs	35.73 35.53 35.77	35.68	0.36	1.02	37.33 36.72 36.83	37.03	0.28	0.75	33.17 33.55 33.23
	72 hrs	35.59 36.28 35.17				36.84 37.08 37.38				33.82 33.58 33.6

Accuplex reference material was run across three different Cobas 6800 instruments (machines s/n 1040 (n=3), s/n 1694 (2 x n=3) and s/n 1161 (n=2)) at the limit of detection of 50 copies/mL. The results are presented in table 12. The machine to machine variation is no more than the variation seen between replicates.

Table 12. Inter-assay variation of the Cobas 6800 systems using the SARS CoV-2 assay against Accuplex reference material at 50 copies/mL on three Cobas systems OBL: 3:2 ratio and 5% triton x-100

Accuplex	Machine	Target 1	Mean	SD	%CV	Target 2	Mean	SD	%CV	IC
50 copies/mL	1040	35.65 35.58 35.41	35.32	0.37	1.06	37.32 38.09 38.69	38.13	0.70	1.84	33. 33. 33. 23 33. 35
	1694	35.08 35.42 35.01 35.44				37.38 37.64 39.73 38.29				33. 21 33. 25

		36.03 34.9				37.66 37.97				33. 22 33. 11 32. 66 33. 53
	1161	35.29 34.72				38.68 37.96				33. 27 33. 86

4. As part of IQC procedures, IQC datapoints have been recorded for viral RNA diluted to a 10⁻⁸ dilution and AccuPlex reference material at a 10⁻¹ dilution (500 copies/mL). The data includes runs on different Cobas 6800 machines over 3 reagent lots, a single on-board lysis, multiple off-board lysis lots and different operators. A summary of the results is presented in table 13.

Table 13. Inter-assay variation using RIPL IQC data for viral RNA and AccuPlex reference material, n=22.

	AccuPlex		Viral RNA	
		33.67	35.16	33.99
Mean	33.67	35.16	33.99	34.96
SD	0.21	0.31	0.21	0.31
%CV	0.62	0.88	0.62	0.89
Min	33.36	34.59	33.39	33.44
Max	34.08	35.80	34.39	35.61
Ct difference	0.72	1.21	1.00	1.28

5. Repeatability: Spike 30 negative samples from different individuals with known amount of agent/positive material (suggested 3x the LLOD), all should be positive.

- From the IFU 09179909001-07EN, Doc Rev 7

The performance of cobas SARS-CoV-2 with prospectively collected nasopharyngeal swab clinical samples was evaluated using 100 individual

negative clinical samples and 50 contrived positive clinical samples collected from patients with signs and symptoms of an upper respiratory infection.

Clinical samples were collected by qualified personnel according to the package insert of the collection device. Samples were handled as described in the package insert of the collection device and stored frozen until use. Samples were tested to be negative by a commercially available nucleic acid test for the qualitative detection of microorganisms associated with common upper respiratory tract infections.

Low positive and moderate positive contrived positive clinical samples were prepared by spiking cultured virus (USA-WA1/2020 strain) into individual negative clinical samples to approximately ~1.5x LoD (Target 1) (25 samples) and ~4x LoD (Target 1) (25 samples), respectively.

As shown in Table 14, all low positive and moderate positive samples were positive and all negative samples were negative in the background of individual clinical sample matrix.

Table 14. Clinical evaluation with nasopharyngeal swabs using contrived spiked samples

Sample concentration	N	Target 1		Target 2	
		% positive (95% CI)	Mean Ct	% positive (95% CI)	Mean Ct
~1.5 x LOD	25	100 (86.7-100)	31.6	100 (86.7-100)	33.2
~4 x LOD	25	100 (86.7-100)	31.1	100 (86.7-100)	32.4
Negative	100	0 (n/a)	n/a	0 (n/a)	n/a

Analytical specificity (Interferences and cross-reactions)

- Cross-reactivity to non-target samples/organisms. A range of samples either direct clinical samples or spiked samples that are known positives for other diseases, both closely related (i.e., other coronaviruses), syndromic diseases (i.e., other respiratory viruses and bacteria) and common diseases (i.e. HIV, HBV, HCV, VZV, EBV, CMV) should be tested
- *In Silico Analysis*
 - From the IFU 09179909001-07EN, Doc Rev 7
- The in silico analysis for possible cross-reactions with all the organisms listed in Table 15 was conducted by mapping primers in cobas SARS-CoV-2 individually to the sequences downloaded from NCBI and GISAID databases. If any two of the primers were mapped to a sequence on opposite strands with short distance apart, potential amplifications were flagged. No potential unintended cross reactivity is expected based on this in silico analysis.

Table 15. In silico analysis for SARS CoV-2

Strain	In Silico Analysis for % Identity to Target 1 (nCoV)	In Silico Analysis for % Identity to Target 2 (Pan-Sarbecovirus 1)
CoV 229E	74.47	No alignment was found*
CoV OC43	72.26	No alignment was found*
CoV HKU1	76.52	No alignment was found*
CoV NL63	71.32	No alignment was found*
SARS CoV	95.04	100
MERS	No alignment was found*	No alignment was found*
AdV	No alignment was found*	No alignment was found*

Strain	In Silico Analysis for % Identity to Target 1 (nCoV)	In Silico Analysis for % Identity to Target 2 (Pan-Sarbecovirus 1)
HMPV	No alignment was found*	No alignment was found*
HPIV1	No alignment was found*	No alignment was found*
HPIV2	No alignment was found*	No alignment was found*
HPIV3	No alignment was found*	No alignment was found*
HPIV4	No alignment was found*	No alignment was found*
Flu A	No alignment was found*	No alignment was found*
Flu B	No alignment was found*	No alignment was found*
EV	No alignment was found*	No alignment was found*
RSV	No alignment was found*	No alignment was found*
RV	No alignment was found*	No alignment was found*
Chlamydia pneumoniae	No alignment was found*	No alignment was found*
Haemophilus influenzae	No alignment was found*	No alignment was found*
Legionella pneumophila	No alignment was found*	No alignment was found*
MTB B=Mycobacterium bovis sbsp. Bovis	No alignment was found*	No alignment was found*
Streptococcus pneumoniae	No alignment was found*	No alignment was found*
Streptococcus pyogenes	No alignment was found*	No alignment was found*
Bordetella	No alignment was found*	No alignment was found*

Strain	In Silico Analysis for % Identity to Target 1 (nCoV)	In Silico Analysis for % Identity to Target 2 (Pan-Sarbecovirus 1)
pertussis		found*
Mycoplasma pneumoniae	No alignment was found*	No alignment was found*
Influenza C	No alignment was found*	No alignment was found*
Parechovirus	No alignment was found*	No alignment was found*
Candida albicans	No alignment was found*	No alignment was found*
Corynebacterium diphtheriae	No alignment was found*	No alignment was found*
Legionella non-pneumophila	No alignment was found*	No alignment was found*
Bacillus anthracis	No alignment was found*	No alignment was found*
Moraxella catarrhalis	No alignment was found*	No alignment was found*
Neisseria elongate and meningitides	No alignment was found*	No alignment was found*
Pseudomonas aeruginosa	No alignment was found*	No alignment was found*
Staphylococcus epidermidis	No alignment was found*	No alignment was found*
Staphylococcus salivarius	No alignment was found*	No alignment was found*
Leptospira	No alignment was found*	No alignment was found*
Chlamydia psittaci	No alignment was found*	No alignment was found*
Coxiella burnetii	No alignment was found*	No alignment was found*
Staphylococcus aureus	No alignment was found*	No alignment was found*

Note: * The amplicon sequences were blasted against all the exclusive sequences with very low stringency cutoff (50% and 100bp). No alignment were found passing the cutoff and no concerns for cross-reactivity were observed.

6. Wet testing

- From the IFU 09179909001-07EN, Doc Rev 7

7. Cross-reactivity of cobas SARS-CoV-2 was evaluated by testing whole organisms. As listed in Table 16, a panel of multiple unique sub-species of microorganisms were tested. High titre stocks of the potentially cross-reacting microorganisms were spiked into negative simulated clinical matrix to a concentration level of 1.0E+05 units/mL for viruses and 1.0E+06 units/mL for other microorganisms, unless otherwise noted.

8. None of the organisms tested interfered with cobas SARS-CoV-2 performance by generating false positive result.

Table 16. Cross-reactivity test results

Microorganism	Concentration	Target 1 result	Target 2 result
Human coronavirus 229E	1.0E+05 TCID ₅₀ /mL	Negative	Negative
Human coronavirus OC43	1.0E+05 TCID ₅₀ /mL	Negative	Negative
Human coronavirus HKU1	1.0E+05 cp/mL	Negative	Negative
Human coronavirus NL63	1.0E+05 TCID ₅₀ /mL	Negative	Negative
MERS coronavirus	1.0E+05 genome equivalents/mL	Negative	Negative
SARS coronavirus	1.0E+05 PFU/mL	Negative	Positive
Adenovirus B (Type34)	1.0E+05 TCID ₅₀ /mL	Negative	Negative
Human metapneumovirus (hMPV)	1.0E+05 TCID ₅₀ /mL	Negative	Negative

Microorganism	Concentration	Target 1 result	Target 2 result
Parainfluenza virus Type 1	1.0E+05 TCID ⁵⁰ /mL	Negative	Negative
Parainfluenza virus Type 2	1.0E+05 TCID ⁵⁰ /mL	Negative	Negative
Parainfluenza virus Type 3	1.0E+05 TCID ⁵⁰ /mL	Negative	Negative
Parainfluenza virus Type 4	1.0E+05 TCID ⁵⁰ /mL	Negative	Negative
Influenza A (H1N1)	1.0E+05 TCID ⁵⁰ /mL	Negative	Negative
Influenza B	1.0E+05 TCID ⁵⁰ /mL	Negative	Negative
Enterovirus E (Type 1)	1.0E+05 TCID ⁵⁰ /mL	Negative	Negative
Respiratory syncytial virus	1.0E+05 PFU/mL	Negative	Negative
Rhinovirus	1.0E+05 TCID ⁵⁰ /mL	Negative	Negative
Chlamydia pneumoniae	1.0E+06 CFU/mL	Negative	Negative
Haemophilus influenzae	1.0E+06 CFU/mL	Negative	Negative
Legionella pneumophila	1.0E+06 CFU/mL	Negative	Negative
Mycobacterium tuberculosis	1.0E+06 CFU/mL	Negative	Negative
Streptococcus pneumoniae	1.0E+06 CFU/mL	Negative	Negative
Streptococcus pyogenes	1.0E+06 CFU/mL	Negative	Negative
Bordetella pertussis	1.0E+06 CFU/mL	Negative	Negative
Mycoplasma pneumoniae	1.0E+06 CFU/mL	Negative	Negative
Pooled human nasal wash	5-50%	Negative	Negative

- From Lab 2

A limited specificity panel of other respiratory pathogens was run. The results are presented in Table 17.

Table 17. Specificity panel of Lab 2 for other respiratory pathogens

Sample type	Target 1	Target 2	IC
Amplirun total atypical bacterial pneumonia control	ND	ND	33.43
PIV1	ND	ND	33.52
PIV2	ND	ND	33.24
PIV3	ND	ND	33.71
PIV4	ND	ND	33.01
RSV A2	ND	ND	34.29
Human coxsackie B4	ND	ND	33.34
HMPV	ND	ND	33.22
Human parechovirus	ND	ND	33.07
Human rhinovirus GA	ND	ND	33,01
A Cal/07/09 H1N1	ND	ND	33.39
A/Switz/15 H3N2	ND	ND	33.28
B/Phuket (Yam)	ND	ND	33.85
B/Brisbane (Vic)	ND	ND	33.59
NTC	ND	ND	33.69
NTC	ND	ND	33.51

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

1. Samples selected for this validation will be appropriate to the assay. Low medium and high viral load samples will be equally distributed to avoid increasing or lowering DSe and DSp.
2. Diagnostic sensitivity: Confirmed clinical samples from patients (positive RT-qPCR result) should be used. Preferably, depending on the availability of samples, ~150 samples should be included to align with MHRA TPP. Clinical sensitivity (95% CI) and positive predictive value (PPV) should be calculated in comparison with a CE marked reference method that itself has 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 must be included in the validation report.

- From Lab 1

158 RT-PCR positive nasopharyngeal and 250 RT-PCR negative nasopharyngeal samples, confirmed using the Nonacus VirPath SARS CoV-2 Multiplex PCR (C3COV187), were simultaneously run on the Cobas SARS CoV-2 assay using a 6800 System and TaqPath COVID-19 CE-IVD RT-PCR assay. For the TaqPath assay, samples were extracted using the MagMAX Viral/Pathogen Nucleic Acid Isolation kit (ThermoFisher Scientific) on the KingFisher Flex system as per manufacturer recommendation for the TaqPath RT-PCR assay with amplification on the QuantStudio 5 Real-Time PCR System (ThermoFisher Scientific). The Cobas SARS CoV-2 assay was run as per manufacturer's instructions.

The results are summarised in table 18.

Table 18. Diagnostic sensitivity and specificity of the Roche cobas SARS CoV-2 assay compared to TaqPath SARS CoV-2 assay.

		TaqPath PCR		Total
		Positive	negative	
Cobas 6800/8800 SARS CoV-2	Positive	157	0	157
	Negative	1	250	251
Total		158	250	408

1 sample was positive by the TaqPath assay but not detected on the Cobas assay. This sample had a Ct value of 36.4 and 37.2 in the TaqPath assay Orf1ab and N gene targets respectively.

Diagnostic sensitivity = 99.4% (95% CI 96.5-99.9, 157/158 samples)

Positive predictive value (PPV) = 100%

Table 19 shows the spread of the CT values compared to the TaqPath comparator assay, which demonstrated a spread of values across the low, medium and high viral load range.

Table 19: Spread of Ct values of Cobas SARS CoV-2 assay compared to TaqPath SARS CoV-2 assay

CT value range	Positive on Cobas /positive on comparator	Sensitivity (%)
≤25 (low)	53/53	100.0
25-<30 (medium)	35/51	68.6
≥30 (high)	31/54	57.4
30-35	28/45	62.2
>35	3/9	33.3

This meets the acceptable criteria in the MHRA TPP for Laboratory Based SARS CoV-2 Viral Detection Tests (<https://www.gov.uk/government/publications/how-tests-and-testing-kits-for-coronavirus-covid-19-work/target-product-profile-laboratory-based-sars-cov-2-viraldetection-tests>) for RNA extracted assays, with sensitivity of greater than 95% (with 95% two-sided confidence interval entirely above 90%). The desirable criteria in the TPP states a sensitivity of greater than 99% (with 95% two-sided confidence interval entirely above 97%). The sensitivity also meets the TVG criteria for Category 1 performance (sensitivity >97%) and the requirement for test to release, which requires a sensitivity greater than 99%.

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 (NPV) 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.

See table 18 above for details.

Diagnostic specificity = 100% (95% CI 97.8-99.9, 250/250 samples)

Negative predictive value (NPV) = 99.6%.

This meets the desired criteria in the MHRA TPP for Laboratory Based SARS CoV-2 Viral Detection Tests (<https://www.gov.uk/government/publications/how-tests-and-testing-kits-for-coronavirus-covid-19-work/target-product-profile-laboratory-based-sars-cov-2-viral-detection-tests>) for RNA extracted assays, with specificity of greater than 99% (with 95% two-sided confidence interval entirely above 97%). The specificity also meets the TVG criteria for Category 1 performance (specificity >99%) and the requirement for test to release, which requires a specificity greater than 99%.

Summary

The Roche Cobas SARS CoV-2 assay has been independently validated by two laboratories in England. Both laboratories have UKAS accreditation. Lab 1 restricted their validation to diagnostic sensitivity, specificity and limit of detection. The data from Lab 2 covered analytical sensitivity and specificity. The assay meets the MHRA TPP for Laboratory Based SARS CoV-2 Viral Detection tests for RNA extracted assays with a diagnostic sensitivity of 99.4% (95% CI 96.5-99.9) and a diagnostic specificity of 100% (95% CI 97.8-99.9). This meets the requirement for DHSC Technologies Validation Group (TVG) Category 1 and DHSC Test to Release criteria for greater than 99% sensitivity and specificity.

The limit of detection was found by both independent labs to be 50 copies/mL for both targets. This meets the desired criteria of ≤ 100 copies/mL in the MHRA TPP for Laboratory Based SARS CoV-2 tests for RNA extracted assays. It also meets the TVG desired criteria of ≤ 100 copies/mL for Category 1 tests and the Test to Release criteria of ≤ 1000 copies/mL.

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