510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number

K223783

B Applicant

Roche Molecular Systems, Inc.

C Proprietary and Established Names

cobas SARS-CoV-2 Nucleic acid test for use on the cobas Liat System

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QWR	Class II	21 CFR 866.3982 - Simple Point-Of-Care Device to Detect SARS-CoV-2 Nucleic Acid Targets From Clinical Specimens In Near-Patient Settings	MI-Microbiology

II Submission/Device Overview:

A Purpose for Submission:
New device

B Measurand:
SARS-CoV-2 nucleic acids

C Type of Test:
The cobas SARS-CoV-2 Nucleic acid test for use on the cobas Liat System (cobas SARS-CoV-2) is a RT-PCR test for use with cobas Liat System for the qualitative in vitro detection and identification of nucleic acids from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in nasopharyngeal, and anterior nasal swabs from individuals with signs and symptoms of upper respiratory tract infection, or those suspected of COVID-19. The automation, small footprint, and easy-to-use system enables it to be used as a point of care (CLIA waived setting) test as well as in laboratory setting.

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The cobas SARS-CoV-2 Nucleic acid test for use on the cobas Liat System (cobas SARS-CoV-2) is an automated, real-time reverse transcriptase polymerase chain reaction (RT-PCR) test intended for the rapid *in vitro* qualitative detection of nucleic acid from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in anterior nasal (nasal) and nasopharyngeal swab specimens collected from individuals with signs and symptoms of respiratory tract infection (i.e., symptomatic). Additionally, this test is intended to be used with nasal and nasopharyngeal swab specimens collected from individuals without signs and symptoms suspected of COVID-19 (i.e., asymptomatic).

The cobas SARS-CoV-2 performed on the cobas Liat System is intended for use as an aid in the diagnosis of COVID-19 if used in conjunction with other clinical, epidemiologic, and laboratory findings. SARS-CoV-2 RNA is generally detectable in nasal and nasopharyngeal swab specimens during the acute phase of infection.

Positive results are indicative of the presence of SARS-CoV-2 RNA. Positive results do not rule out co-infection with other microorganisms.

A negative result from an asymptomatic individual is presumptive. Additionally, a negative result obtained with a nasal swab collected from an asymptomatic patient should be followed up by testing at least twice over three days with at least 48 hours between tests. Negative results do not preclude SARS-CoV-2 infection.

The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

This test is intended for prescription use only and can be used in Point-of-Care settings.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

IVD- For *in vitro* Diagnostic Use Only

D Special Instrument Requirements:

cobas Liat System.

IV Device/System Characteristics:

A Device Description

The cobas SARS-CoV-2 Nucleic acid test for use on the cobas Liat System uses real-time reverse transcriptase polymerase chain reaction (RT-PCR) technology for qualitative amplification, detection and differentiation of specific RNA sequences within the genomes of SARS-CoV-2, from nasopharyngeal and anterior nasal swabs. The assay uses unitized reagents in a self-contained disposable Liat Tube that holds the sample preparation and PCR reagents, and

in which the nucleic acid extraction and amplification/detection processes take place. Each Liat Tube houses reagents in pre-packed tube segments that are separated by frangible seals, and which are ruptured sequentially by actuators within the cobas Liat System to effect sample processing, including viral lysis, RNA recovery and removal of inhibitors, RT-PCR amplification, and detection. The Liat Analyzer controls the reaction volume, temperature, and the duration of each process step.

To perform the assay the operator performs the following three steps:

1. Transfers an aliquot of a swab sample in transport medium into a cobas SARS-CoV-2 assay tube,
2. Scans the relevant tube and sample identification barcodes, and
3. Inserts the tube into the cobas Liat Analyzer for automated processing and result interpretation.

An embedded microprocessor coordinates the functions of the cobas Liat Analyzer to move the sample from one segment of the tube to another, and to control the reaction volume, temperature and duration of each step.

The cobas SARS-CoV-2 test kit contains sufficient reagents to process 20 specimens or quality control samples. Positive and negative external controls are provided separately from the assay reagents in the cobas SARS-CoV-2 Quality Control Kit. The Positive Control consists of non-infectious plasmid DNA (microbial) containing the SARS-CoV-2-specific target sequences. The Negative Control comprises transport medium without target virus or nucleic acid.

Specimens are collected from patients using sterile flocked swabs with a synthetic tip using standard collection technique in transport media. A provided transfer pipette is used to transfer approximately 0.2 mL of the specimen into the cobas SARS-CoV-2 assay tube. The sample volume is adjusted by the analyzer if more sample is loaded. The barcode on the cobas SARS-CoV-2 assay tube is then scanned which prompts the Liat Analyzer to open immediately and allows insertion of the Liat Tube. All the steps of the test are controlled by the instrument. After a run time of 20 minutes the results can be viewed in the View Results window and are printed directly through a USB connected printer.

Table 1: Interpretation of Results for the cobas SARS-CoV-2 Test

Result Report	**Interpretation**
SARS-CoV-2 Not Detected	Negative test for SARS-CoV-2 (no SARS-CoV-2 RNA detected)
SARS-CoV-2 Detected	Positive test for SARS-CoV-2 (SARS-CoV-2 RNA present)
Assay Invalid	Presence or absence of SARS-CoV-2 cannot be determined. Repeat assay with same sample or, if possible, collect new sample for testing.
Assay Aborted by System	Run failed or aborted by system. Repeat assay with same sample or, if possible, collect new sample for testing.
Assay aborted by script: Script aborted	Run failed or aborted by script. Repeat assay with same sample or, if possible, collect new sample for testing.
Assay Aborted by User	Run aborted by user.

B Principle of Operation:

The cobas SARS-CoV-2 assay is performed on the cobas Liat Analyzer which automates and integrates sample purification, nucleic acid amplification, and detection of the target sequences in biological samples using real-time RT-PCR assay. The assay targets both, the ORF1 a/b non-structural region and structural nucleocapsid protein (N) gene that are unique to SARS-CoV-2. An Internal Process Control (IPC) is also included. The IPC is present to control for adequate processing of the target virus through steps of sample purification, nucleic acid amplification, and to monitor the presence of inhibitors in the RT-PCR processes.

Before using a new lot of cobas SARS-CoV-2 assay tubes (Liat Tubes), a Lot Validation procedure must be performed on the cobas Liat Analyzer. The procedure includes running a Negative Control sample and a Positive Control sample. Both the positive and negative control sample should be "accepted" before patient samples can be processed.

C Instrument Description Information:

1. Instrument Name:
   cobas Liat Analyzer which includes cobas Liat System Software (Core) Version 3.3 or higher.

2. Specimen Identification:
   Specimen identification can be done by scanning the barcode present on the assay tube before placing it in the Liat Analyzer.

3. Specimen Sampling and Handling:
   Samples are collected using sterile flocked swab with a synthetic tip using standard collection technique in transport media.

4. Calibration:
   Lot validation procedure needs to be performed before a new lot of Liat Tubes is used.

5. Quality Control:
   The assay tube contains an internal positive control which monitors the entire automated test process, including sample preparation (nucleic acid extraction), amplification and detection steps for a given sample. A separate external positive and negative control (as cobas SARS-CoV-2 Quality Control Kit) is also provided with each kit which is used for Liat Tubes lot validation. Additional controls can be purchased separately.

V Substantial Equivalence Information:

A Predicate Device Name(s):

ID NOW COVID-19 2.0

B Predicate 510(k) Number(s):
K221925

C Comparison with Predicate(s):

Device & Predicate Device(s):	**K223783**	**K221925**
Device Trade Name	cobas SARS-CoV-2 Nucleic acid test for use on the cobas Liat System	ID NOW COVID-19 2.0
General Device Characteristic Similarities		
Intended Use/Indications For Use	The cobas SARS-CoV-2 Nucleic acid test for use on the cobas Liat System (cobas SARS-CoV-2) is an automated, real-time reverse transcriptase polymerase chain reaction (RT-PCR) test intended for the rapid *in vitro* qualitative detection of nucleic acid from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in anterior nasal (nasal) and nasopharyngeal swab specimens collected from individuals with signs and symptoms of respiratory tract infection (i.e., symptomatic). Additionally, this test is intended to be used with nasal and nasopharyngeal swab specimens collected from individuals without signs and symptoms suspected of COVID-19 (i.e., asymptomatic).	ID NOW COVID-19 2.0 performed on the ID NOW Instrument is a rapid molecular *in vitro* diagnostic test utilizing an isothermal nucleic acid amplification technology (NAAT) intended for the qualitative detection of nucleic acid from SARS-CoV-2 in direct anterior nasal (nasal) or nasopharyngeal swabs from individuals with signs and symptoms of respiratory tract infection. ID NOW COVID-19 2.0 performed on the ID NOW Instrument is intended for use as an aid in the diagnosis of COVID-19 if used in conjunction with other clinical, epidemiologic, and laboratory findings. SARS-CoV-2 RNA is generally detectable in nasal and nasopharyngeal swab specimens during the acute phase of infection.

	The cobas SARS-CoV-2 performed on the cobas Liat System is intended for use as an aid in the diagnosis of COVID-19 if used in conjunction with other clinical, epidemiologic, and laboratory findings. SARS-CoV-2 RNA is generally detectable in nasal and nasopharyngeal swab specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA. Positive results do not rule out co-infection with other microorganisms. A negative result from an asymptomatic individual is presumptive. Additionally, a negative result obtained with a nasal swab collected from an asymptomatic patient should be followed up by testing at least twice over three days with at least 48 hours between tests. Negative results do not preclude SARS-CoV-2 infection. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. This test is intended for prescription use only	Positive results are indicative of the presence of SARS-CoV-2 RNA. Positive results do not preclude co-infection with bacteria or other viruses and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. A negative test result is presumptive, and it is recommended these results be confirmed by another molecular SARS-CoV-2 assay. Negative results do not preclude SARS- CoV-2 infection and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. This test is intended for prescription use only and can be used in Point-of-Care settings.

	and can be used in Point-of-Care settings.	
Sample Types	Nasopharyngeal and anterior nasal swabs.	Nasopharyngeal and anterior nasal swabs.
Sample Preparation and Amplification Technology	Automated	Same
Controls Used	• Internal Control (a process control for sample purification, nucleic acid amplification, and for monitoring presence of inhibitors) • External Positive and Negative Controls	• Internal Control (verifying assay reagents) • External Positive and Negative Controls
General Device Characteristic Differences		
Instrumentation	cobas Liat Analyzer	ID NOW Instrument
Amplification Technology	Real-time RT-PCR for detecting the presence/absence of viral RNA in clinical specimens	Isothermal nucleic acid amplification for detecting the presence/absence of viral RNA in clinical specimens
Analyte Targets	SARS-CoV-2 ORF1 a/b non-structural region and SARS-CoV-2 nucleocapsid protein gene	RdRp gene of SARS-CoV-2 RNA
Detection Chemistry	Assay using different reporter dyes for target and Internal Control	Labeled molecular beacon probes

VI Standards/Guidance Documents Referenced:

- Class II Special Controls as per 21 CFR 866.3982.
- FDA-2020-D-0987 Policy for Evaluating Impact of Viral Mutations on COVID-19 Tests (Revised Jan-2023)
- Recommendations for Dual 510(k) and CLIA Waiver by Application Studies
- CLSI EP12 - Ed3 Finally Published (Evaluation of Qualitative, Binary Output Examination Performance)

- CLSI EP15-A3:2014 - User Verification of Precision and Estimation of Bias; Approved Guideline— Third Edition
- CLSI EP25-A:2009 - Evaluation of Stability of In Vitro Diagnostic Reagents

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

A reproducibility study was conducted assessing the total variability of the cobas SARS-CoV-2 assay across operators, study sites, testing days, cobas Liat Analyzers, and cobas Liat assay tube lots. The study was conducted at three CLIA waived sites using three different reagent lots with 3-member reproducibility panels (three replicates for each panel member) were tested by two operators on each of five testing days (days did not need to be consecutive), for a total of 810 tests (3 sites × 3 lots/site × 5 days/lot × 2 operators/day × 3 panel members/operator ×3 replicates/panel member). The reproducibility panel samples (negative, low positive (1-2x LoD), and moderate positive (3-5x LoD)) were prepared using a strain of SARS-CoV-2 diluted in simulated clinical matrix. The panels were provided to the sites with coded sample identification numbers to reduce bias. Each sample was processed according to the cobas SARS-CoV-2 instructions for use. Analysis of the Ct signal variability for the positive panel members is presented below.

Table 2: Summary of Reproducibility Results (Positive Panel Members)

Panel Member	n/N[a]	Mean Ct[b]	Site to Site		Lot to Lot		Day to Day		Operator to Operator		Within Run (Between Replicates)		Total	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD[b]	CV%[c]
Low Positive (SARS-CoV-2)	264/266	33.4	0.08	0.2	0.00	0.0	0.00	0.0	0.10	0.3	0.95	2.9	0.96	2.9
Moderate Positive (SARS-CoV-2)	268/268	32.5	0.00	0.0	0.00	0.0	0.25	0.8	0.00	0.0	0.48	1.5	0.54	1.7

Ct: cycle threshold, CV%: percent coefficient of variation, LOD: limit of detection, SARS-CoV-2: severe acute respiratory syndrome coronavirus 2, SD: standard deviation.

[a]n is the number of positive tests, which contribute Ct values to the analysis. N is the total number of valid tests for the panel member.

[b]The Mean and Total Standard Deviation was estimated using the PROC MIXED procedure.

[c]Total CV (%) = (SD/Mean) *100.

Percent agreement with expected results, demonstrated in the Reproducibility study, is shown below.

Table 3: SARS-CoV-2 Reproducibility (Percent Agreement with Expected Results)

Sample	Total number of valid test runs	Site 1	Site 2	Site 3	All sites	
		Agreement with Expected Results	Agreement with Expected Results	Agreement with Expected Results	Avg. Ct ± SD (%CV)	Agreement(n/N) and (95% CI)
Negative	268	100.0% (90/90)	100.0% (88/88)	98.9% (89/90) *	-	99.6% (267/268) (97.9%-99.9%)
SARS-CoV-2 Low Positive	266	100.0% (89/89)	100.0% (90/90)	97.7% (85/87)	33.4±0.96 (2.9%)	99.2% (264/266) (97.3%-99.8%)
SARS-CoV-2 Moderate Positive	268	100.0% (88/88)	100.0% (90/90)	100.0% (90/90)	32.5±0.54 (1.7%)	100.0% (268/268) (98.6%-100.0%)

*One negative sample yielded a "Detected" result; remnant volume from this negative sample was retested twice using the same assay tube lot and yielded a "Not Detected" result as expected.

2. Linearity:
   Not applicable; this is a qualitative assay.

3. Analytical Specificity/Interference:
   Cross-reactivity/microbial interference was evaluated by testing microorganisms in the absence and presence of 3x LoD SARS-CoV-2 cultured virus in pooled negative clinical nasopharyngeal swab matrix. Three replicates of negative and SARS-CoV-2 positive samples per microorganism were tested using one lot of cobas SARS-CoV-2 assay tubes. The testing concentrations for potentially interfering microorganisms were $\geq 10^5$ units/mL for viruses and $\geq 10^6$ units/mL for other microorganisms, unless otherwise noted in the table below. Microorganisms were tested in groups of 4 or less. Negative clinical matrix and contrived positive matrix spiked with SARS-CoV-2 at 3x LoD, without microorganisms, were tested as negative sample control (NSC) and positive sample control (PSC). All testing was performed using cobas Liat system software version 3.3.1. The summary of results from the cross-reactivity testing is shown below.

Table 4: Cross-reactivity/Microbial Interference: list of organisms tested

Description	Concentration Tested	Description	Concentration Tested*
Human coronavirus 229E	2.80E+05	*Aspergillus Flavus var. flavus*	1.00E+06
Human Coronavirus HKU1	1.38E+07	*Bordetella parapertussis*	1.00E+06
Human coronavirus OC43	3.16E+05	*Bordetella pertussis*	1.74E+06
Human Coronavirus, NL63	1.38E+06	*Candida albicans*	1.58E+07
SARS Coronavirus	1.00E+05	*Chlamydia pneumoniae*	6.88E+06
SARS Coronavirus	1.00E+04	*Corynebacterium flavescens*	1.00E+06
MERS Coronavirus	1.50E+07	*Escherichia coli*	1.00E+06

Adenovirus	2.88E+05	*Fusobacterium necrophorum subsp. necrophprum*	1.00E+06
Cytomegalovirus	1.00E+05	*Haemophilus influenzae*	2.00E+06
Enterovirus Type 71	1.05E+05	*Lactobacillus crispatus*	1.00E+06
Epstein-Barr virus	1.00E+05	*Legionella pneumophila*	1.38E+08
Human Metapneumovirus (hMPV)	1.60E+05	*Moraxella catarrhalis*	1.00E+06
Influenza A (Brisbane 59/07) H1N1	1.00E+05	*Mycobacterium tuberculosis*	5.75E+06
Influenza A (Kansas-14/2017)	1.99E+07	*Mycoplasma genitalium*	1.00E+06
Influenza B (Colorado-06/2017)	6.10E+08	*Mycoplasma pneumoniae*	3.45E+06
Influenza B (Florida/04/06)	1.00E+05	Nasal Wash	1:10
Measles	1.00E+05	*Neisseria flava*	1.00E+06
Mumps	1.00E+05	*Neisseria meningitidis*	1.00E+06
Parainfluenza Virus (hPIV)	1.60E+05	*Pneumocystis jirovecii*	1.59E+07
Parainfluenza Virus Type 1	1.26E+05	*Pneumocystis jirovecii* (Clinical sample)	1:10
Parainfluenza Virus Type 3	3.45E+05	*Pseudomonas aeruginosa*	2.03E+07
Parainfluenza Virus Type 4A	2.88E+05	*Staphylococcus aureus*	1.00E+06
Respiratory Syncytial Virus Type A	1.26E+05	*Staphylococcus epidermis*	1.20E+07
Rhinovirus	5.50E+05	*Streptococcus pneumoniae*	1.22E+06
		Streptococcus pyogenes	6.25E+06
		Streptococcus salivarius	6.63E+06

* TCID50/mL, EID50/mL, cp/mL PFU/mL, genome equiv/mL for viruses; CFU/mL, IFU/mL for bacteria and fungi.
** SARS CoV-1 at 1e5 pfu/mL or higher may interfere with SARS-CoV-2 detection. It did not interfere with the SARS-CoV-2 detection at 1e4 pfu/mL.

In the cross-reactivity study, all microorganisms at the tested concentrations showed 3/3 valid negative results.
In the microbial interference study all samples showed 3/3 valid positive results, except for SARS Coronavirus, Urbani at 10^5 pfu/mL, where all 3 replicates generated valid negative results. When SARS Coronavirus, Urbani was diluted to 10^4 pfu/mL, 3 valid positive results were obtained indicating that SARS Coronavirus, Urbani at concentrations $>10^4$ pfu/mL may interfere with SARS-CoV-2 detection. This will be included in the Limitations section.

Interference

cobas SARS-CoV-2 assay was tested for potential interference by endogenous and exogenous substances that may be present in respiratory specimens. Each substance was tested, by introducing interferents into pooled negative nasopharyngeal swab specimens in UTM and tested in three replicates with and without the SARS-CoV-2 at 3x LoD. All samples spiked with SARS-CoV-2 returned positive results and all samples that were not spiked with SARS-CoV-2 returned negative results in the presence of the substances at the concentrations tested, as shown below.

Table 5: Interference Study with Endogenous and Exogenous Substances

Potential Interferent	Active Ingredient	Concentration Tested
Mucin	Purified mucin protein	5 mg/mL
Human Whole Blood	Endogenous-	5% (v/v)
Peripheral blood mononuclear cell (PBMC)	Endogenous	1.0E+06 cells/mL
Nasal spray - Afrin / Anefrin	Oxymetazoline	5% (v/v)
Nasal corticosteroids - Flonase	Fluticasone	5% (v/v)
Nasal gel - Zicam	Galphimia glauca, Histaminum hydrochloricum, Luffa operculata, Sulphur	5% (v/v)
Throat lozenges, oral anesthetic and analgesic - Cepacol	Benzocaine, Menthol	5 mg/mL
Antibiotic, nasal ointment - Bactroban	Mupirocin	5 mg/mL
Antiviral drug - Relenza	Zanamivir	5 mg/mL
Antiviral drug - Tamiflu	Oseltamivir	7.5 mg/mL
Antimicrobial, systemic	Tobramycin	4 µg/mL
Influenza vaccine - FluMist	Live Quadrivalent 2022-2023: • A/Victoria/1/2020 (H1N1) (an A/Victoria/2570/2019 (H1N1) pdm09 - like virus), • /Norway/16606/2021 (H3N2) (an A/Darwin/9/2021 (H3N2) - like virus), • B/Phuket/3073/2013 (B/Yamagata lineage), and • B/Austria/1359417/2021 (B/Victoria lineage) lineage)	5.93E+06 FFU/mL

4. Assay Reportable Range:

   Not applicable; this is a qualitative assay.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Assay Controls:

cobas SARS-CoV-2 Nucleic acid test for use on the cobas Liat System contains two sets of controls: Internal process controls and external assay quality control.
The Internal Process Controls (IPC) is to monitor the performance of the cobas SARS-CoV-2 assay sample processing and PCR amplification/detection under simulated process and reagent failures. The external positive and negative controls monitor the assay process and reagent/assay tube failure.

6. Detection Limit (LoD):

The Limit of Detection (LoD) in NPS samples was determined using SARS-CoV-2 (USAWA1/2020) culture diluted into clinical negative nasopharyngeal swab matrix. LoD is defined as the lowest concentration that can be detected at a rate of at least 95%. Tentative LoD was determined using two five-member panels and three lots of cobas SARS-CoV-2 Assay Tubes over two days. The LoD by 95% hit rate for SARS-CoV-2 is 0.012 TCID50/mL or 12 copies/mL.

Table 6: Results of SARS-CoV-2 LoD Determination

Strain	Concentration [TCID50/mL]	Concentration [copies/mL] *	Total valid results (replicates)	Hit rate [%]	Mean Ct
USA-WA1/2020	0.048	49	10	100%	33.03
	0.024	24	20	100%	33.56
	0.012	12	20	95%	34.74
	0.006	6	20	90%	35.41
	0.003	3	20	55%	35.45

* Viral stock was previously titered in copies/mL using ddPCR.

Limit of detection (LoD) of the device was also evaluated using three panels of six concentration levels, 24 replicates per concentration level with three lots of cobas SARS-CoV-2 Assay Tubes over three days using WHO First International Standard for SARS-CoV-2 RNA. WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146) was determined according to the WHO NIBSC code: 20/146 Instructions for Use (Version 1.0, Dated 14/12/2020). Three panels of six concentration levels, 24 replicates per concentration level with three lots of cobas SARS-CoV-2 Assay Tubes over three days were tested. The LoD, the lowest concentration which yielded a positive rate of ≥95%, was determined to be 30 IU/mL.

Table 7: Hit Rate and mean Ct Results of SARS-CoV-2 LoD Determination

Strain	Concentration [IU/mL]	Total Valid Results (replicates)	Total Positive Results	Hit Rate [%]	Mean Ct
WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146)	120	24	24	100%	32.74
	60	24	24	100%	33.81
	30	24	24	100%	34.28
	20	24	21	88%	34.97
	15	24	19	79%	35.48
	7.5	24	9	38%	36.05

7. Matrix Equivalency Study:

   Because reproducibility study was conducted using simulated matrix, equivalency between simulated and real clinical matrix was evaluated. SARS-CoV-2 target was diluted to make the test panel of three concentration levels (0x, 2x and 5x LoD) in each of three matrices (simulated clinical matrix, nasopharyngeal swab specimen and nasal swab specimen). Ten replicates were tested at 0x and 5x LoD for each specimen type whereas 30 replicates were tested at 2x LoD.

   The results showed the performance of the assay with simulated clinical matrix, nasopharyngeal swab, and nasal swab matrices is equivalent.

   Collection media equivalency (UTM, M4RT, and Saline (0.9% and 0.85%) Media Types

   Equivalency study between collection media types was executed by using positive samples, prepared with SARS-CoV-2 inactivated viral culture, spiked at ~3x LOD concentration into pooled negative nasopharyngeal swab clinical specimens, collected in UTM, M4RT, 0.85% saline, and 0.9% saline. For negative samples, pooled negative nasopharyngeal swab clinical specimens were used for each media type.
   The results from the matrix equivalency study demonstrated that 0.9%, 0.85% physiological saline and M4RT are equivalent collection media. Although these collection media have been shown analytically to be compatible with the cobas SARS-CoV-2 assay, the assay performance with these collection media has not been evaluated in the clinical study.

8. Analytical Reactivity:
   Wet testing

   The inclusivity study evaluates the assay ability to detect SARS-CoV-2 isolates/variants. In this study, 16 SARS-CoV-2 isolates/variants were tested. The clinically relevant isolates/variants were tested as inactivated viruses diluted into pooled clinical negative nasopharyngeal swab matrices; each virus was tested in three replicates. The isolates/variants tested in the study are listed below, showing the concentrations where the virus was detected at 100%, i.e., in 3 out of 3 replicates.

Table 10: Summary of SARS-CoV-2 Inclusivity Testing

Isolate/Variant	Pango Lineage	WHO Label	Lowest Concentration Detected (cp/mL)
Italy-INMI1	not listed	N/A	5.0E+00
Hong Kong/VM20001061/2020	A	N/A	2.0E+01
UK variant	B.1.1.7	Alpha	5.0E+00
South Africa Variant	B.1.351	Beta	2.0E+01
USA/COR-22-063113/2022	BA5.5	Omicron	6.0E+00
USA/GA-EHC-2811C/2021	BA.1	Omicron	1.5E+00
hCoV-19/USA/MD-HP40900/2022	B.1.1.529, XBB.1.5	Omicron	6.0E+00
hCoV-19/USA/MD-HP38861/2022	B.1.1.529, BQ.1.1	Omicron	1.2E+01
hCoV-19/USA/MD-HP38288/2022	B.1.1.529, BF.7	Omicron	1.2E+01
hCoV-19/USA/MD-HP30386/2022	B.1.1.529, BA.4	Omicron	6.0E+00
USA/MD-HP24556/2022	BA.2.3	Omicron	1.2E+01
USA/MD-HP20874/2021	B.1.1.529	Omicron	6.0E+00
USA/CA-Stanford-15_S02/2021	B.1.617.1	Kappa	1.2E+01
USA/NY-Wadsworth-21025952/2021	B.1.526	Iota	1.2E+01
USA/PHC658/2021	B.1.617.2	Delta	3.6E+01
Japan/TY7-503/2021 (Brazil P.1)	P.1	Gamma	3.6E+01

In silico testing

In silico analysis of the oligo sets for SARS-CoV-2 (taxonomy ID 2697049) have been continuously performed since the onset of the pandemic and cobas SARS-CoV-2 assay will detect all analyzed SARS-CoV-2 sequences in the GISAID (>14 M) database (as of 15th November 2023).

The analysis showed that the assay is predicted to detect all sequences from the GISAID database. Mismatches to the primer and probe sequences were observed at a frequency of less than 1% for each stratified time window. As the cobas SARS-CoV-2 test is a dual-target assay, both targets (i.e., ORF1 a/b and N-gene) are detected in the same fluorescent channel and both contribute to the result call.

All relevant variants are detected by cobas SARS-CoV-2 assay. The mutations in the spike gene do not impact the tests as this gene is not used as a target region. Furthermore, none of the

ORF1a/b, N or E gene mutations reported to date are predicted to lead to a false negative result with cobas SARS-CoV-2 test.

Table 11: *In silico* analysis of cobas SARS-CoV-2 assay oligos design as of November 15th, 2023

Assay	Orf1ab				N gene				Orf1ab & N gene			
Database	NCBI		GISAID		NCBI		GISAID		NCBI		GISAID	
total	45731	100.00%	250487	100.00%	45731	100.00%	250487	100.00%	45731	100.00%	250487	100.00%
With mismatch	375	0.82%	1078	0.43%	1007	2.20%	3122	1.25%	9	0.02%	19	0.01%
dCp>5 or Tm<65	0	0.00%	4	0.00%	0	0.00%	9	0.00%	0	0.00%	0	0.00%

The results from *in silico* analysis show that cobas SARS-CoV-2 assay can detect the current circulating sub variants of SARS-CoV-2.

9. Assay Cut-Off:

   The PCR reaction for the internal process control and target analyte occur in distinct optical channels within the entire assay tube chamber; as a result, signal detection can be assessed separately. The optical channel that specifically detects SARS-CoV-2, the acceptable signal detection of either or both SARS-CoV-2-specific targets (ORF1a/b or N) would constitute a positive result for SARS-CoV-2 target channel. A final result is determined for the targets based on the combination of the target result call, internal process control result call, curve validity (including Ct and Amplitude cutoffs), and the sample input volume.

10. Carry-Over:

    A carry-over study was previously conducted in support of K153544 (cobas Influenza A/B & RSV test for the Liat System) which demonstrated that there was no carry-over or cross contamination observed on the Liat Analyzer. The cobas SARS-CoV-2 for the Liat System instrumentation and workflow are identical to the cobas Influenza A/B & RSV for the Liat System, therefore, additional carry-over studies were not performed.

B Comparison Studies:

1. Method Comparison with Predicate Device:

   See clinical studies below.

C Clinical Studies:

1. Clinical Study Design:
   The performance of cobas SARS-CoV-2 assay was evaluated in a study with prospectively collected samples from February to June 2022 at 10 geographically diverse intended use clinical sites that represented non-laboratory based near-patient testing settings (e.g., emergency rooms, out-patient clinics, and physician's offices). Specimens were prospectively collected from individuals with signs and symptoms (symptomatic) or without

signs and symptoms (asymptomatic) of COVID-19. The asymptomatic subjects consisted of both high and low risk individuals in terms of SARS-CoV-2 exposure.

Each participant provided a nasopharyngeal swab (NPS) from one nostril (the other nostril was used for SOC NPS collection) and nasal swab (NS, anterior nares) specimens from both the nostrils. As part of a dual collection, NS specimens were self-collected by patients under the healthcare provider (HCP) supervision, while the other 50% were collected by the healthcare provider. The swabs were placed in pre-specified collection tubes. To supplement the prospective data for the SARS-CoV-2 symptomatic claim for NPS and NS, archived frozen positive and negative NPS (n=46) and NS (n=46) specimens, collected prospectively and sequentially between March 29 and May 26, 2021, were included in the study. The retrospective positive and negative specimens were incorporated for testing into the standard POC workflow and tested along with the other point-of-care collected specimens. The study used a composite comparator method consisting of three highly sensitive EUA SARS-CoV-2 molecular assays. The composite comparator result was defined by the concordant results from two comparator assays (test A and test B). In case of discordance between the initial two comparator assays, the sample was tested by a third assay (test C) and the result of the third test determined the composite comparator result (2-out-of-3 rule).

Based on the submitted information, 1874 evaluable NPS samples and 1872 evaluable NS samples were included in the analysis for the performance evaluation of the cobas SARS-CoV-2 assay. Of these, 673 NPS specimens were collected from individuals with signs and symptoms of respiratory tract infection and 1201 were from asymptomatic individuals (413 suspected of SARS-CoV-2 infection due to recent exposure or other reasons and 788 from individuals without symptoms or other reasons to suspect COVID-19). Among the NS specimens tested in the study, 674 were collected from individuals with signs and symptoms of respiratory tract infection and 1198 were from asymptomatic individuals (411 suspected of SARS-CoV-2 infection due to recent exposure or other reasons and 787 from individuals without symptoms or other reasons to suspect COVID-19).

The demographic and sample characteristics of the 1862 subjects from whom fresh specimens were obtained are summarized in Table 6. Of these evaluable subjects, 790 were males (42.4%) and 1072 were females (57.6%). The median age was 37 years, with the oldest subject being 86 years old and the youngest subject(s) being less than 1 years old.

Table 12: Subject Demographics-Prospective symptomatic population.

Characteristics	**Study Population**
Total, N	1,862
Age (years)	
Mean	37.1
Standard Deviation	19.06
Median	37.0
Range (minimum - maximum)	0.0 - 86.0
Age group (years), n (%)	
<= 18	342 (18.4%)

19 to 39	667 (35.8%)
40 to 64	713 (38.3%)
>= 65	140 (7.5%)
Sex at Birth, n (%)	
Male	790 (42.4%)
Female	1072 (57.6%)

2. Clinical Performance Estimates

Clinical Performance in Symptomatic Subjects

The clinical performance of the cobas SARS-CoV-2 Nucleic acid test for use on the cobas Liat System, expressed as positive percent agreement (PPA) and negative percent agreement (NPA) with the composite comparator result, testing NPS and NS specimens from patients with symptoms of respiratory tract infection, is shown in Tables 13 and 14 below, respectively.

Table 13: Clinical performance of cobas SARS-CoV-2 on the cobas Liat System with NPS specimens (symptomatic patients)

	Composite Comparator SARS-CoV-2 Result	
cobas SARS-CoV-2 on cobas Liat System	Positive	Negative
Positive	125	3
Negative	6	539
PPA = 95.4% (95% CI: 90.4% - 97.9%) NPA = 99.5% (95% CI: 98.4% - 99.8%)		

Table 14: Clinical performance of cobas SARS-CoV-2 on the cobas Liat System with NS specimens (symptomatic patients)

	Composite Comparator Method SARS-CoV-2 Result	
cobas SARS-CoV-2 on cobas Liat System	Positive	Negative
Positive	129	1
Negative	5	539
PPA =96.3% (95% CI: 91.6% - 98.4%) NPA = 99.8% (95% CI: 98.96% - 99.97%)		

Clinical Performance in Asymptomatic Subjects

The clinical performance of the cobas SARS-CoV-2 Nucleic acid test for use on the Liat System, testing specimens from patients without symptoms of respiratory tract infection, testing NPS and NS specimens, is shown in Tables 15 and 16, respectively. The asymptomatic subjects consisted both of the high risk and low risk individuals.

Table 15: Clinical performance of cobas SARS-CoV-2 on the cobas Liat System with NPS specimens (asymptomatic patients)

	Composite Comparator Method SARS-CoV-2 Result	
cobas SARS-CoV-2 on cobas Liat System	Positive	Negative
Positive	52	5
Negative	2	1142
PPA =96.3% (95% CI: 87.5% - 98.98%) NPA = 99.6% (95% CI: 98.98% - 99.8%)		

Table 16: Clinical performance of cobas SARS-CoV-2 on the cobas Liat System with NS specimens (asymptomatic patients)

	Composite Comparator Method SARS-CoV-2 Result	
cobas SARS-CoV-2 on cobas Liat System	Positive	Negative
Positive	45	1
Negative	5	1147
PPA =90.0% (95% CI: 78.6% - 95.7%) NPA = 99.9% (95% CI: 99.5% - 99.98%)		

D Clinical Cut-Off:
Not applicable

E Expected Values:
The SARS-CoV-2 positivity rate observed during the clinical study, as determined by the cobas SARS CoV-2 assay, is presented by enrollment site in Table 17 below.

Table 17: Positivity Rate by the cobas SARS-CoV-2 Test on the Liat System

		NPS Specimens			NS Specimens		
Clinical Site ID	**Site Location**	**Total No.**	**No. Positive for SARS-CoV-2**	**Positivity (%)**	**Total No.**	**No. Positive for SARS-CoV-2**	**Positivity (%)**
	Overall	1828	162	8.9%	1826	153	8.4%
1	Albuquerque, NM	222	2	0.9%	222	0	0.0%
2	Vienna, VA	390	60	15.4%	390	61	15.6%
3	Northridge, CA	97	0	0.0%	96	0	0.0%
4	Savannah, GA	385	20	5.2%	384	18	4.7%
5	North Miami, FL	343	23	6.7%	342	18	5.3%
6	Indianapolis, IN	9	1	11.1%	8	1	12.5%
7	Las Vegas, NV	105	1	1.0%	105	1	1.0%
8	Evanston, IL	131	34	26.0%	131	32	24.4%
9	Seneca, SC	30	1	3.3%	33	2	6.1%
10	Rochester, NY	116	20	17.2%	115	20	17.4%

F Other Supportive Instrument Performance Characteristics Data:
Not applicable

VIII Proposed Labeling:

The labeling is acceptable and supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.