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

I Background Information:

A 510(k) Number

K243346

B Applicant

Roche Molecular Systems, Inc.

C Proprietary and Established Names

cobas liat SARS-CoV-2 v2 nucleic acid test

D Regulatory Information

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

II Submission/Device Overview:

A Purpose for Submission:

The purpose of this submission is to show that the cobas liat SARS-CoV-2 v2 nucleic acid test is substantially equivalent to cobas SARS-CoV-2 Nucleic acid test for use on the cobas Liat System (K223783).

B Measurand:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA. The assay targets both the ORF1 a/b non-structural region and membrane protein gene that are unique to SARS-CoV-2.

C Type of Test:

This assay is a nucleic acid assay for the qualitative detection of SARS-CoV-2 RNA through nucleic acid extraction, amplification, and detection using real-time RT-PCR. All steps of the

assay are automated within the cobas liat system, after scanning the specimen ID barcode, scanning the assay tube barcode, and the manual addition of sample into the assay tube.

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The cobas liat SARS-CoV-2 v2 nucleic acid test is an automated real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleic acids in anterior nasal (nasal) and nasopharyngeal swab specimens collected from individuals exhibiting 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 of COVID-19 (i.e., asymptomatic).

The cobas liat SARS-CoV-2 v2 nucleic acid test is intended for use as an aid in the diagnosis of COVID-19 if used in conjunction with other clinical and epidemiological information and laboratory findings. SARS-CoV-2 RNA is generally detectable in nasal swab 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. Negative results do not preclude SARS-CoV-2 infection. Negative results must be combined with clinical observations, patient history, and epidemiological information. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

A negative result from an asymptomatic individual is presumptive. Additionally, a negative result obtained with a nasal or nasopharyngeal swab collected from an asymptomatic individual should be followed up by testing at least twice over three days with at least 48 hours between tests.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only
For *in vitro* diagnostic use

D Special Instrument Requirements:

For use with the cobas liat system, only

IV Device/System Characteristics:

A Device Description:

cobas liat SARS-CoV-2 v2 nucleic acid test uses real-time reverse transcriptase polymerase chain reaction (RT-PCR) technology to detect SARS-CoV-2 virus from nasopharyngeal and nasal swabs in approximately 20 minutes. The assay targets both the ORF1 a/b non-structural region and membrane protein gene that are unique to SARS-CoV-2. An Internal Control (IC) is

included to control for adequate processing of the target viruses through all steps of the assay process and to monitor the presence of inhibitors in the RT-PCR processes. The test is performed on the cobas liat analyzer which automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence in biological samples using real-time PCR assays.

B Principle of Operation:

The cobas liat SARS-CoV-2 v2 nucleic acid test uses nasopharyngeal and nasal swabs collected in universal transport media (UTM), universal viral transport (UVT) and is compatible with other viral transport media (VTM) such as M4RT, M4, M5, M6 and 0.9% saline. The cobas liat system software includes step-by-step on-screen instructions that guide the user through the process of starting a run on the instrument.

First, the operator scans the barcode on cobas liat SARS-CoV-2 v2 assay tube to identify the test; then, the sample barcode is scanned to link the sample ID with the assay run. Next, the operator transfers ~200 µL of the sample into the cobas liat SARS-CoV-2 v2 assay tube using the provided transfer pipette. The operator scans the assay tube barcode again before inserting the cobas liat SARS-CoV-2 v2 assay tube into the cobas liat analyzer. Once the assay tube is inserted into the cobas liat analyzer, the analyzer automatically performs all NAAT processes and displays interpreted results in approximately 20 minutes.

A report of the interpreted results can be viewed in the View Results window on the LCD touch screen integrated with the cobas liat analyzer. The report can be also printed directly through a USB connected printer.

C Instrument Description Information:

1. Instrument Name:
   cobas liat analyzer with cobas liat system software version 3.4 or higher.

2. Specimen Identification:
   Specimen identification is either entered manually or via barcode.

3. Specimen Sampling and Handling:
   Nasopharyngeal swab (NPS) or nasal swab (NS) specimens collected in 3 mL viral transport media or 0.9% physiological saline.

4. Calibration:
   The analyzer performs self-diagnostics during startup (initialization) and utilizes an advanced error diagnostics system to monitor the analyzer's performance during an assay. Under normal operation, the analyzer alerts the operator if a malfunction or error is detected. The analyzer requires no adjustment or calibration from the operator.

5. Quality Control:
The cobas liat SARS-CoV-2 v2 nucleic acid test contains an Internal Control (IC) comprised of a buffer, antimicrobial agent, and a non-target RNA construct containing primer- and probe-specific regions that serves as a full-process control. The IC is used to monitor the entire automated test process, including sample preparation (nucleic acid extraction), amplification, and detection steps and is included in each assay tube of the candidate test.

External positive and negative controls are included with cobas liat SARS-CoV-2, Influenza A/B & RSV Control Kit and are packaged and sold separately from the assay kit. The positive control (PC) contains a buffer, antimicrobial agent, and non-infectious armored RNA sequences of all target analytes. The PC is needed for a lot validation procedure. The PC will be invalid if the PC run does not detect target analytes and/or IC signal are outside of allowable ranges. The negative control (NC) contains only a buffer and antimicrobial agent. The NC is needed for a lot validation procedure (i.e., before using a new lot of the candidate test). The NC will be invalid if the NC run detects target analytes. The external controls were validated in the analytical, clinical, and flex studies.

V Substantial Equivalence Information:

A Predicate Device Name(s):
cobas SARS-CoV-2 Nucleic acid test for use on the cobas Liat System

B Predicate 510(k) Number(s):
K223783

C Comparison with Predicate(s):

Device & Predicate Device(s):	K243346	K223783
Device Trade Name	cobas liat SARS-CoV-2 v2 nucleic acid test	cobas SARS-CoV-2 for use on the cobas Liat System
Regulation Name	Same	21 CFR 866.3982
Product Code	Same	QWR
Prescription Use Only	Same	Yes
Intended Use	The cobas liat SARS-CoV-2 v2 nucleic acid test is an automated real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleic acids in anterior nasal (nasal) and nasopharyngeal swab specimens collected from individuals exhibiting signs and symptoms of respiratory tract	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

	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 of COVID-19 (i.e., asymptomatic). The cobas liat SARS-CoV-2 v2 nucleic acid test is intended for use as an aid in the diagnosis of COVID-19 if used in conjunction with other clinical and epidemiological information and laboratory findings. SARS-CoV-2 RNA is generally detectable in nasal swab 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. Negative results do not preclude SARS-CoV-2 infection. Negative results must be combined with clinical observations, patient history, and epidemiological information. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. A negative result from an asymptomatic individual is presumptive. Additionally, a negative result obtained with a nasal or nasopharyngeal swab collected from an asymptomatic individual should be followed up by testing at least twice over three days with at least 48 hours between tests.	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 setting.
Sample Type	Same	Nasopharyngeal and anterior nasal swabs

Target Analyte	SARS-CoV-2 ORF1 a/b non-structural region and SARS-CoV-2 membrane protein gene	SARS-CoV-2 ORF1 a/b non-structural region and SARS-CoV-2 nucleocapsid protein gene
Ancillary Collection Kits	• Copan FLOQSwab with UTM • BD UVT with flocked swab • Sterile flocked swabs with a synthetic tip with other viral transport media (VTM) – M4RT, M4, M5 and M6 • 0.9% Saline	• Copan FLOQSwabs with UTM, UVT and other swabs with other viral transport media (VTM) – e.g., M4RT, M4, M5 and M6 • 0.9% and 0.85% Saline
Amplification Technology	Same	Real-time PCR
Detection Chemistry	Same	Assay using different reporter dyes for target and control
Controls Used	Sample processing control (IC), External positive and negative control	Internal Control (a process control for sample purification, nucleic acid amplification, and for monitoring presence of inhibitors) External Positive and Negative Controls
Instrumentation	Same	cobas liat system

VI Standards/Guidance Documents Referenced:

Class II Special Controls as per 21 CFR 866.3982.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

Analytical studies including reproducibility, inclusivity (reactivity), cross-reactivity and microbial interference utilized simulated nasopharyngeal matrix (S-UTM) to prepare the contrived specimens for testing. Equivalency between using natural and simulated clinical matrices was demonstrated in the Matrix Equivalency Study described in section VII.B.2 below.

1. Precision/Reproducibility:
   A reproducibility study was conducted assessing the total variability of the cobas liat SARS-CoV-2 v2 nucleic acid assay across operators, study sites, testing days, cobas liat analyzers, and cobas liat assay tube lots. The cobas liat SARS-CoV-2 v2 assay was evaluated at three CLIA waived sites. Two (2) operators at each of the three sites tested a 3-member reproducibility panel in triplicate on five different days across three (3) reagent lots, for a total of ~810 tests (3 sites × 3 lots/site × 5 day/lot × 2 operators/day × 3 panel members/operator × 3 replicates/panel member), ~270 tests/panel member. Each site utilized

a minimum of three liat analyzers. The reproducibility panel contained a true negative, a low SARS-CoV-2 positive and a moderate SARS-CoV-2 positive member.

The reproducibility panel samples were prepared by spiking SARS-CoV-2 (USA-WA1/2020, inactivated) into negative simulated nasopharyngeal matrix (S-UTM). The moderate positive and low positive concentrations used corresponded to 5x LoD and 2x LoD, respectively. The true negative sample was comprised of S-UTM.

As shown in **Table 1**, the cobas liat SARS-CoV-2 v2 nucleic acid test demonstrated 100% agreement with the moderate and low positive panel members. The negative panel member also yielded 100% agreement with the expected results. This performance is acceptable and demonstrates acceptable assay reproducibility.

Table 1. Reproducibility Study- Qualitative Results

Target	Panel Conc.	% Agreement with Expected Results/ (n Agreement/N Tested) (95% CI)			
		Site 1	Site 2	Site 3	Overall
Negative	0	100% (86/86) (95.7-100)	100% (89/89) (95.9-100)	100% (90/90) (95.9-100)	100% (265/265) (98.6-100)
SARS-CoV-2[a]	Low Positive (2x LoD)	100% (90/90) (95.9-100)	100% (90/90) (95.9-100)	100% (90/90) (95.9-100)	100% (270-270) (98.6-100)
	Mod. Positive (5x LoD)	100% (88/88) (95.9-100)	100% (89/89) (95.9-100)	100% (90/90) (95.9-100)	100% (267/267) (98.6-100)

Mod = moderate, Conc= Concentration
[a] Inactivated virus

The total Ct variability, as measured by the standard deviation, was less than or equal to 1.18. These results, shown in **Table 2**, indicate that the reproducibility of the cobas liat SARS-CoV-2 v2 nucleic acid test is acceptable.

Table 2. Reproducibility Study- Ct Analysis Results

Viral Target	Panel Member Conc.	n/N[a]	Mean Ct	Between Sites		Between Lots		Between Days		Between Operators		Within-Run (Residual)		Total	
				SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
SARS-CoV-2[b]	2x LoD	270/270	33.8	0.00	0.0	0.38	1.1	0.17	0.5	0.36	1.1	1.03	3.0	1.16	3.4
SARS-CoV-2[b]	5x LoD	267/267	32.4	0.13	0.4	0.54	1.7	0.27	0.8	0.00	0.0	1.00	3.1	1.18	3.6

Ct = cycle threshold; LoD = Limit of Detection; SARS-CoV-2 = Severe acute respiratory syndrome coronavirus-2; SD = standard deviation; CV% = percent coefficient of variation.
[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] Inactivated virus

2. Linearity:

Not applicable.

3. Analytical Specificity/Interference:

Analytical Reactivity (Inclusivity)

a. *In silico*

The inclusivity of the cobas liat SARS-CoV-2 v2 assay was evaluated using *in silico* analysis of the forward primers, reverse primers, and probes for the SARS-CoV-2 target in relation to sequences available in the NCBI and GISAID gene databases. For both SARS-CoV-2 gene targets taken together, *in silico* analysis on January 15, 2025 (>7.94M sequences in NCBI and >15.04M sequences in GISAID) indicates that the majority of sequence variants are predicted to be detected, with only <0.04% of sequences in NCBI and <0.05% of sequences in GISAID, with any mismatch in primer/probe binding sites for both targets. *In silico* analysis also indicates that the cobas liat SARS-CoV-2 v2 assay's SARS-CoV-2 test detects all emerging new variant strains.

No sequences in the NCBI database, and three unique sequences in GISAID (0.00002%) had mismatches that were predicted to affect detection and performance of the test. Due to the low percentage representation in the databases of mismatches and potential for sequencing errors, the cobas liat SARS-CoV-2 v2 assay is expected to have high inclusivity for the SAR-CoV-2 target.

b. Wet-Testing

The inclusivity study evaluates the ability of the test to detect SARS-CoV-2 isolates/variants. The reactivity/inclusivity was evaluated with ten (10) SARS-CoV-2 isolates/variants. Viral suspensions were prepared at ~3x LoD for each strain individually in S-UTM and tested in triplicate to evaluate inclusivity. If < 100% hit rate was observed, the concentration was doubled until 3/3 replicates were detected.

The detected concentrations for the SARS-CoV-2 variants tested with the cobas liat SARS-CoV-2 v2 nucleic acid test are shown in **Table 3**. All ten (10) lineages were detected at concentration 0.105 $TCID_{50}$/mL, which is ~3x LoD for the SARS-CoV-2 USA-WA1/2020 strain.

Table 3. SARS-CoV-2 Inclusivity Strains Tested and Concentration Detected

Lineage/Subtype	Isolate/Variant[a,b]	$TCID_{50}$/mL	Relative LoD	Replicates Detected/ Tested
Alpha	Hong Kong/VM20001061/2020	0.105	3x	3/3
Beta, B.1.595_2020 (was B.1.2)	NY-Wadsworth-33126-01/2020	0.105	3x	3/3
Delta, B.1.617.2	USA/MD-HP05285/2021	0.105	3x	3/3
Epsilon, B.1.427	USA/CA/VRLC009/2021	0.105	3x	3/3
Gamma, P.1	Japan/TY7-503/2021	0.105	3x	3/3

Lineage/Subtype	Isolate/Variant[a,b]	$TCID_{50}$/mL	Relative LoD	Replicates Detected/ Tested
Iota, B.1.526_2021	USA/NY-Wadsworth-21025952-01/2021	0.105	3x	3/3
Kappa, B.1.617.1	USA/CA-Stanford-15_S02/2021	0.105	3x	3/3
Omicron, B.1.1.529, CH.1.1	USA/MD-HP41275/2022	0.105	3x	3/3
Omicron, B.1.1.529, XBB.1.5	USA/MD-HP40900/2022	0.105	3x	3/3
Zeta, P2_2021	USA/NY-Wadsworth-21006055-01/2021	0.105	3x	3/3

[a] All strains tested were inactivated virus.
[b] These strains are in addition to the SARS-CoV-2 USA-WA1/2020 and inactivated SARS-CoV-2/ 20/146, v3, 11/2021 used in the analytical sensitivity study.

Cross-Reactivity/ Microbial Interference

Cross-reactivity and microbial interference of the cobas liat SARS-CoV-2 v2 nucleic acid test was evaluated by testing fifty-one (51) strains of bacteria, fungi, and viruses for cross-reactivity and interference as well as nasal wash containing normal respiratory flora. The effect of non-target microorganisms on the performance of the cobas liat SARS-CoV-2 v2 nucleic acid test was tested by introducing non-target microorganisms into S-UTM spiked with and without inactivated SARS-CoV-2 (USA-WA1/2020) at ~3x LoD. Three (3) replicates in target positive background and three (3) replicates in target negative background were tested for each non-target microorganism. Five (5) replicates of interference control and five (5) replicates of specificity control were tested for the study, and all generated the expected results. Of the 51 total microorganisms tested, 48 were tested at concentrations of at least 1.0e+6 units/mL (bacteria and fungi) or at least 1.0e+5 units/mL (viruses), and the remaining three organisms (SARS Coronavirus, Urbani, Human Rhinovirus Type 1A, and Human Parainfluenza Virus Type 4A) were tested at the highest concentration possible. Clinical specimens containing Human Coronavirus HKU1 and *Pneumocystis jirovecii* were also tested. As summarized in **Table 4**, no cross-reactivity or microbial interference was observed at the concentrations tested, and no invalid results were obtained.

Table 4. Cross-reactivity and Microbial Interference Results for Non-Target Microorganisms

Microorganism	Concentration (unit/mL)	Negative Sample (Specificity Test)	Positive Sample (Interference Test)
		SARS-CoV-2 n Detected/N Tested	SARS-CoV-2 n Detected/N Tested
Adenovirus Type 1[c]	1.00E+05	0/3	3/3
Cytomegalovirus	1.00E+05	0/3	3/3
Epstein-Barr virus[c]	1.00E+05	0/3	3/3
Human Coronavirus OC43	1.00E+05	0/3	3/3
Human Coronavirus 229E	1.00E+05	0/3	3/3
	8.00E+04	0/3	3/3
Human Rhinovirus Type 1A[a,c]	7.05E+04	0/3	3/3
	7.05E+03	0/3	3/3
Measles	1.00E+05	0/3	3/3
Human Enterovirus 68	1.00E+05	0/3	3/3
Human Parainfluenza Virus Type 2	1.00E+05	0/3	3/3

Microorganism	Concentration (unit/mL)	Negative Sample (Specificity Test)	Positive Sample (Interference Test)
		SARS-CoV-2 n Detected/N Tested	SARS-CoV-2 n Detected/N Tested
Human Parainfluenza Virus Type 3	1.00E+05	0/3	3/3
Human Coronavirus HKU1	NA[b]	0/3	3/3
SARS Coronavirus, Urbani[a,c]	2.85E+04	0/3	3/3
Human Coronavirus NL63[c]	1.00E+05	0/3	3/3
	1.78E+04	0/3	3/3
MERS-Coronavirus[c]	1.00E+05	0/3	3/3
	2.09E+04	0/3	3/3
Adenovirus Type 7	1.00E+05	0/3	3/3
Human Parainfluenza Virus Type 4A[a,c]	5.85E+04	0/3	3/3
	5.85E+03	0/3	3/3
Human Parainfluenza Virus Type 1[c]	1.00E+05	0/3	3/3
Human Metapneumovirus 27	1.00E+05	0/3	3/3
Mumps	1.00E+05	0/3	3/3
Human Rhinovirus B	1.00E+05	0/3	3/3
RSV (Long/ Subtype A)	1.00E+05	0/3	3/3
RSV (9320/ Subtype B)	1.00E+05	0/3	3/3
Influenza A (Brisbane/02/2018)	1.00E+05	0/3	3/3
Influenza A (Darwin/6/2021)	1.00E+05	0/3	3/3
Influenza B (Austria/1359417/2021)	1.00E+05	0/3	3/3
Influenza B (Phuket/3073/2013)	1.00E+05	0/3	3/3
Nasal Wash	NA	0/3	3/3
Bordetella pertussis	1.00E+06	0/3	3/3
Corynebacterium flavescens	1.00E+06	0/3	3/3
Escherichia coli	1.00E+06	0/3	3/3
Haemophilus Influenzae	1.00E+06	0/3	3/3
Lactobacillus crispatus	1.00E+06	0/3	3/3
Legionella pneumophila	1.00E+06	0/3	3/3
Moraxella catarrhalis	1.00E+06	0/3	3/3
Mycoplasma pneumoniae	1.00E+06	0/3	3/3
Neisseria elongate	1.00E+06	0/3	3/3
Neisseria meningitidis	1.00E+06	0/3	3/3
Pseudomonas aeruginosa	1.00E+06	0/3	3/3
Staphylococcus aureus	1.00E+06	0/3	3/3
Staphylococcus epidermidis	1.00E+06	0/3	3/3
Streptococcus pneumoniae	1.00E+06	0/3	3/3
Streptococcus pyogenes	1.00E+06	0/3	3/3
Streptococcus salivarius	1.00E+06	0/3	3/3
Chlamydophila pneumoniae	1.00E+06	0/3	3/3
Fusobacterium necrophorum subsp. Necrophorum	1.00E+06	0/3	3/3
Neisseria flava	1.00E+06	0/3	3/3
Bordetella parapertussis	1.00E+06	0/3	3/3
Mycobacterium tuberculosis	1.00E+06	0/3	3/3
Mycoplasm gentialium[c]	1.00E+06	0/3	3/3
Candida albicans	1.00E+06	0/3	3/3
Aspergillus flavus var. flavus	1.00E+06	0/3	3/3
	2.90E+05	0/3	3/3
Pneumocystis jirovecii	NA[b]	0/3	3/3

[a] Tested at highest concentration possible per stock concentration.
[b] Positive clinical specimen was used and the concentration was unknown.
[c] Inactivated virus.

Interfering Substances

Three (3) endogenous substances and nine (9) exogenous substances that may be present in nasopharyngeal and nasal swab clinical specimens were tested with the cobas liat SARS-CoV-2 v2 nucleic acid test to assess potential interference with the assay. Five (5) replicates of each substance were tested individually in the presence and absence of inactivated SARS-CoV-2 (USA-WA1/2020) formulated at approximately 3x LoD in S-UTM.

As summarized in **Table 5**, none of the substances at the concentrations tested caused false positive or invalid results in the absence of SARS-CoV-2. Furthermore, none of the substances at the concentrations tested caused false negative or invalid results in the presence of SARS-CoV-2, except for one invalid result obtained with Cepacol. The one (1) invalid replicate was retested and obtained a positive result for SARS-CoV-2, indicating that the invalid result was not attributable to interference. Potentially interfering substances that may be present in clinical respiratory specimens did not interfere with the performance of the cobas liat SARS-CoV-2 v2 nucleic acid test at the concentrations tested.

Table 5. Summary of Interfering Substances Testing Results

Substance	Concentration Tested	SARS-CoV-2 Absent	SARS-CoV-2 Present
		Valid Negative Results/ Total # of Valid Results	Valid Positive Results/ Total # of Valid Results
Human Cells (PBMC)	1.00E+06 cell/mL	5/5	5/5
Mucin	5 mg/mL	5/5	5/5
Human Whole Blood	5% v/v	5/5	5/5
NNSC (Target Negative No Substance Control)	N/A	5/5	0/5
PNSC (Target Positive No Substance Control)	N/A	0/5	5/5
Nasal spray - Afrin / Anefrin	15% (v/v)	5/5	5/5
Nasal corticosteroids - Flonase	5% (v/v)	5/5	5/5
Nasal gel - Zicam	5% (v/v)	5/5	5/5
Throat lozenges, oral anesthetic and analgesic – Cepacol	5 mg/mL	5/5	5/5[a]
Antibiotic, nasal ointment - Bactroban mupirocin ointment	5 mg/mL	5/5	5/5
Antiviral drug - Relenza	5 mg/mL	5/5	5/5
Antiviral drug - Tamiflu	7.5 mg/mL	5/5	5/5
Antimicrobial, systemic - Tobramycin	4 ug/mL	5/5	5/5
Intranasal Vaccine – FluMist	6.25% (v/v)	5/5	5/5

[a] One invalid result was obtained, and was detected for all targets upon repeat.

4. Assay Reportable Range:
Not applicable; this is a qualitative assay.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):
   a. **Controls**
   The assay contains an Internal Control (IC) added to each test specimen and external positive and negative controls. For more information, see Section IV.C.5. Quality Control, above.

   b. **Sample Stability**
   Stability studies have been performed to support the following claims:

   - Primary NPS or NS specimens collected in transport media (UTM, UVT, M4, M4RT, M5, and M6) may be stored up to 4 hours at room temperature (15-30°C), up to 72 hours refrigerated (2-8°C), or frozen at ≤-70°C if not tested within 72 hours of collection.
   - Primary NPS or NS specimens collected in 0.9% physiological saline may be stored up to 4 hours at room temperature (15-30°C) or up to 72 hours refrigerated (2-8°C).
   - Specimens transferred into the cobas liat SARS-CoV-2 v2 nucleic acid test assay tube may be stored up to 4 hours at room temperature (15-30°C).

6. Detection Limit:
Limit of detection (LoD) studies determine the lowest detectable concentration of SARS-CoV-2 at which equal to or greater than 95% of all replicates test positive. Two strains of SARS-CoV-2 were evaluated. To determine the LoD, panels were formulated using inactivated virus diluted in pooled negative nasopharyngeal swab matrix. Twenty-one replicates per lot of assay tubes per dilution were tested for five or six 2-fold dilutions using three lots of assay tubes. The strains evaluated, as well as their corresponding LoD values are shown in **Table 6**.

Table 6. LoD determination for SARS-CoV-2 strains

Virus	Strain	Concentration at LoD	n Detected/ N Tested (Mean Ct)
SARS-CoV-2*	USA-WA1/2020	0.0350 $TCID_{50}$/mL	20/21 (35.5)
SARS-CoV-2*	SARS-CoV-2/20/146, v3,11/2021	65.1 IU/mL	21/21 (34.9)

* Inactivated virus

7. Assay Cut-Off:
A target result call is determined separately for the SARS-CoV-2 channel and the internal control (IC) channel based on the combination of curve call, Ct, and other validity features. The cobas liat SARS-CoV-2 v2 nucleic acid test Ct cutoffs are described in **Table 7**.

Table 7. cobas liat SARS-CoV-2 v2 nucleic acid test Ct Cut-offs for Positive/Negative Results Determination

Target	Positive	Negative
SARS-CoV-2	≤ 45	> 45
IC	≤ 34.5	> 34.5*

* IC values that are greater than 34.5 render the sample with an invalid result if target is not detected.

8. Accuracy (Instrument):
   Not applicable

9. Carry-Over:
   A carry-over study for the cobas Influenza A/B & RSV test for the liat system was conducted and reviewed previously. Please refer to K153544 for details.

B Comparison Studies:

1. Method Comparison with Predicate Device:
   Not applicable

2. Matrix Comparison:
   The equivalence of samples prepared in natural nasopharyngeal swab (NPS), nasal swab (NS), and simulated nasopharyngeal swab (S-UTM) matrices was verified by using inactivated SARS-CoV-2 (USA-WA1/2020). Samples were prepared at 5x LoD (0.175 $TCID_{50}$/mL) and 2x LoD (0.070 $TCID_{50}$/mL) as well as one SARS-CoV-2 negative level in S-UTM and each clinical swab matrices in UTM. Ten (10) replicates at 5x LoD, 30 at 2x LoD, and ten (10) target negative replicates were tested in S-UTM, natural clinical NPS, and natural clinical NS matrices.

   As summarized in **Table 8**, all positive contrived samples tested positive for SARS-CoV-2 at both testing levels and all target negative replicates tested negative for SARS-CoV-2. Therefore, equivalency has been demonstrated between natural clinical NPS, NS and S-UTM matrices for the cobas liat SARS-CoV-2 v2 nucleic acid test.

Table 8. Summary of Matrix Equivalency Study Results

Matrix	**Concentration**	**SARS-CoV-2**		
		n Detected/ N Tested	**% Positive**	**Mean Ct**
NPS UTM	2x LoD	30/30	100%	33.51
	5x LoD	10/10	100%	31.88
	Negative	0/10	0%	N/A
NS UTM	2x LoD	30/30	100%	34.01
	5x LoD	10/10	100%	32.07
	Negative	0/10	0%	N/A

Matrix	Concentration	SARS-CoV-2		
		n Detected/ N Tested	% Positive	Mean Ct
S-UTM	2x LoD	30/30	100%	34.11
	5x LoD	10/10	100%	32.75
	Negative	0/10	0%	N/A

3. Collection Media Equivalency (UTM, M4RT and 0.9% physiological saline)

To compare the performance of the cobas liat SARS-CoV-2 v2 nucleic acid test with different media, NPS specimens were used as the representative specimen type. NPS matrix pools collected in Universal Transport Media (UTM), Remel M4RT, and 0.9% saline were spiked with inactivated SARS-CoV-2 (USA-WA1/2020) at levels of 5x LoD (0.175 $TCID_{50}$/mL) and 2x LoD (0.070 $TCID_{50}$/mL). A target negative level was also included for evaluation. Ten (10) replicates at 5x LoD, 30 replicates at 2x LoD, and ten (10) target-negative replicates were tested in each of the three (3) media types.

As summarized in **Table 9**, all ten (10) replicates at 5x LoD and all 30 replicates at 2x LoD tested positive for SARS-CoV-2 in each collection media type. The ten (10) replicates of negative samples were negative for SARS-CoV-2 as well in each media type. Therefore, equivalency has been demonstrated between UTM, M4RT and 0.9% saline collection media types for the cobas liat SARS-CoV-2 v2 nucleic acid test.

Table 9. Summary of Media Equivalency Study Results

Matrix	Concentration	SARS-CoV-2		
		n Detected/ N Tested	% Positive	Mean Ct
UTM	2x LoD	30/30	100%	33.51
	5x LoD	10/10	100%	31.88
	Negative	0/10	0%	N/A
M4RT	2x LoD	30/30	100%	34.48
	5x LoD	10/10	100%	33.17
	Negative	0/10	0%	N/A
0.9% Saline	2x LoD	30/30	100%	34.03
	5x LoD	10/10	100%	31.81
	Negative	0/10	0%	N/A

C Clinical Studies:

1. Clinical Sensitivity:

The clinical performance of the cobas liat SARS-CoV-2 v2 nucleic acid test for the detection of SARS-CoV-2 was evaluated using subject matched paired prospective clinical nasopharyngeal swab (NPS) and anterior nasal swab (NS) specimens collected from individuals with or without signs and symptoms of respiratory viral infection. NS specimens were either collected by a healthcare provider or self-collected under the supervision of a healthcare provider. Testing of clinical samples was performed with the cobas lait SARS-

CoV-2 v2 nucleic acid test at 14 point-of-care healthcare facilities (e.g., emergency rooms, outpatient clinics, and physician offices) by untrained operators. The comparator method for SARS-CoV-2 results from the candidate device were compared to results from an acceptable FDA-cleared molecular assay. Performance for NPS was assessed against comparator results from testing an aliquot of the same NPS specimen and performance for NS was established against comparator results from testing an aliquot of the same NS specimen.

Subject matched paired NPS and NS samples were prospectively collected by 41 intended use operators from September 2023 to May 2024. In total, 4,446 prospectively collected paired NPS and NS specimens from individuals with or without signs and symptoms of respiratory viral infection were collected for the evaluation of cobas liat SARS-CoV-2 v2 nucleic acid test. Four (4) subjects were withdrawn due to not meeting the study eligibility criteria.

Symptomatic Cohort
In total, 1,730 prospectively collected paired NPS and NS specimens from symptomatic subjects were collected for the evaluation of cobas liat SARS-CoV-2 v2 nucleic acid test. One (1) subject was withdrawn due to not meeting the study eligibility criteria. Of the 1,729 prospective symptomatic subjects enrolled, 1,705 NPS specimens were evaluable in the SARS-CoV-2 analysis, 19 were non-evaluable due to missing candidate test results due to protocol deviations, and five (5) were non-evaluable due to specimen handling issues. Of the 1,729 prospective symptomatic subjects enrolled, 1,706 NS specimens were evaluable in the SARS-CoV-2 analysis, 22 were non-evaluable due to missing or invalid candidate test results, and one (1) was non-evaluable due to specimen handling issues.

Asymptomatic Cohort
In total, 2,716 prospectively collected paired NPS and NS specimens from asymptomatic subjects were collected for the evaluation of cobas liat SARS-CoV-2 v2 nucleic acid test. Three (3) subjects were withdrawn due to not meeting the study eligibility criteria. Of the 2,713 prospective asymptomatic subjects enrolled, 2,697 NPS specimens were evaluable in the SARS-CoV-2 analyses, 12 were non-evaluable due to missing candidate test results due to protocol deviations, one (1) sample observed a failed test, and three (3) were non-evaluable due to specimen handling issues. 2,700 NS specimens were evaluable in the SARS-CoV-2 analyses, ten (10) were non-evaluable due to missing or invalid candidate test results and three (3) were non-evaluable due to specimen handling issues **Table 10** below provides a summary of the demographic information for subjects included in the performance analysis from the prospective clinical study.

Table 10. Demographics of Prospectively Enrolled Individuals that were Included in the Performance Analysis

Age Group (Years)	Symptomatic Subjects Overall N(%) (N=1729)	Asymptomatic Subjects Overall N(%) (N=2013)
<1	33 (1.9%)	11 (0.4%)
1 - <18	434 (25.1%)	205 (7.6%)
18 - <30	381 (22.0%)	791 (29.2%)
30 - <40	246 (14.2%)	453 (16.7%)
40 - <50	200 (11.6%)	393 (14.5%)

50 - <60	181 (10.5%)	394 (14.5%)
>=60	254 (14.7%)	466 (17.2%)

A summary of the cobas liat SARS-CoV-2 v2 nucleic acid test prospective clinical study performance for symptomatic and asymptomatic individuals is provided in **Tables 11 and 12,** respectively.

Positive Percent Agreement (PPA) for the NPS specimen tested on the candidate and comparator device and NS specimen tested on the candidate and comparator device was calculated as 100% × (a / (a + c)). True positive (a) indicates that both the cobas liat SARS-CoV-2 v2 nucleic acid test and the comparator method had a positive result for SARS-CoV-2, and false negative (c) indicates that the cobas liat SARS-CoV-2 v2 nucleic acid test was negative while the comparator result was positive for SARS-CoV-2. Negative Percent Agreement (NPA) for the NPS specimen tested on the candidate and comparator device and NS specimen tested on the candidate and comparator device was calculated as 100% × (b / (b + d)). True negative (b) indicates that both the cobas liat SARS-CoV-2 v2 nucleic acid test and the comparator method had negative results for SARS-CoV-2, and false positive (d) indicates that the cobas liat SARS-CoV-2 v2 nucleic acid test was positive for SARS-CoV-2 while the comparator result was negative. Specimens that obtained discordant results underwent additional testing with an FDA cleared molecular test, when sufficient sample volume remained.

Table 11. Clinical Performance of cobas liat SARS-CoV-2 v2 nucleic acid test in Symptomatic Subjects

Specimen Type	Total (N)	Positive Percent Agreement			Negative Percent Agreement		
		a/ (a+c)	%	95% CI	b/ (b+d)	%	95% CI
NPS	1705	207/219[1]	94.5	90.7-96.8	1451/1486[2]	97.6	96.7-98.3
NS	1706	208/215[3]	96.7	93.2-98.4	1449/1491[4]	97.2	96.2-97.9

N = Total number of paired samples, CI = Confidence Interval, NPS = Nasopharyngeal swab, NS = Anterior Nasal Swab
Note: a = number of samples where both the cobas liat SARS-CoV-2 v2 nucleic acid test and the comparator tests are positive; b = number of samples where the cobas liat SARS-CoV-2 v2 nucleic acid test is positive and the comparator is negative; c = number of samples where the cobas liat SARS-CoV-2 v2 nucleic acid test is negative and the comparator is positive; d = number of samples where both the cobas liat SARS-CoV-2 v2 nucleic acid test and the comparator tests are negative.
[1] Of 12 NPS specimens negative for SARS-CoV-2 on cobas liat and positive on the comparator, eight (8) were positive for SARS-CoV-2 and four (4) were negative by an FDA cleared SARS-CoV-2 molecular test.
[2] Of 35 NPS specimens positive for SARS-CoV-2 on cobas liat and negative on the comparator, 12 were positive for SARS-CoV-2 and 23 were negative by an FDA cleared SARS-CoV-2 molecular test.
[3] Of seven (7) NS specimens negative for SARS-CoV-2 on cobas liat and positive on the comparator, six (6) were positive for SARS-CoV-2 and one (1) was negative by an FDA cleared SARS-CoV-2 molecular test.
[4] Of 42 NS specimens positive for SARS-CoV-2 on cobas liat and negative on the comparator, eight (8) were positive for SARS-CoV-2 and 34 were negative by an FDA cleared SARS-CoV-2 molecular test.

Table 12. Clinical Performance of cobas liat SARS-CoV-2 v2 nucleic acid test in Asymptomatic Subjects

Specimen type	Total (N)	Positive Percent Agreement			Negative Percent Agreement		
		a/ (a+c)	%	95% CI	d/ (b+d)	%	95% CI
NPS	2697	62/72[1]	86.1	76.3-92.3	2569/2625[2]	97.9	97.2-98.4
NS	2700	51/57[3]	89.5	78.9-95.1	2597/2643[4]	98.3	97.7-98.7

PPA = Positive Percent Agreement; CI = Confidence Interval; NPA = Negative Percent Agreement; NPS = Nasopharyngeal swab; NS = Nasal Swab.
Note: N = Total number of specimens; a = number of samples where both the cobas liat SARS-CoV-2 v2 nucleic acid test and the comparator tests are positive; b = number of samples where the cobas liat SARS-CoV-2 v2 nucleic acid test is positive and the comparator is negative; c = number of samples where the cobas liat SARS-CoV-2 v2 nucleic acid test is negative and the comparator is positive; d = number of samples where both the cobas liat SARS-CoV-2 v2 nucleic acid test and the comparator tests are negative.
[1] Of ten (10) NPS specimens negative for SARS-CoV-2 on cobas liat and positive on the comparator, six (6) were positive for SARS-CoV-2 and four (4) were negative by an FDA cleared SARS-CoV-2 molecular test.
[2] Of 56 NPS specimens positive for SARS-CoV-2 on cobas liat and negative on the comparator, 17 were positive for SARS-CoV-2 and 39 were negative by an FDA cleared SARS-CoV-2 molecular test.
[3] Of six (6) NS specimens negative for SARS-CoV-2 on cobas liat and positive on the comparator, three (3) were positive for SARS-CoV-2 and three (3) were negative by an FDA cleared SARS-CoV-2 molecular test.
[4] Of 46 NS specimens positive for SARS-CoV-2 on cobas liat and negative on the comparator, six (6) were positive for SARS-CoV-2 and 40 were negative by an FDA cleared SARS-CoV-2 molecular test.

2. Clinical Specificity:
   See Section "Clinical Sensitivity" above

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):
   Not applicable

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

The cobas liat SARS-CoV-2 v2 nucleic acid test prospective clinical study included a total of 4,446 prospectively collected NPS and NS specimens. Of the 1,730 specimens collected from symptomatic individuals 1,705 NPS specimens and 1,706 NS specimens were evaluable. Of the 2,716 specimens collected from asymptomatic individuals, 2,697 NPS specimens and 2,700 NS specimens were evaluable. The number and percentage of cases positive for SARS-CoV-2 as determined by the cobas liat SARS-CoV-2 v2 nucleic acid test are presented in **Table 13**, stratified by collection site.

Table 13. cobas liat SARS-CoV-2 v2 nucleic acid test – Expected Values by Specimen Collection Site for NPS and NS Specimens

Site	SARS-CoV-2 % Positivity (n Positive/N Total Samples)			
	Symptomatic Subjects		Asymptomatic Subjects	
	NPS	NS	NPS	NS
Site 1	6.0% (4/67)	7.5% (5/67)	3.9% (11/283)	4.2% (12/283)
Site 2	12.2% (5/41)	12.2% (5/41)	3.6% (11/309)	2.6% (8/309)
Site 3	6.1% (2/33)	3.0% (1/33)	0.0% (0/0)	0.0% (0/0)
Site 4	0.0% (0/42)	0.0% (0/42)	0.0% (0/55)	1.8% (1/56)
Site 5	17.5% (54/308)	21.5% (66/307)	2.9% (2/70)	7.0% (5/71)
Site 6	20.0% (34/171)	20.3% (35/173)	12.1% (21/173)	8.6% (15/175)
Site 7	7.1% (13/184)	8.1% (15/185)	2.3% (1/43)	2.3% (1/43)
Site 8	3.0% (2/66)	3.0% (2/66)	1.9% (2/107)	2.8% (3/106)
Site 9	26.1% (42/161)	26.1% (42/161)	4.4% (11/252)	5.6% (14/251)
Site 10	17.3% (29/168)	16.7% (28/168)	9.9% (18/182)	3.9% (7/181)
Site 11	14.5% (29/200)	12.9% (26/201)	1.7% (5/294)	1.4% (4/294)

Site 12	3.9% (6/154)	3.9% (6/152)	2.6% (9/343)	1.7% (6/344)
Site 13	14.3% (8/56)	12.5% (7/56)	5.1% (15/293)	2.7% (8/294)
Site 14	25.9% (14/54)	22.2% (12/54)	4.1% (12/293)	4.4% (13/293)
All	14.2% (242/1705)	14.7% (250/1706)	4.4% (118/2697)	3.6% (97/2700)

F Other Supportive Instrument Performance Characteristics Data:
Not applicable.

VIII Proposed Labeling:

The labeling 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.