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

I Background Information:

A 510(k) Number

K243400

B Applicant

Roche Molecular Systems, Inc.

C Proprietary and Established Names

cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QOF	Class II	21 CFR 866.3981 - Device To Detect And Identify Nucleic Acid Targets In Respiratory Specimens From Microbial Agents That Cause The SARS-CoV-2 Respiratory Infection And Other Microbial Agents When In A Multi-Target Test	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 & Influenza A/B v2 nucleic acid test is substantially equivalent to Hologic Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay (K241240).

B Measurand:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A and influenza B RNA. The assay targets both the ORF1 a/b non-structural region and membrane protein gene that are unique to SARS-CoV-2, a well-conserved region of the matrix gene of influenza A and the nonstructural protein 1 (NS1) gene of influenza B.

C Type of Test:

This assay is a multiplex nucleic acid assay for the qualitative detection and differentiation of SARS-CoV-2, influenza A and influenza B 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 & Influenza A/B v2 nucleic acid test is an automated rapid multiplex real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the simultaneous qualitative detection and differentiation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A virus and influenza B virus nucleic acids in anterior nasal (nasal) and nasopharyngeal swab specimens from individuals exhibiting signs and symptoms of respiratory tract infection. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2 and influenza can be similar. This test is intended to aid in the differential diagnosis of SARS-CoV-2, influenza A and influenza B infections in humans and is not intended to detect influenza C virus infections.

Nucleic acids from the viral organisms identified by this test are generally detectable in nasopharyngeal and nasal swab specimens during the acute phase of infection. The detection and identification of specific viral nucleic acids from individuals exhibiting signs and symptoms of respiratory tract infection are indicative of the presence of the identified virus, and aid in diagnosis if used in conjunction with other clinical and epidemiological information and laboratory findings.

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

Positive results do not rule out coinfection with other organisms. The organism(s) detected by the cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test may not be the definite cause of disease. Negative results do not preclude SARS-CoV-2, influenza A virus or influenza B virus infections.

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 & Influenza A/B v2 nucleic acid test uses real-time reverse transcriptase polymerase chain reaction (RT-PCR) technology to detect and differentiate between SARS-CoV-2, influenza A and influenza B viruses 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, a well-conserved region of the matrix gene of influenza A and the nonstructural protein 1 (NS1) gene of influenza B. 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 & Influenza A/B 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 & Influenza A/B 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 & Influenza A/B v2 assay tube using the provided transfer pipette. The operator scans the assay tube barcode again before inserting the cobas liat SARS-CoV-2 & Influenza A/B 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 & Influenza A/B 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):
Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay

B Predicate 510(k) Number(s):
K241240

C Comparison with Predicate(s):

Device & Predicate Device:	K243400	K241240
Device Trade Name	cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test	Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay
Regulation Name	Same	21 CFR 866.3981
Product Code	Same	QOF
Prescription Use Only	Same	Yes

Intended Use	The cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test is an automated rapid multiplex real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the simultaneous qualitative detection and differentiation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A virus and influenza B virus nucleic acids in anterior nasal (nasal) and nasopharyngeal swab specimens from individuals exhibiting signs and symptoms of respiratory tract infection. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2 and influenza can be similar. This test is intended to aid in the differential diagnosis of SARS-CoV-2, influenza A and influenza B infections in humans and is not intended to detect influenza C virus infections. Nucleic acids from the viral organisms identified by this test are generally detectable in nasopharyngeal and nasal swab specimens during the acute phase of infection. The detection and identification of specific viral nucleic acids from individuals exhibiting signs and symptoms of respiratory tract infection are indicative of the presence of the identified virus, and aid in diagnosis if used in conjunction with other clinical and epidemiological information, and laboratory findings. The results of this test should not be used as the sole basis for diagnosis,	The Panther Fusion SARS-CoV-2/Flu A/B/RSV assay is a fully automated multiplexed real-time polymerase chain reaction (RT-PCR) in vitro diagnostic test intended for the qualitative detection and differentiation of severe acute respiratory syndrome coronavirus 2 (SARS- CoV-2), influenza A virus (Flu A), influenza B virus (Flu B), and respiratory syncytial virus (RSV). Nucleic acids are isolated and purified from nasopharyngeal (NP) swab specimens and anterior nasal (AN) swab specimens obtained from individuals exhibiting signs and symptoms of a respiratory tract infection. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2, influenza, and RSV can be similar. This assay is intended to aid in the differential diagnosis of SARS-CoV-2, Flu A, Flu B, and RSV infections in humans and is not intended to detect influenza C virus infections. Nucleic acids from the viral organisms identified by this test are generally detectable in NP and AN swab specimens during the acute phase of infection. The detection and identification of specific viral nucleic acids from individuals exhibiting signs and symptoms of respiratory tract infection are indicative of the presence of the identified virus and aids in diagnosis if used in conjunction with other clinical and epidemiological information, and

	treatment, or other patient management decisions. Positive results do not rule out coinfection with other organisms. The organism(s) detected by the cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test may not be the definite cause of disease. Negative results do not preclude SARS-CoV-2, influenza A virus or influenza B virus infections.	laboratory findings. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Positive results do not rule out coinfection with other organisms. The organism(s) detected by the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay may not be the definite cause of disease. Negative results do not preclude SARS-CoV-2, influenza A virus, influenza B virus, or RSV infections. This assay is designed for use on the Panther Fusion System. The Hologic RespDirect Collection Kit is cleared for NP swab specimens only for testing with the Panther Fusion SARSCoV-2/Flu A/B/RSV assay.
Sample Type	Same	Nasopharyngeal and anterior nasal swabs
Analyte	Same	SARS-CoV-2, Flu A, Flu B, RSV RNA
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	• RespDirect Swab, intended for collection of NPS specimens with UTM/VTM or eSTM • Enhanced Direct Load Tube (eDLT), containing enhanced specimen transport media (eSTM).
Time to result	About 15 minutes	~ 2.5 hours
Amplification Technology	Same	Real-time PCR
Detection Chemistry	Same	Assay using different reporter dyes for target and control
Controls Used	Same	Sample processing control (IC), Positive and negative control
Instrumentation	cobas liat system	Panther Fusion System

VI Standards/Guidance Documents Referenced:

Class II Special Controls as per 21 CFR 866.3981.

VII Performance Characteristics:

A Analytical Performance:

Analytical studies including reproducibility, inclusivity (reactivity), cross-reactivity, microbial interference, and competitive inhibition 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.

Analytical studies including reproducibility, microbial interference, interfering substances, competitive inhibition, sample stability, kit stability, limit of detection (LoD), matrix equivalency, and media equivalency utilized co-formulated (multi-spiked) samples for testing. A study was conducted to demonstrated that the LoD of each strain individually was equivalent to the co-spiked LoD.

1. Precision/Reproducibility:

A reproducibility study was conducted assessing the total variability of the cobas liat SARS-CoV-2 & Influenza A/B 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 & Influenza A/B 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 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 positive and a moderate positive member co-formulated with SARS-CoV-2, influenza A and influenza B.

The reproducibility panel samples were prepared by spiking SARS-CoV-2 (USA-WA1/2020, inactivated), influenza A virus (Darwin/6/2021) and influenza B virus (Austria/1359417/2021) of known titer into negative simulated nasopharyngeal matrix (S-UTM). The moderate positive and low positive concentrations used for each of the strains 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 & Influenza A/B v2 nucleic acid test demonstrated 100% agreement for each target analyte with the moderate positive panel members. For low positive panel members, the assay yielded 100% agreement for SARS-CoV-2 and 99.6% agreement for influenza A and influenza B. All negative panel members 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[b]	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)
Influenza A	Low positive (2x LoD)	100% (90/90) (95.9-100)	100% (90/90) (95.9-100)	98.9% (88/89)[a] (93.9-99.8)	99.6% (268/269) (97.9-99.9)
	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)
Influenza B	Low positive (2x LoD)	100% (90/90) (95.9-100)	98.9% (89/90) (94.0-99.8)	100% (89/89)[a] (95.9-100)	99.6% (268/269) (97.9-99.9)
	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

Note: Results are shown only for the intended targets. Panel members were all co-spiked with all targets, so results are presented three times

[a] One valid test obtained an invalid results for the specified target.

[b] Inactivated virus

The total Ct variability, as measured by the standard deviation, was less than or equal to 1.18 across all target viruses and concentrations. These results, shown in **Table 2**, indicate that the reproducibility of the cobas liat SARS-CoV-2 & Influenza A/B 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
Influenza A	2x LoD	268/269	34.8	0.11	0.3	0.08	0.2	0.13	0.4	0.00	0.0	0.62	1.8	0.65	1.9
Influenza A	5x LoD	267/267	33.8	0.00	0.0	0.05	0.2	0.00	0.0	0.11	0.3	0.39	1.2	0.41	1.2
Influenza B	2x LoD	268/269	33.8	0.04	0.1	0.29	0.9	0.00	0.0	0.13	0.4	0.66	2.0	0.73	2.2
Influenza B	5x LoD	267/267	32.8	0.05	0.2	0.47	1.4	0.12	0.4	0.00	0.0	0.52	1.6	0.71	2.2

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 & Influenza A/B 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 & Influenza A/B 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 & Influenza A/B 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, influenza A and influenza B isolates/variants. The reactivity/inclusivity was evaluated with ten (10) SARS-CoV-2, 28 influenza A (15 H1N1, 10 H3N2, 1 H5N1, 1 H5N2 and 1 H7N9) and ten (10) influenza B (5 Victoria and 5 Yamagata) 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.

SARS-CoV-2

The detected concentrations for the SARS-CoV-2 variants tested with the cobas liat SARS-CoV-2 & Influenza A/B 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
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.

Influenza A

Ten (10) influenza A H1N1 and 10 influenza A H3N2 strains were detected near the 0.885 $TCID_{50}$/mL concentration, which is the ~3x LoD concentration that was established for the influenza A/Darwin/6/2021 strain. Some of the strains, A/Christ Church/16/2020, A/Swine/Iowa/15/30, A/Sydney/5/2021, and A/Darwin/9/2021 required testing at 1.77 $TCID_{50}$/mL (~6x LoD) and one strain A/Victoria/2570/2019 required testing at 3.54 $TCID_{50}$/mL (~12x LoD) to pass acceptance criteria. Additionally, the avian influenza H7N9 strain A/northern shoveler/Mississippi/11OS145/2011 was detected at 3.54 $CEID_{50}$/mL (~12x LoD). The influenza A isolates/variants tested in the study and the lowest concentrations detected are listed in **Table 4**.

Table 4. Influenza A Inclusivity Strains Tested and Concentration Detected.

Lineage/ Subtype	Isolate/Variant[a]	Test Concentration ($TCID_{50}$/mL)[d]	Relative LoD	Replicates Detected/ Tested
H1N1	A/Brisbane/59/07	0.885	3x	3/3
	A/Christ Church/16/2020	1.77 EID_{50}/mL	6x	3/3
	A/Denver/01/57	0.885	3x	3/3
	A/England/221740513/2022[b]	75 copies/mL[c]	1x	3/3
	A/England/224020815/2022[b]	91.5 copies/mL[c]	1x	3/3
	A/Fort Monmouth/01/47	0.885	3x	3/3
	A/Malaya/302/54	0.885	3x	3/3

Lineage/ Subtype	Isolate/Variant[a]	Test Concentration ($TCID_{50}$/mL)[d]	Relative LoD	Replicates Detected/ Tested
	A/New Caledonia/20/99	0.885	3x	3/3
	A/Swine/Iowa/15/30	1.77 $CEID_{50}$/mL	6x	3/3
	A/Sydney/5/2021[e]	1.77	6x	3/3
	A/Victoria/2570/2019	3.54 EID_{50}/mL	12x	3/3
	A/WS/33	0.885	3x	3/3
	A/Brisbane/14/2023	75 copies/mL[c]	1x	3/3
	A/Townsville/1A/2023	75 copies/mL[c]	1x	3/3
	A/ Townsville/2A/2023	75 copies/mL[c]	1x	3/3
H3N2	A/Aichi/2/68	0.885 $CEID_{50}$/mL	3x	3/3
	A/Brisbane/10/07	0.885	3x	3/3
	A/Cambodia/E0826360/2020	0.885	3x	3/3
	A/Darwin/9/2021	1.77	6x	3/3
	A/Hong Kong/8/68	0.885	3x	3/3
	A/H3/Perth/16/09	0.885	3x	3/3
	A/Tasmania/503/2020	0.885 EID_{50}/mL	3x	3/3
	A/Victoria/3/75	0.885 $CEID_{50}$/mL	3x	3/3
	A/Wisconsin/67/2005	0.885	3x	3/3
	A/Singapore/INFIMH-16-0019/2016	0.885	3x	3/3
H5N1	A/mallard/Wisconsin/2576/2009	0.885 $CEID_{50}$/mL	3x	3/3
H5N2	A/ruddy turnstone/New Jersey/828212/2001	0.885 $CEID_{50}$/mL	3x	3/3
H7N9	A/northern shoveler/Mississippi/ 11OS145/2011	3.54 $CEID_{50}$/mL	12x	3/3

[a] These strains are in addition to the influenza A Darwin/6/2021 and Brisbane/02/2018 strains used in the analytical sensitivity study.
[b] A/England/221740513/2022 GISAID ID is EPI_ISL_14387941 and for A/England/224020815/2022 GISAID ID is EPI_ISL_15803829.
[c] The concentrations tested are near the 1x LoD value (69 copies/mL) determined by digital PCR for the influenza A/Darwin/6/2021 strain.
[d] Concentrations are shown in $TCID_{50}$/mL unless otherwise indicated.
[e] Inactivated virus.

Influenza B

Five (5) influenza B Victoria lineage and five influenza B Yamagata lineage strains were detected at the 2.937 $TCID_{50}$/mL concentration, which is the ~3x LoD concentration that was established for the influenza B/Austria/1359417/2021 strain. The influenza B isolates/variants tested in the study and the lowest concentrations detected are listed in **Table 5**.

Table 5: Results of Testing Influenza B Isolate/Variants

Lineage/ Subtype	Isolate/Variant[a]	Test Concentration ($TCID_{50}$/mL) [b]	Relative LoD	Replicates Detected/ Tested
Victoria	B/Brisbane/60/2008	2.937	3x	3/3
Victoria	B/Colorado/06/2017	2.937	3x	3/3
Victoria	B/Malaysia/2506/04	2.937	3x	3/3
Victoria	B/Michigan/09/2011	2.937 EID_{50}/mL	3x	3/3
Victoria	B/Washington/02/2019	2.937	3x	3/3
Yamagata	B/Florida/04/06	2.937	3x	3/3
Yamagata	B/Massachusetts/2/2012	2.937	3x	3/3
Yamagata	B/Texas/6/2011	2.937	3x	3/3
Yamagata	B/Texas/81/2016	2.937 EID_{50}/mL	3x	3/3
Yamagata	B/Wisconsin/1/2010	2.937	3x	3/3

[a] These strains are in addition to the influenza B Austria/1359417/2021 and Phuket/3073/2013 strains used in the analytical sensitivity study.
[b] Concentrations are shown in $TCID_{50}$/mL unless otherwise indicated.

This study demonstrates that the cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test is able to detect multiple strains/variants for all three (3) virus targets.

Cross-Reactivity/ Microbial Interference

Cross-reactivity and microbial interference of the cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test was evaluated by testing forty-seven (47) 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 & Influenza A/B v2 nucleic acid test was tested by introducing non-target microorganisms into S-UTM spiked with and without co-formulated SARS-CoV-2 (USA-WA1/2020, inactivated), influenza A (Darwin/6/2021) and influenza B (Austria/1359417/2021) viruses 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 47 total microorganisms tested, 44 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 6**, no cross-reactivity or microbial interference was observed at the concentrations tested, and no invalid results were obtained.

Table 6. Cross-reactivity and Microbial Interference Results for Non-Target Microorganisms

Microorganism	Concentration (unit/mL)	Negative Sample (Specificity Test) Target Result: n Detected/N Tested			Positive Sample (Interference Test) Target Result: n Detected/N Tested		
		SARS-CoV-2	Influenza A	Influenza B	SARS-CoV-2	Influenza A	Influenza B
Adenovirus Type 1[c]	1.00E+05	0/3	0/3	0/3	3/3	3/3	3/3
Cytomegalovirus	1.00E+05	0/3	0/3	0/3	3/3	3/3	3/3
Epstein-Barr virus[c]	1.00E+05	0/3	0/3	0/3	3/3	3/3	3/3
Human Coronavirus OC43	1.00E+05	0/3	0/3	0/3	3/3	3/3	3/3
Human Coronavirus 229E	1.00E+05	0/3	0/3	0/3	3/3	3/3	3/3
	8.00E+04	0/3	0/3	0/3	3/3	3/3	3/3
Human Rhinovirus Type 1A[a,c]	7.05E+04	0/3	0/3	0/3	3/3	3/3	3/3
	7.05E+03	0/3	0/3	0/3	3/3	3/3	3/3
Measles	1.00E+05	0/3	0/3	0/3	3/3	3/3	3/3
Human Enterovirus 68	1.00E+05	0/3	0/3	0/3	3/3	3/3	3/3
Human Parainfluenza Virus Type 2	1.00E+05	0/3	0/3	0/3	3/3	3/3	3/3
Human Parainfluenza Virus Type 3	1.00E+05	0/3	0/3	0/3	3/3	3/3	3/3
Human Coronavirus HKU1	NA[b]	0/3	0/3	0/3	3/3	3/3	3/3
SARS Coronavirus, Urbani[a,c]	2.85E+04	0/3	0/3	0/3	3/3	3/3	3/3
Human Coronavirus NL63[c]	1.00E+05	0/3	0/3	0/3	3/3	3/3	3/3
	1.78E+04	0/3	0/3	0/3	3/3	3/3	3/3
MERS-Coronavirus[c]	1.00E+05	0/3	0/3	0/3	3/3	3/3	3/3
	2.09E+04	0/3	0/3	0/3	3/3	3/3	3/3
Adenovirus Type 7	1.00E+05	0/3	0/3	0/3	3/3	3/3	3/3
Human Parainfluenza Virus Type 4A[a,c]	5.85E+04	0/3	0/3	0/3	3/3	3/3	3/3
	5.85E+03	0/3	0/3	0/3	3/3	3/3	3/3
Human Parainfluenza Virus Type 1[c]	1.00E+05	0/3	0/3	0/3	3/3	3/3	3/3
Human Metapneumovirus 27	1.00E+05	0/3	0/3	0/3	3/3	3/3	3/3
Mumps	1.00E+05	0/3	0/3	0/3	3/3	3/3	3/3
Human Rhinovirus B	1.00E+05	0/3	0/3	0/3	3/3	3/3	3/3
RSV (Long/ Subtype A)	1.00E+05	0/3	0/3	0/3	3/3	3/3	3/3
RSV (9320/ Subtype B)	1.00E+05	0/3	0/3	0/3	3/3	3/3	3/3
Nasal Wash	NA	0/3	0/3	0/3	3/3	3/3	3/3
Bordetella pertussis	1.00E+06	0/3	0/3	0/3	3/3	3/3	3/3
Corynebacterium flavescens	1.00E+06	0/3	0/3	0/3	3/3	3/3	3/3

Microorganism	Concentration (unit/mL)	Negative Sample (Specificity Test)			Positive Sample (Interference Test)		
		Target Result: n Detected/N Tested			Target Result: n Detected/N Tested		
		SARS-CoV-2	Influenza A	Influenza B	SARS-CoV-2	Influenza A	Influenza B
Escherichia coli	1.00E+06	0/3	0/3	0/3	3/3	3/3	3/3
Haemophilus Influenzae	1.00E+06	0/3	0/3	0/3	3/3	3/3	3/3
Lactobacillus crispatus	1.00E+06	0/3	0/3	0/3	3/3	3/3	3/3
Legionella pneumophila	1.00E+06	0/3	0/3	0/3	3/3	3/3	3/3
Moraxella catarrhalis	1.00E+06	0/3	0/3	0/3	3/3	3/3	3/3
Mycoplasma pneumoniae	1.00E+06	0/3	0/3	0/3	3/3	3/3	3/3
Neisseria elongate	1.00E+06	0/3	0/3	0/3	3/3	3/3	3/3
Neisseria meningitidis	1.00E+06	0/3	0/3	0/3	3/3	3/3	3/3
Pseudomonas aeruginosa	1.00E+06	0/3	0/3	0/3	3/3	3/3	3/3
Staphylococcus aureus	1.00E+06	0/3	0/3	0/3	3/3	3/3	3/3
Staphylococcus epidermidis	1.00E+06	0/3	0/3	0/3	3/3	3/3	3/3
Streptococcus pneumoniae	1.00E+06	0/3	0/3	0/3	3/3	3/3	3/3
Streptococcus pyogenes	1.00E+06	0/3	0/3	0/3	3/3	3/3	3/3
Streptococcus salivarius	1.00E+06	0/3	0/3	0/3	3/3	3/3	3/3
Chlamydophila pneumoniae	1.00E+06	0/3	0/3	0/3	3/3	3/3	3/3
Fusobacterium necrophorum subsp. Necrophorum	1.00E+06	0/3	0/3	0/3	3/3	3/3	3/3
Neisseria flava	1.00E+06	0/3	0/3	0/3	3/3	3/3	3/3
Bordetella parapertussis	1.00E+06	0/3	0/3	0/3	3/3	3/3	3/3
Mycobacterium tuberculosis	1.00E+06	0/3	0/3	0/3	3/3	3/3	3/3
Mycoplasm gentialium[c]	1.00E+06	0/3	0/3	0/3	3/3	3/3	3/3
Candida albicans	1.00E+06	0/3	0/3	0/3	3/3	3/3	3/3
Aspergillus flavus var. flavus	1.00E+06	0/3	0/3	0/3	3/3	3/3	3/3
	2.90E+05	0/3	0/3	0/3	3/3	3/3	3/3
Pneumocystis jirovecii	NA[b]	0/3	0/3	0/3	3/3	3/3	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 & Influenza A/B v2 nucleic acid test to assess potential interference with the assay. Five replicates of each substance were tested individually in the presence and absence of a representative strain for each of SARS-CoV-2 (USA-WA1/2020, inactivated), influenza A (Darwin/6/2021) and influenza B (Austria/1359417/2021) viruses. The viral stocks were co-formulated in S-UTM matrix to approximately three times (~3x) the limit of detection (LoD) of the test.

As summarized in **Table 7**, none of the substances at the concentrations tested caused false positive or invalid results in the absence of target organism, except for FluMist, which is expected to be positive for influenza A and influenza B. Furthermore, none of the substances at the concentrations tested caused false negative or invalid results in the presence of target organism, except for one invalid result obtained with Cepacol. The one invalid replicate was retested and obtained positive results for all targets, 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 & Influenza A/B v2 nucleic acid test at the concentrations tested.

Table 7. Summary of Interfering Substances Testing Results

Substance	Concentration Tested	SARS-CoV-2, Influenza A, Influenza B Absent	SARS-CoV-2, Influenza A, Influenza B 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

Substance	Concentration Tested	SARS-CoV-2, Influenza A, Influenza B Absent	SARS-CoV-2, Influenza A, Influenza B Present
		Valid Negative Results/ Total # of Valid Results	Valid Positive Results/ Total # of Valid Results
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[b]	5/5

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

[b] FluMist is expected to give positive (detected) results for influenza A and influenza B targets. 5/5 represents the SARS-CoV-2 target.

Competitive Inhibition

Low concentrations of targets were mixed with a high concentration of the other target and tested with the cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test to assess competitive inhibition between influenza A, influenza B and SARS-CoV-2. The low level concentrations of each target were at approximately 3x LoD, while the high level concentrations were >1.0E+05 $TCID_{50}$/mL (**Table 8**). Co-formulated sample panels with a total of three (3) different combinations of influenza A, influenza B and SARS-CoV-2 at low and high concentration levels were prepared and tested. Five (5) replicates per co-formulated sample panel were tested.

Table 8. Viral Strains and Concentrations tested for Competitive Inhibition

Assay Target	Strain	3x LoD ($TCID_{50}$/mL)	High Concentration ($TCID_{50}$/mL)
SARS-CoV-2	USA WA-1/2020 (inactivated)	0.105	1.14E+05
Influenza A	A/Darwin/6/2021	0.885	1.48E+06
Influenza B	B/Austria/1359417/2021	2.937	9.79E+06

All runs generated five (5) out of five (5) detected results for the SARS-CoV-2, influenza A and influenza B targets under the conditions tested. No competitive inhibition was observed at the concentrations tested. The detection results for three (3) co-formulated sample panels tested with the cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test are summarized in **Table 9**.

Table 9. Competitive Inhibition Results Summary

Panel #	Target Level			n Detected/ N Tested (% Positive)		
	SARS CoV-2	Influenza A	Influenza B	SARS-CoV-2	Influenza A	Influenza B
1	High	3x LoD	3x LoD	5/5 (100%)	5/5 (100%)	5/5 (100%)
2	3x LoD	High	3x LoD	5/5 (100%)	5/5 (100%)	5/5 (100%)
3	3x LoD	3x LoD	High	5/5 (100%)	5/5 (100%)	5/5 (100%)

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 & Influenza A/B 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, influenza A and influenza B at which equal to or greater than 95% of all replicates test positive.

   Two strains for each target (SARS-CoV-2, influenza A and influenza B) were evaluated. To determine the LoD for each target, co-spiked panels were formulated using cultured viral material or 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 10**.

Table 10. LoD determination for SARS-CoV-2, influenza A and influenza B 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)
Influenza A	Darwin/6/2021 (H3N2)	0.295 $TCID_{50}$/mL	21/21 (35.0)
Influenza A	Brisbane/02/2018 (H1N1pdm09)	0.00325 $TCID_{50}$/mL	21/21 (36.4)
Influenza B	B/Austria/1359417/2021	0.979 $TCID_{50}$/mL	20/21 (34.6)
Influenza B	Phuket/3073/2013	0.183 $TCID_{50}$/mL	21/21 (34.1)

* Inactivated virus

A separate study was performed to demonstrate that the LoD of each strain individually was equivalent to the co-spiked LoD.

7. Assay Cut-Off:
A target result call is determined separately for each target channel (i.e., SARS-CoV-2, influenza A, influenza B) and the internal control (IC) channel based on the combination of curve call, Ct, and other validity features. The cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test Ct cutoffs are described in **Table 11**.

Table 11. cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test Ct Cut-offs for Positive/Negative Results Determination

Target	Positive	Negative
SARS-CoV-2	≤ 45	> 45
Influenza A	≤ 45	> 45
Influenza B	≤ 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 a co-formulated panel consisting of a representative strain of cultured influenza A, influenza B and inactivated SARS-CoV-2 (**Table 12**). Two co-formulated levels of 5x LoD and 2x LoD as well as one target negative level were prepared 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.

Table 12. Viral Stains and Concentrations Tested

Strain Name	5x LoD concentration ($TCID_{50}$/mL)	2x LoD concentration ($TCID_{50}$/mL)
A/Darwin/6/2021	1.475	0.590
B/Austria/1359417/2021	4.895	1.958
SARS WA1/2020 (inactivated)	0.175	0.070

As summarized in **Table 13**, all positive contrived samples tested positive for all targets at both testing levels and all target negative replicates tested negative for all targets. Therefore, equivalency has been demonstrated between natural clinical NPS, NS and S-UTM matrices for the cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test.

Table 13. Summary of Matrix Equivalency Study Results

Matrix	Concentration	n Detected/ N Tested, % Positive (Mean Ct)		
		Influenza A	Influenza B	SARS-CoV-2
NPS UTM	2x LoD	30/30, 100% (34.80)	30/30, 100% (33.88)	30/30, 100% (33.51)
	5x LoD	10/10, 100% (33.33)	10/10, 100% (32.77)	10/10, 100% (31.88)
	Negative	0/10, (0%) (N/A)	0/10, 0% (N/A)	0/10, 0% (N/A)
NS UTM	2x LoD	30/30, 100% (34.74)	30/30, 100% (34.12)	30/30, 100% (34.01)
	5x LoD	10/10, 100% (33.23)	10/10, 100% (32.66)	10/10, 100% (32.07)
	Negative	0/10, 0% (N/A)	0/10, 0% (N/A)	0/10, 0% (N/A)
S-UTM	2x LoD	30/30, 100% (34.95)	30/30, 100% (34.17)	30/30, 100% (34.11)
	5x LoD	10/10, 100% (33.63)	10/10, 100% (33.04)	10/10, 100% (32.75)
	Negative	0/10, 0% (N/A)	0/10, 0% (N/A)	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 & Influenza A/B 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 a co-formulated virus panel consisting of representative strains of cultured influenza A, influenza B and inactivated SARS-CoV-2 at levels of 5x LoD and 2x LoD (**Table 12** above). 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 14**, all ten (10) replicates at 5x LoD and all 30 replicates at 2x LoD tested positive for each target in each collection media type. The ten (10) replicates of negative samples were negative for all targets 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 & Influenza A/B v2 nucleic acid test.

Table 14. Summary of Media Equivalency Study Results

Matrix	**Concentration**	**n Detected/ N Tested, % Positive (Mean Ct)**		
		Influenza A	**Influenza B**	**SARS-CoV-2**
UTM	2x LoD	30/30, 100% (34.80)	30/30, 100% (33.88)	30/30, 100% (33.51)
	5x LoD	10/10, 100% (33.33)	10/10, 100% (32.77)	10/10, 100% (31.88)
	Negative	0/10, (0%) (N/A)	0/10, 0% (N/A)	0/10, 0% (N/A)
M4RT	2x LoD	30/30, 100% (34.94)	30/30, 100% (34.51)	30/30, 100% (34.48)
	5x LoD	10/10, 100% (33.56)	10/10, 100% (33.57)	10/10, 100% (33.17)
	Negative	0/10, 0% (N/A)	0/10, 0% (N/A)	0/10, 0% (N/A)
0.9% saline	2x LoD	30/30, 100% (34.83)	30/30, 100% (34.30)	30/30, 100% (34.03)
	5x LoD	10/10, 100% (33.37)	10/10, 100% (32.70)	10/10, 100% (31.81)
	Negative	0/10, 0% (N/A)	0/10, 0% (N/A)	0/10, 0% (N/A)

C Clinical Studies:

1. Clinical Sensitivity:

a. Prospective Study

The clinical performance of the cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test for the detection of SARS-CoV-2, influenza A and influenza B was evaluated using subject matched paired prospective clinical nasopharyngeal swab (NPS) and anterior nasal swab (NS) specimens collected from individuals with 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 liat SARS-CoV-2 & Influenza A/B 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 all four target results from the candidate device was 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, 1,730 prospectively collected paired NPS and NS specimens were collected for the evaluation of cobas liat SARS-CoV-2 & Influenza A/B 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, influenza A and influenza B analyses, 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 and influenza B analyses, 22 were non-evaluable due to missing or invalid candidate test results, and one (1) was non-evaluable to specimen handling issues. Two additional NS specimens obtained inconclusive comparator results for influenza A, leaving 1,704 NS specimens in the influenza A analysis.

Table 15 below provides a summary of the demographic information for subjects included in the performance analysis from the prospective clinical study.

Table 15. Demographics of Subjects from Prospective Population that were Included in the Performance Analysis

Age Group (Years)	Overall N(%) (N=1729)
<1	33 (1.9%)
1 - <18	434 (25.1%)
18 - <30	381 (22.0%)
30 - <40	246 (14.2%)
40 - <50	200 (11.6%)
50 - <60	181 (10.5%)
>=60	254 (14.7%)

A summary of the cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test prospective clinical study performance is provided in **Table 16**.

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 & Influenza A/B v2 nucleic acid test and the comparator method had a positive result for the specific analyte, and false negative (c) indicates that the cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test was negative while the comparator result was positive. 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 & Influenza A/B v2 nucleic acid test and the comparator method had negative results, and false positive (d) indicates that the cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test was positive while the comparator result was negative. Specimens that obtained discordant results underwent additional testing with an FDA cleared SARS-CoV-2/influenza A/B molecular test, when sufficient sample volume remained.

Table 16. Clinical Performance of the cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test in the Prospective Study

Analyte	Specimen Type	Total (N)	Positive Percent Agreement			Negative Percent Agreement		
			a/ (a+c)	%	95% CI	b/ (b+d)	%	95% CI
SARS-CoV-2	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
Influenza A	NPS	1705	54/54	100.0	93.4-100	1640/1651[5]	99.3	98.8-99.6
	NS	1704	53/53	100.0	93.2-100	1640/1651[6]	99.3	98.8-99.6
Influenza B	NPS	1705	22/22	100.0	85.1-100	1671/1683[7]	99.3	98.8-99.6
	NS	1706	24/24	100.0	86.2-100	1673/1682[8]	99.5	99.0-99.7

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 & Influenza A/B v2 nucleic acid test and the comparator tests are positive; b = number of samples where the cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test is positive and the comparator is negative; c = number of samples where the cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test is negative and the comparator is positive; d = number of samples where both the cobas liat SARS-CoV-2 & Influenza A/B 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/influenza A/B 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/influenza A/B 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/influenza A/B 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/influenza A/B molecular test.

[5] Of 11 NPS specimens positive for influenza A on cobas liat and negative on the comparator, two (2) were positive for influenza A and nine (9) were negative by an FDA cleared SARS-CoV-2/influenza A/B molecular test.

[6] Of 11 NS specimens positive influenza A on cobas liat and negative on the comparator, one (1) was positive for influenza A and ten (10) were negative by an FDA cleared SARS-CoV-2/influenza A/B molecular test.

[7] Of 12 NPS specimens positive influenza B on cobas liat and negative on the comparator, all 12 were negative by an FDA cleared SARS-CoV-2/influenza A/B molecular test.

[8] Of nine (9) NS specimens positive for influenza B on cobas liat and negative on the comparator, all nine (9) were negative by an FDA cleared SARS-CoV-2/influenza A/B molecular test.

Number of samples with positive results for more than one target observed in the prospective cohort as detected by the cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test and comparator method are listed in **Table 17**.

Table 17. Multiple Detection Combination by cobas liat SARS-CoV-2 & Influenza A/B v2 in Prospective Study

Distinct Multiple Detection Combinations		Specimen Type	Total Multiple Detection on cobas liat	Target(s) detected by comparator method (number of samples)
Target 1	Target 2			
Influenza A	Influenza B	NS	2	Negative (2)
Influenza A	Influenza B	NPS	4	Influenza A (3), Negative (1)
Influenza A	SARS-CoV-2	NS	5	Influenza A and SARS-CoV-2 (2), Influenza A (2), SARS-CoV-2 (1)
Influenza A	SARS-CoV-2	NPS	7	Influenza A and SARS-CoV-2 (3), SARS-CoV-2 (2), Negative (2)
Influenza B	SARS-CoV2	NS	3	Influenza B and SARS-CoV-2 (1), Influenza B (2)

b. Retrospective Study (Category III Specimens)

Influenza B was of lower prevalence and was not encountered in sufficiently large numbers during the prospective clinical study to adequately demonstrate assay performance. To supplement the results of the prospective clinical study, retrospective frozen clinical NPS and NS specimens collected in Universal Viral Transport medium (UVT) or Universal Transport Medium (UTM) from individuals with signs and symptoms of respiratory viral infection were tested. Frozen archived (Category III) influenza B positive and negative NPS (n=223) and NS (n=206) specimens obtained between 2019 and 2023 were distributed to 6 sites and tested in the course of the daily workflow. One (1) NPS sample pre-characterized as positive for influenza B was non-evaluable due to missing/invalid results on the comparator method. The comparator method was an acceptable FDA-cleared molecular assay. Available demographic data regarding the individuals from the retrospective study are shown in **Table 18**.

Table 18. Demographics of Subjects from Retrospective Population

Age Group (Years)	Overall N(%) (N=429)
<1	0 (0.00%)
1 - <18	129 (30.07%)
18 - <30	77 (17.95%)
30 - <40	79 (18.41%)
40 - <50	28 (6.53%)
50 - <60	14 (3.26%)

Age Group (Years)	Overall N(%) (N=429)
>=60	23 (5.36%)
Not reported	79 (18.41%)

A summary of the cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test agreement for influenza B with the comparator method is provided in **Table 19**.

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 & Influenza A/B v2 nucleic acid test and the comparator method had a positive result for influenza B, and false negative (c) indicates that the cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test was negative while the comparator result was positive for influenza B. 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 & Influenza A/B v2 nucleic acid test and the comparator method had negative influenza B results, and false positive (d) indicates that the cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test was positive for influenza B 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 19. Agreement of cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test and Comparator test for influenza B in Retrospective Samples

Analyte	Specimen Type	Total (N)	Positive Percent Agreement			Negative Percent Agreement		
			a/ (a+c)	%	95% CI	b/ (b+d)	%	95% CI
Influenza B	NPS	222	34/34	100.0	89.8-100	184/188[1]	97.9	94.7-99.2
	NS	206	34/34	100.0	89.8-100	169/172[2]	98.3	95.0-99.4

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 & Influenza A/B v2 nucleic acid test and the comparator tests are positive; b = number of samples where the cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test is positive and the comparator is negative; c = number of samples where the cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test is negative and the comparator is positive; d = number of samples where both the cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test and the comparator tests are negative.
[1] Of four (4) NPS specimens positive for influenza B on cobas liat and negative on the comparator, all four (4) were negative by an FDA cleared molecular test.
[2] Of three (3) NS specimens positive for influenza B on cobas liat and negative on the comparator, one (1) was positive for influenza B and 2 were negative by an FDA cleared 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 & Influenza A/B v2 nucleic acid test prospective clinical study included a total of 1,729 prospectively collected NPS and NS specimens, of which 1,706 NS specimens and 1,705 NPS specimens were evaluable. The number and percentage of cases positive for SARS-CoV-2, influenza A and influenza B, as determined by the cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test, are presented in **Table 20**, stratified by collection site.

Table 20. cobas liat SARS-CoV-2 & Influenza A/B v2 nucleic acid test – Expected Values by Specimen Collection Site for NPS and NS Specimens in the Prospective Study

Site	% Positivity (n Positive/N Total Samples)					
	SARS-CoV-2		Influenza A		Influenza B	
	NPS	NS	NPS	NS	NPS	NS
Site 1	6.0% (4/67)	7.5% (5/67)	4.5% (3/67)	4.5% (3/67)	0.0% (0/67)	0.0% (0/67)
Site 2	12.2% (5/41)	12.2% (5/41)	2.4% (1/41)	0.0% (0/41)	2.4% (1/41)	2.4% (1/41)
Site 3	6.1% (2/33)	3.0% (1/33)	0.0% (0/33)	0.0% (0/33)	0.0% (0/33)	0.0% (0/33)
Site 4	0.0% (0/42)	0.0% (0/42)	7.1% (3/42)	9.5% (4/42)	0.0% (0/42)	0.0% (0/42)
Site 5	17.5% (54/308)	21.5% (66/307)	0.3% (1/308)	0.0% (0/307)	1.0% (3/308)	0.7% (2/307)
Site 6	19.9% (34/171)	20.2% (35/173)	9.9% (17/171)	10.4% (18/173)	0.0% (0/171)	0.0% (0/173)
Site 7	7.1% (13/184)	8.1% (15/185)	3.3% (6/184)	3.8% (7/185)	8.7% (16/184)	8.1% (15/185)
Site 8	3.0% (2/66)	3.0% (2/66)	1.5% (1/66)	0.0% (0/66)	1.5% (1/66)	1.5% (1/66)
Site 9	26.1% (42/161)	26.1% (42/161)	3.1% (5/161)	3.1% (5/161)	2.5% (4/161)	2.5% (4/161)
Site 10	17.3% (29/168)	16.7% (28/168)	0.6% (1/168)	0.6% (1/168)	0.0% (0/168)	0.0% (0/168)
Site 11	14.5% (29/200)	12.9% (26/201)	1.5% (3/200)	1.0% (2/201)	0.0% (0/200)	0.0% (0/201)
Site 12	3.9% (6/154)	3.9% (6/152)	11.0% (17/154)	11.8% (18/152)	1.9% (3/154)	2.6% (4/152)
Site 13	14.3% (8/56)	12.5% (7/56)	1.8% (1/56)	0.0% (0/56)	10.7% (6/56)	10.7% (6/56)
Site 14	25.9% (14/54)	22.2% (12/54)	11.1% (6/54)	13.0% (7/54)	0.0% (0/54)	0.0% (0/54)
All	14.2% (242/1705)	14.7% (250/1706)	3.8% (65/1705)	3.8% (65/1706)	2.0% (34/1705)	1.9% (33/1706)

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.