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

I Background Information:

A 510(k) Number

K243455

B Applicant

Roche Molecular Systems, Inc.

C Proprietary and Established Names

cobas Respiratory 4-flex for use on the cobas 5800/6800/8800 Systems

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 Respiratory 4-flex is substantially equivalent to the BioFire Respiratory Panel 2.1 (RP2.1) (DEN200031) and to obtain clearance for the cobas Respiratory 4-flex.

B Measurand:

The cobas Respiratory 4-flex detects and identifies nucleic acids from influenza A, influenza B, respiratory syncytial virus (RSV), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

C Type of Test:
A multiplexed, reverse transcription polymerase chain reaction (RT-PCR) test, which utilizes temperature assisted generation of signal (TAGS) technology, intended for use with the automated cobas 5800/6800/8800 Systems for the qualitative *in vitro* detection and identification of influenza A, influenza B, RSV, and SARS-CoV-2 nucleic acids in nasopharyngeal swab (NPS) specimens obtained from individuals with signs and symptoms of respiratory tract infections.

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B Indication(s) for Use:
The cobas Respiratory 4-flex for use on the cobas 5800/6800/8800 Systems is an automated, multiplex, nucleic acid test that utilizes real-time polymerase chain reaction (PCR) technology intended for simultaneous in vitro qualitative detection and differentiation of severe acute respiratory syndrome coronavirus (SARS-CoV-2), influenza A virus, influenza B virus, and respiratory syncytial virus (RSV) in nasopharyngeal swab specimens obtained from individuals with signs and symptoms of respiratory tract infection. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2, influenza A, influenza B and RSV can be similar. This test is intended to aid in the differential diagnosis of SARS-CoV-2, influenza A, influenza 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 nasopharyngeal 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. Negative results do not preclude SARS-CoV-2, influenza A virus, influenza B virus, or RSV infections. Conversely, positive results do not rule out coinfection with other organisms, and the agent(s) detected by the cobas Respiratory 4-flex for use on the cobas 5800/6800/8800 Systems may not be the definite cause of disease.

Special Conditions for Use Statement(s):
Rx - For Prescription Use Only
For in vitro diagnostic Use Only

C Special Instrument Requirements:
For use with the cobas 5800/6800/8800 Systems, only.

IV Device/System Characteristics:

A Device Description:

The cobas Respiratory 4-flex for use on the cobas 5800/6800/8800 Systems (cobas Respiratory 4-flex) is based on fully automated sample preparation (nucleic acid extraction and purification) followed by reverse transcription, PCR amplification, and detection. The cobas Respiratory 4-flex is for use with the fully automated cobas 5800 System, cobas 6800 System, or cobas 8800 System. The cobas 5800 System is designed as one integrated instrument. The cobas 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytical module.

The cobas Respiratory 4-flex has four (4) different reportable targets: influenza A, influenza B, RSV, and SARS-CoV-2. Reporting of these targets is based on detection of one or more of the nucleic acid targets.

Prior to initiating a test on the cobas 5800/6800/8800 Systems, NPS specimens are processed using the cobas omni Secondary Tube. To process specimens, first 0.8 mL of cobas Microbial Inactivation Solution (MIS) is added to the secondary tube. The user then transfers 0.4 mL of NPS specimen (in UTM/UVT) to the secondary tube. Next, the specimen is added to the cobas System and the specimen type "Diluted in cobas MIS" is selected to run the cobas Respiratory 4-flex.

Flexible Result Reporting

To provide flexible reporting strategies, the cobas Respiratory 4-flex has several ordering/result reporting options, which are described independently for each cobas System below.

Cobas 5800

Configuring Target Viral Groups. Each NPS specimen can be tested for any combination of viral targets (i.e., influenza A, influenza B, RSV, and SARS-CoV-2).

Digital Reflex Result Reporting Option. Based on the initial test results, additional targets can be ordered in a predefined timeframe. Results for the additional ordered targets will then be calculated and reported based on the already measured raw data. The sample is not re-processed to obtain additional test results.

Cobas 6800/8800

Configuring Target Viral Groups. Each NPS specimen can be tested for all four panel targets or a predefined subgroup of panel targets using one of the three available Assay Specific Analysis Packages (ASAPs). Reflex testing options are not available when running the cobas Respiratory 4-flex on the cobas 6800/8800 Systems with software version 1.4.

The available ASAPs for these systems include:

- RESP-4FLEX ASAP: Result calculation of all four targets influenza A, influenza B, RSV and SARS-CoV-2.
- RESP-4FLEX-CAB ASAP: Result calculation for targets influenza A, influenza B and SARS-CoV-2.

- RESP-FLEX-FLUR ASAP: Result calculation for targets influenza A, influenza B and RSV.

Regardless of the result reporting strategy implemented, automated data management is performed by the cobas 5800 System and cobas 6800/8800 System software, which assigns results for all tests. Results can be reviewed directly on the System screen and printed as a report.

Interpretation of Results for cobas 5800 and cobas 6800/8800 Systems

To report a patient NPS specimen result, all external positive control targets must be positive and all external negative control targets must be negative. When the external controls yield the expected results, patient specimen results are reported for each of the four (4) assay targets. For each target, a negative, positive, or invalid result is reported according to the interpretative criteria outlined in **Table 1**. A patient specimen can have one more target combinations reported as invalid if at least one target is positive, *and* the RNA IC is not detected.

Table 1. Individual Target Result Interpretation for Patient Specimens

Target Result*	Interpretation
Negative	No target signal detected for the corresponding viral target and RNA IC signal detected.
Positive	Target signal detected for the corresponding viral target and the RNA IC signal may or may not be detected.
Invalid	No target signal detected for the corresponding viral target and the RNA IC signal is not detected. If clinically indicated for the corresponding viral target, repeat assay with same sample. If the result is still invalid, a new specimen should be obtained.

*Shown for each of the four (4) viral targets (influenza A, influenza B, RSV, SARS-CoV-2) individually.

B Principle of Operation:

cobas Respiratory 4-flex is based on a fully automated sample preparation process (nucleic acid extraction and purification) followed by reverse transcription, PCR amplification, and detection. Nucleic acid from patient NPS specimens and added Internal Control RNA (RNA-IC) molecules are simultaneously extracted. Nucleic acid is released by addition of proteinase and lysis reagent to the specimen. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris, and potential PCR inhibitors, are removed with subsequent wash steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature. External controls (positive and negative) are processed in the same way.

Selective amplification of target nucleic acid from the specimen is achieved using target-specific forward and reverse primers detecting conserved viral genome regions, as shown in **Table 2**.

Table 2. cobas Respiratory 4-flex Target Regions

Targeted Organism	Targeted Gene (Symbol)
Influenza A	Matrix protein 1 (M1)
Influenza B	Non-structural protein NS-1/2 (NS1/NEP)
RSV	Matrix protein (M)
SARS-CoV-2	ORF1 ab polyprotein (ORF1ab) and ORF 1a polyprotein (ORF1a)

Selective amplification of RNA-IC is achieved using non-competitive, sequence specific forward and reverse primers, which have no homology with the viral-target specific genomes. Amplified target is detected by the cleavage of fluorescently labeled oligonucleotide probes. Temperature assisted generation of signal (TAGS) technology is introduced to differentiate up to three targets per fluorescence channel and the RNA-IC, in each well. A thermostable DNA polymerase enzyme is used for amplification.

Multiplicity of target detection is enabled with temperature-dependent quenching of cleaved fluorescent target-specific probes. This is achieved by separating signals from probes into introduced thermal channels, where fluorescence is acquired at two additional fixed temperatures for each amplification cycle. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5' to 3' exonuclease activity of the DNA polymerase, resulting in separation of the reporter and quencher dyes, and the generation of a fluorescent signal. Conventional probes release fluorescence signal immediately upon separation of reported from quencher. TAGS probes rely on temperature dependent fluorescence activation, requiring both nuclease cleavage during the extension phase, as well as an increase in reaction temperature, to activate the otherwise dormant fluorophore. For this reason, during each PCR cycle the test captures fluorescence in five available fluorescence channels in combination with three thermal channels (detection of fluorescence at three defined temperatures: T1, T2, and T3), which enables simultaneous detection and differentiation of the amplified viral targets and the RNA-IC.

The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythymidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA amplicon. Any contaminating amplicons from previous PCR runs are destroyed by the AmpErase enzyme (uracil-N-glycosylase), which is included in the PCR mix, when heated in the first thermal cycling step. However, newly formed amplicons are not destroyed since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

C Instrument Description Information:

1. Instrument Name:
   cobas 5800 System with software 1.0.5 or higher using cobas 5800 RESP-4FLEX ASAP v1.1.0, and cobas 6800/8800 Systems with software 1.4.7 or higher using cobas RESP-4FLEX, RESP-4FLEX-CAB and RESP-4FLEX-FLUR ASAP v12.1.0.

2. Specimen Identification:
   Specimen identification information can be configured in an automated fashion or manually entered.

3. Specimen Sampling and Handling:
   Nasopharyngeal swab (NPS) specimens collected in Copan UTM or BD UVT.

4. Calibration:
   The user does not perform calibration of the cobas 5800/6800/8800 Systems.

5. Quality Control:
   The RNA Internal Control (RNA-IC) consists of MS2 bacteriophage harboring a non-target RNA sequence. The RNA-IC, which is introduced into each NPS specimen during sample

processing, is used to monitor the entire sample preparation and reverse transcription/PCR amplification process. In addition, the test utilizes external controls – cobas Respiratory flex Positive Control (low titer positive control consisting of non-infectious plasmid DNA) and cobas Buffer Negative Control, provided separately.

V Substantial Equivalence Information:

A Predicate Device Name(s):
BioFire Respiratory Panel 2.1 (RP2.1)

B Predicate 510(k) Number(s):
DEN200031

C Comparison with Predicate(s):

Device & Predicate Device(s):	K243455	DEN200031
Device Trade Name	cobas Respiratory 4-flex for use on the cobas 5800/6800/8800 Systems	BioFire Respiratory Panel 2.1 (RP2.1)
Similarities		
Regulation Number	Same	21 CFR 866.3981
Regulation Name	Same	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.
Primary Product Code	Same	QOF
Intended Use/Indications For Use	The cobas Respiratory 4-flex for use on the cobas 5800/6800/8800 Systems is an automated, multiplex, nucleic acid test that utilizes real-time polymerase chain reaction (PCR) technology intended for simultaneous in vitro qualitative detection and differentiation of severe acute respiratory syndrome coronavirus (SARS-CoV-2), influenza A virus, influenza B virus, and respiratory syncytial virus (RSV) in nasopharyngeal swab specimens obtained from individuals with signs and symptoms of respiratory tract infection. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2, influenza A, influenza B and RSV can be similar. This test is intended to aid in the differential diagnosis of SARS-CoV-2, influenza A, influenza B, and RSV infections in humans and is not	The BioFire Respiratory Panel 2.1 (RP2.1) is a PCR-based multiplexed nucleic acid test intended for use with the BioFire FilmArray 2.0 or BioFire FilmArray Torch systems for the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections, including COVID-19. The following organism types and subtypes are identified using the BioFire RP2.1: Adenovirus, Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43,

	intended to detect influenza C virus infections. Nucleic acids from the viral organisms identified by this test are generally detectable in nasopharyngeal 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. Negative results do not preclude SARS-CoV-2, influenza A virus, influenza B virus, or RSV infections. Conversely, positive results do not rule out coinfection with other organisms, and the agent(s) detected by the cobas Respiratory 4-flex for use on the cobas 5800/6800/8800 Systems may not be the definite cause of disease.	Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2), Human Metapneumovirus, Human Rhinovirus/Enterovirus, Influenza A, including subtypes H1, H1-2009, and H3, Influenza B, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, Respiratory Syncytial Virus, *Bordetella parapertussis* (IS1001), *Bordetella pertussis* (ptxP), *Chlamydia pneumoniae*, and *Mycoplasma pneumonia* Nucleic acids from the respiratory viral and bacterial organisms identified by this test are generally detectable in NPS specimens during the acute phase of infection. The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and/or symptoms of respiratory infection is indicative of the presence of the identified microorganism and aids in the diagnosis of respiratory infection if used in conjunction with other clinical and epidemiological information. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test, or lower respiratory tract infection that may not be detected by an NPS specimen. Positive results do not rule out coinfection with other organisms. The agent(s) detected by the BioFire RP2.1 may not be the definite cause of disease. Additional laboratory testing (e.g. bacterial and viral culture, immunofluorescence, and radiography) may be necessary when

		evaluating a patient with possible respiratory tract infection.
Differences		
Analyte Targets	Influenza A, Influenza B, Respiratory Syncytial Virus, and Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2)	Adenovirus, Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43, Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2), Human Metapneumovirus, Human Rhinovirus/Enterovirus, Influenza A, including subtypes H1, H1-2009, and H3, Influenza B, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, Respiratory Syncytial Virus, *Bordetella parapertussis* (IS1001), *Bordetella pertussis* (ptxP), *Chlamydia pneumoniae*, and *Mycoplasma pneumonia*
Sample Preparation Procedure	Automated by cobas 5800/6800/8800 Systems	Automated by BioFire FilmArray 2.0 or BioFire FilmArray Torch Systems
Detection Chemistry	PCR amplification and detection, consisting of TaqMan probes with fluorescent dyes. Multiplicity of target detection is enabled with temperature-dependent quenching of cleaved fluorescent target-specific probes.	Two Step Nested multiplex PCR: • Reverse transcription, followed by a multiplexed first stage PCR reaction (PCR1). • Multiple simultaneous second-stage PCR reactions (PCR2) to amplify sequences within the PCR1 products using fluorescence double stranded binding dye. Endpoint melting curve data to detect target-specific amplicons.
Controls	Sample processing internal control (IC) and external positive and external negative controls.	Two process controls: • RNA Process Control (IC). • PCR2 Control (A positive result indicates that PCR2 was successful).
Result Analysis	Based on PCR cycle threshold analysis.	Endpoint melting curve data to detect target specific amplicons.

VI Standards/Guidance Documents Referenced:

Standards

- CLSI EP17-A2. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures.
- CLSI EP07-A3. Interference Testing in Clinical Chemistry.
- ISO 14971:2019. Medical devices – Application of risk management to medical devices.
- IEC 62366-1:2015+AMD1:2020 CSV. Medical devices – Application of usability engineering to medical devices.
- IEC 62304:2006. Medical device software – Software life-cycle processes
- ISO 15223-1:2021. Medical devices – Symbols to be used with information to be supplied by the manufacturer – Part 1: General requirements.
- ISO 13485:2016. Medical devices – Quality management t systems – Requirements for regulatory purposes.
- ISO 20916:2019. In vitro diagnostic medical devices – Clinical performance studies using specimens from human subjects – Good study practice.

Special Controls

- Class II Special Controls as per 21 CFR 866.3981

Guidance Documents

- Respiratory Viral Panel Multiplex Nucleic Acid Assay – Class II Special Controls Guidance for Industry and FDA Staff (October 2009).
- Instrumentation for Clinical Multiplex Test Systems – Class II Special Controls Guidance for Industry and FDA Staff (March 2005).
- Cybersecurity in Medical Devices: Quality System Considerations and Content of Premarket Submissions Guidance for Industry and Food and Drug Administration Staff (September 2023).
- Off-The-Shelf Software Use in Medical Devices Guidance for Industry and Food and Drug Administration Staff (August 2023).
- Content of Premarket Submissions for Device Software Functions Guidance for Industry and Food and Drug Administration Staff (June 2023).

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

   a. Within-Laboratory Precision

   Within-laboratory precision was evaluated at a single site using the cobas Respiratory 4-flex run on the cobas 5800 System and cobas 6800/8800 System. Contrived panels containing known quantities of representative target analytes were prepared in simulated NPS matrix. The cultured influenza A, influenza B, RSV and inactivated SARS-CoV-2 material that was used to generate the positive panel members are denoted in **Table 3**. The contrived positive panels were co-formulated with target analytes at a low positive

concentration (~1x LoD) and moderate concentration (~3x LoD). A negative panel was also included in the study. The study was conducted by five (5) operators using three test cassette lots over the course of 12 non-consecutive days, on three cobas 5800 Systems and three cobas 6800/8800 Systems. Each panel member was tested in triplicate twice per day over 12 different days. In total, 216 replicates were collected per panel member.

Table 3. Viral Strains Used to Evaluate Within-Laboratory Precision

Target Channel	Organism	Viral Strain	Vendor: Catalog No.
RSV	RSV A	Respiratory Syncytial Virus A2	Microbiologics: Lot: D2126D
Influenza A	Influenza A (H3N2)	A/Darwin/6/2021	Microbiologics: SD-DRW0621A
SARS-CoV-2	SARS-CoV-2[1]	England/02/2020	NIBSC[2]: 20/146
Influenza B	Influenza B (Victoria lineage)	B/Austria/1359417/2021	Microbiologics: SD-AT1721A

[1]Inactivated virus
[2] NIBSC - National Institute for Biological Standards and Control

The qualitative results (i.e., % agreement with expected results) from the study are illustrated in **Table 4**.

Table 4. Within-Laboratory Precision Study – Qualitative Results

Target	Level	Positive Results	Total Results	Positivity %	Two-sided 95% CI Lower Bound	Two-sided 95% CI Upper Bound
Influenza A (H3N2)	~3x LoD	216	216	100	98.31	100
	~1x LoD	216	216	100	98.31	100
Influenza B (Victoria)	~3x LoD	216	216	100	98.31	100
	~1x LoD	215	216	99.54	97.45	99.99
RSV A	~3x LoD	216	216	100	98.31	100
	~1x LoD	214	216	99.07	96.70	99.89
SARS-CoV-2	~3x LoD	216	216	100	98.31	100
	~1x LoD	216	216	100	98.31	100
N/A	Blank	0	216	0	0.00	3.36

All low positive panel members exhibited a detection rate >99% and all moderate positive panel members exhibited a detection rate of 100%. The negative panel was negative 100% of the time. The results of the study demonstrate acceptable assay variability.

The means, standard deviations, and coefficients of variation (%) for cycle threshold (Ct) values by target analyte and expected concentration (Positive Panel Members) are shown in **Table 5**.

Table 5. Precision - standard deviations and coefficients of variation of Ct values

Target	Level	Hit rate %	Mean Ct	Instrument-to-Instrument		Lot-to-Lot		Day-to-Day		Run-to-Run		Within Run		Total	
				SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
Influenza A (H3N2)	~3x LoD	100	37.33	0.08	0.22	0.08	0.21	0.00	0.00	0.07	0.20	0.48	1.28	0.50	1.33
	~1x LoD	100	39.06	0.13	0.34	0.14	0.35	0.23	0.59	0.00	0.00	1.02	2.60	1.06	2.71
Influenza B (Victoria)	~3x LoD	100	34.61	0.04	0.11	0.09	0.26	0.00	0.00	0.00	0.00	0.22	0.64	0.24	0.69
	~1x LoD	99.54	35.34	0.04	0.12	0.08	0.23	0.00	0.00	0.00	0.00	0.24	0.69	0.26	0.73
RSV A	~3x LoD	100	33.20	0.06	0.18	0.08	0.25	0.04	0.11	0.00	0.00	0.19	0.58	0.22	0.66
	~1x LoD	99.07	33.62	0.04	0.11	0.05	0.16	0.02	0.06	0.02	0.06	0.24	0.70	0.25	0.73
SARS-CoV-2	~3x LoD	100	35.62	0.03	0.09	0.00	0.00	0.03	009	0.00	0.00	0.32	0.89	0.32	0.90
	~1x LoD	100	36.48	0.00	0.00	0.00	0.00	0.03	0.09	0.00	0.00	0.41	1.13	0.42	1.14

Note: SD= standard deviation, CV= coefficient of variation, Ct= cycle threshold. LoD limit of detection

b. Reproducibility

A reproducibility study was conducted at three testing sites (one internal) using the cobas Respiratory 4-flex run on the cobas 5800 System and cobas 6800/8800 System. The study incorporated potential sources of variation introduced by site (three (3) testing sites), day (six (6) different days), operator (nine (9) operators), test cassette lot (three (3) total, >2 per site), instrument (five (5) cobas 6800/8800 Systems and four (4) cobas 5800 Systems, with at least one (1) cobas 5800 or cobas 6800/8800 System per site).

The same contrived co-formulated panels used to evaluate precision (see **Table 3**) were included in the reproducibility study. Each panel member was tested in triplicate, twice per day. In total, 504 replicates were collected per panel member (72 at site 1 and 216 at both sites 2 and 3). The qualitative results of the study are illustrated in **Table 6**.

Table 6. Reproducibility Study- Qualitative Results

Target Channel	Level	% Agreement with Expected Results (n Detected/ N Tested)			
		Site 1	Site 2	Site 3	Overall (95% CI)
NA	Negative	100% (72/72)	99.1% (214/216)	100% (216/216)	99.6% (502/504) (98.6-99.9%)
Influenza B		100% (72/72)	99.1% (214/216)	99.5% (215/216)	99.4% (501/504) (98.3-100%)

Target Channel	Level	% Agreement with Expected Results (n Detected/ N Tested)			
		Site 1	Site 2	Site 3	Overall (95% CI)
RSV	~1x LoD	100% (72/72)	99.5% (215/216)	99.1% (214/216)	99.4% (501/504) (98.3-99.8%)
Influenza A		100% (72/72)	100% (216/216)	100% (216/216)	100% (504/504) (99.3-100%)
SARS-CoV-2		100% (72/72)	100% (216/216)	100% (216/216)	100% (504/504) (99.3-100%)
Influenza B	~3x LoD	100% (72/72)	100% (215/215)	100% (216/216)	100% (503/503)[a] (99.3-100%)
RSV		100% (72/72)	100% (216/216)	100% (216/216)	100% (504/504) (99.3-100%)
Influenza A		100% (72/72)	100% (216/216)	100% (216/216)	100% (504/504) (99.3-100%)
SARS-CoV-2		100% (72/72)	100% (216/216)	100% (216/216)	100% (504/504) (99.3-100%)

[a]At site 2, a single invalid result was obtained for influenza B, resulting in 503 samples with valid results.

The cobas Respiratory 4-flex results showed acceptable lot-to-lot, instrument-to-instrument (site), day-to-day, and between run variation for the ~1x LoD, and ~~3x LoD panel members (**Table 7**). Additionally, the cobas 5800 and cobas 6800/8800 Systems demonstrated equivalent reproducibility.

Table 7. Reproducibility - Standard Deviations and Coefficients of Variation of Ct Values

Target Virus	Level	(n/N)[a]	Mean Ct	Total		Site		Lot		Day		Run		Within Run	
				SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
Negative	0	502/504	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc
Influenza A	~1× LoD	504/504	39.1	1.03	2.63	0.00	0.00	0.19	0.47	0.00	0.00	0.21	0.54	0.99	2.52
	~3× LoD	504/504	37.4	0.50	1.34	0.04	0.10	0.00	0.00	0.12	0.33	0.00	0.00	0.49	1.30
Influenza B	~1× LoD	501/504	35.4	0.28	0.79	0.08	0.22	0.07	0.19	0.03	0.09	0.00	0.00	0.26	0.72
	~3× LoD	503/503[b]	34.7	0.25	0.72	0.11	0.30	0.04	0.12	0.04	0.11	0.05	0.13	0.21	0.62
RSV	~1× LoD	501/504	33.8	0.31	0.91	0.12	0.36	0.11	0.34	0.05	0.15	0.07	0.22	0.24	0.72
	~3× LoD	504/504	33.4	0.29	0.88	0.15	0.44	0.13	0.38	0.06	0.19	0.00	0.00	0.21	0.64
SARS-CoV-2	~1× LoD	504/504	36.4	0.41	1.12	0.04	0.11	0.02	0.06	0.00	0.00	0.00	0.00	0.41	1.11
	~3× LoD	504/504	35.6	0.31	0.88	0.01	0.03	0.00	0.00	0.00	0.00	0.07	0.20	0.30	0.85

Ct = cycle threshold, LoD = limit of detection, SD = standard deviation, CV (%) = percent coefficient of variation, nc = not calculable, RSV= respiratory syncytial virus, SARS-CoV-2= severe acute respiratory syndrome coronavirus 2.
[a] n is the number of tests which matched the expected results. N is the total number of valid tests for the panel member.
[b] A single invalid result was obtained for influenza B, resulting in 503 samples with valid results.

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

3. Analytical Specificity/Interference:

Analytical Reactivity (Inclusivity)
The inclusivity of the cobas Respiratory 4-flex was evaluated using a combination of *in silico* analysis of publicly available sequence information and laboratory testing of contrived specimens containing viral isolates that were selected to represent phylogenetic, geographic, and temporal diversity.

a. Wet-Testing
This study was performed to determine the analytical reactivity of the cobas Respiratory 4-flex with clinically relevant strains, serotypes, or subtypes of the target species. The inclusivity panel was prepared by spiking various target microorganism strains/serotypes/subtypes encompassing temporal and geographical diversity into simulated NPS matrix at a concentration of ~3x LoD and were tested in triplicate. Strains that did not yield 100% reactivity at ~3x LoD were prepared at higher concentrations and tested in triplicate until 100% reactivity was observed. The strains evaluated and the results of inclusivity testing are shown in **Tables 8-11**, below.

Table 8. Strains of Influenza A Evaluated for Inclusivity

Subtype	Strain/Isolate	Source	Catalogue No.	Concentration		% Detected (# Detected/#Tested)
				Cp/mL	xLoD	
H1N1	A/New Caledonia/20/99	Zeptometrix	0810036CF	165	~3x	100% (3/3)
	A/Brisbane/59/07	Zeptometrix	0810244CF	165	~3x	100% (3/3)
H1N1 pdm09	A/California/07/09	Zeptometrix	0810165CF	165	~3x	100% (3/3)
	A/NY/03/09	Zeptometrix	0810249CF	165	~3x	100% (3/3)
	A/Victoria/2570/2019	Microbiologics	SD-VIC219A-7	165	~3x	100% (3/3)
	A/Wisconsin/588/2019	Microbiologics	SD-WA519A-8	165	~3x	100% (3/3)
	A/Victoria/4897/2022	Microbiologics	SD-VIC9722B	165	~3x	100% (3/3)
	A/Wisconsin/67/2022	Microbiologics	SD-WI6722MS 1B	330	~6x	100% (3/3)
	A/England/73/2022	UHSA	N/A	357	~6.8x	100% (3/3)
	A/England/55/2022	UHSA	N/A	357	~6.8x	100% (3/3)
H3	A/Port Chalmers/1/73	ATCC	VR-810	165	~3x	100% (3/3)
	A/Texas/50/12	Zeptometrix	0810238CF	242.7	~4.4x	100% (3/3)
	A/Victoria/3/75	ATCC	VR-822	165	~3x	100% (3/3)
	A/Wisconsin/67/05	Zeptometrix	810252CF	165	~3x	100% (3/3)
	A/Darwin/9/2021	Microbiologics	SD-DRW921-6	165	~3x	100% (3/3)
	A/Hong Kong/8/68	Zeptometrix	0810250CF	165	~3x	100% (3/3)
	A/Perth/16/09	Zeptometrix	0810251CF	165	~3x	100% (3/3)
	A/Hong Kong/4801/14	Zeptometrix	0810526CF	165	~3x	100% (3/3)
	A/Kansas/14/17	Zeptometrix	0810586CF	165	~3x	100% (3/3)
	A/Switzerland/9715293/13	Zeptometrix	0810511CF	165	~3x	100% (3/3)

Subtype	Strain/Isolate	Source	Catalogue No.	Concentration		% Detected (# Detected/#Tested)
				Cp/mL	xLoD	
H5	A/mallard/Wisconsin/2576/2009 (H5N1)	BEI	NR-31131	165	~3x	100% (3/3)
	A/duck/Singapore/645/1997 (H5N3)	BEI	NR-3558	165	~3x	100% (3/3)
	A/ruddy turnstone/New Jersey/828212/2001 (N5N2)	BEI	NR-44298	165	~3x	100% (3/3)
H7	A/northern pintail/Illinois/10OS3959/2010 (H7N3)	BEI	NR-35979	165	~3x	100% (3/3)
	H7N9, A/northern shoveler/Mississippi/11OS145/2011	BEI	NR-36001	165	~3x	100% (3/3)
	H7N8, A/mallard/Ohio/11 OS2033/2011	BEI	NR-36008	165	~3x	100% (3/3)
H9	H9N7, A/shorebird/Delaware Bay/31/1996	BEI	NR-45171	165	~3x	100% (3/3)

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Table 9. Strains of Influenza B Evaluated for Inclusivity

Lineage	Strain/Isolate	Source	Catalogue No.	Concentration		% Detected (# Detected/#Tested)
				Cp/mL	xLoD	
Victoria	B/Colorado/6/17	Zeptometrix	0810573CF	1779	~3x	100% (3/3)
	B/Hong Kong/5/72	ATCC	VR-823	1779	~3x	100% (3/3)
	B/Brisbane/60/08	Zeptometrix	0810254CF	1779	~3x	100% (3/3)
	B/Florida/02/06	Zeptometrix	0810037CF	1779	~3x	100% (3/3)
Yamagata	B/Florida/07/04	Zeptometrix	0810256CF	1779	~3x	100% (3/3)
	B/Massachusetts/2/2012	ATCC	VR-1813	1779	~3x	100% (3/3)
	B/Wisconsin/1/2010	ATCC	VR-1883	1779	~3x	100% (3/3)
	B/Florida/4/2006	ATCC	VR-1804	1779	~3x	100% (3/3)
	B/Texas/6/11	Zeptometrix	0810242CF	1779	~3x	100% (3/3)
Unknown	B/Allen/45	ATCC	VR-102	1779	~3x	100% (3/3)
	B/Lee/40	ATCC	VR-101	1779	~3x	100% (3/3)
	B/Taiwan/2/62	ATCC	VR-295	1779	~3x	100% (3/3)

Table 10. Strains of RSV Evaluated for Inclusivity

Subtype	Strain/ Isolate	Source	Catalogue No.	Concentration		% Detected (# Detected/#Tested)
				Cp/mL	xLoD	
A	2006 Isolate	Zeptometrix	0810040ACF-CL	18510	~3x	100% (3/3)
	02/2015	Zeptometrix	0810475CF	18510	~3x	100% (3/3)
	A2	ATCC	VR-1540	18510	~3x	100% (3/3)
B	CH93(18)-18	Zeptometrix	0810040CF-CL	18510	~3x	100% (3/3)
	9320	Zeptometrix	VPL-030	18510	~3x	100% (3/3)
	WV/14617/85	ATCC	VR-1400	18510	~3x	100% (3/3)
	18537	ATCC	VR-1580	18510	~3x	100% (3/3)

Table 11. Strains of SARS-CoV-2 Evaluated for Inclusivity

Lineage	Strain/Isolate	Source	Catalogue No.	Concentration		% Detected (# Detected/#Tested)
				Cp/mL	xLoD	
B.1.1.7	England/204820464/2020	Zeptometrix	0810614CFHI-CL	312	~3x	100% (3/3)
B.1.351	South Africa/KRISPK005325/2020	Zeptometrix	0810613CFHI-CL	312	~3x	100% (3/3)
B.1.617.2	USA/PHC658/2021	Zeptometrix	0810624CFHI-CL	312	~3x	100% (3/3)
B.1.1.529	USA/MDHP20874/2021	Zeptometrix	0810642CFHI-CL	312	~3x	100% (3/3)
P.1	Japan/TY7-503/2021	Zeptometrix	0810616CFHI-CL	312	~3x	100% (3/3)
-	USA-WA1/2020	Zeptometrix	0810587CFHI	312	~3x	100% (3/3)

b. *In silico*

SARS-CoV-2 In Silico Analysis

The inclusivity of the cobas Respiratory 4-flex was evaluated using *in silico* analysis of the forward primers, reverse primers, and probes for the SARS-CoV-2 target in relation to all sequences available in the NCBI and GISAID gene databases. For both SARS-CoV-2 gene targets taken together, *in silico* analysis on June 13, 2025 (>8.32M sequences in NCBI and >15.96M sequences in GISAID) indicates that the majority of sequence variants are predicted to be detected, with only <0.07% of sequences in NCBI and <0.06% of sequences in GISAID, with any mismatch in primer/probe binding sites for both targets. *In silico* analysis indicates that the cobas Respiratory 4-flex SARS-CoV-2 test detects all known variant strains.

No sequences in the NCBI database, and seven (7) unique sequences in GISAID (<0.00005%) had mismatches that were predicted to affect detection and performance of the test. Due to the low percentage representation in the databases of mismatches, performance of the cobas Respiratory 4-flex is not expected to be impacted.

Cross-Reactivity and Microbial Interference

Cross-reactivity and microbial interference of the cobas Respiratory 4-flex was evaluated by testing a panel of bacteria, fungi, and viruses commonly found in the respiratory tract for cross-reactivity and interference. The effect of non-target microorganisms on the performance of the cobas Respiratory 4-flex was tested by introducing non-target microorganisms into simulated NPS matrix spiked with and without co-formulated SARS-CoV-2, influenza A, influenza B and RSV viruses (**Table 3**) 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. Bacteria and fungi were spiked at 1.0e+6 units/mL and viruses at 1.0e+5 units/mL, or the highest concentration possible. As summarized in **Table 12**, no cross-reactivity or microbial interference was observed at the concentrations tested, and no invalid results were obtained.

Table 12. Cross-reactivity and Microbial Interference Results for Non-Target Microorganisms

Microorganism	Concentration	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	RSV	SARS-CoV-2	Influenza A	Influenza B	RSV
Aspergillus flavus	1.00E+06 CFU/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Bordetella parapertussis	1.00E+06 CFU/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Bordetella pertussis	1.00E+06 CFU/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Candida albicans	1.00E+06 CFU/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Chlamydia pneumoniae	1.00E+06 IFU/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Corynebacterium diphtheriae	1.00E+06 CFU/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Cytomegalovirus	1.00E+05 $TCID_{50}$/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Epstein Barr virus	1.00E+05 cp/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Escherichia coli	1.00E+06 CFU/vial	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Fusobacterium necrophorum	1.00E+06 CFU/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Haemophilus influenzae	1.00E+06 CFU/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Lactobacillus acidophilus	1.00E+06 CFU/vial	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Legionella pneumophila	1.00E+06 CFU/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Measles virus	1.00E+05 $TCID_{50}$/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
MERS-coronavirus*	1.00E+05 cp/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Moraxella catarrhalis	1.00E+06 CFU/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Mumps virus	1.00E+05 $TCID_{50}$/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Mycobacterium bovis	1.00E+06 CFU/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Mycoplasma genitalium	1.00E+06 CFU/vial	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Mycoplasma pneumoniae	1.00E+06 CCU/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Neisseria elongata	1.00E+06 CFU/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Neisseria meningitidis	1.00E+06 CFU/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Pneumocystis jirovecii	5.00E+03 organisms/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Pooled nasal wash	NA	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3

Microorganism	Concentration	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	RSV	SARS-CoV-2	Influenza A	Influenza B	RSV
Pseudomonas aeruginosa	1.00E+06 CFU/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
SARS-coronavirus (SARS-CoV)*	1.00E+05 cp/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Staphylococcus aureus	1.00E+06 CFU/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Staphylococcus epidermidis	1.00E+06 CFU/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Streptococcus pneumoniae	1.00E+06 CFU/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Streptococcus pyogenes	1.00E+06 CFU/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Streptococcus salivarius	1.00E+06 CFU/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3

CFU = Colony Forming Units; CCU = Colony Changing Units; IFU= Inclusion Forming Units; $TCID_{50}$ = Median Tissue Culture Infectious Dose.
*Inactivated virus was used for testing

Interfering Substances

An analytical study was performed to assess the potential inhibitory effects of endogenous and exogenous substances that may be commonly found in NPS specimens. All substances in **Table 13** were evaluated in the presence and absence of target organisms (**Table 3**) co-formulated at ~3x LoD. Testing for each condition was performed in replicates of ten.

None of the evaluated substances, at the concentrations tested, interfered with detection of the candidate assay targets, except for FluMist and snuff tobacco. Specifically, in the absence of target organisms, FluMist caused positive influenza A and influenza B results and snuff tobacco caused invalid results for all target organisms. The FluMist results were anticipated, as FluMist contains both influenza A and influenza B viruses.

Table 13. Substances Evaluated for Interference

Substance	Active Ingredient	Concentration Tested	SARS-CoV-2, Influenza A, Influenza B, RSV Absent	SARS-CoV-2, Influenza A, Influenza B, RSV Present
			Valid Negative Results/ Total # of Valid Results	Valid Positive Results/ Total # of Valid Results
Mucin	NA	0.3% w/v	10/10	10/10
		0.5% w/v	10/10	10/10
Human Whole Blood	NA	1.5% (v/v)	10/10	10/10
		3.0% (v/v)	10/10	10/10
NNSC (Target Negative No Substance Control)	NA	NA	10/10	0/10

Substance	Active Ingredient	Concentration Tested	SARS-CoV-2, Influenza A, Influenza B, RSV Absent	SARS-CoV-2, Influenza A, Influenza B, RSV Present
			Valid Negative Results/ Total # of Valid Results	Valid Positive Results/ Total # of Valid Results
AXOTIDE Diskus Multidose 250 mcg	Fluticasone propionate	0.167 mg/mL	10/10	10/10
BACTROBAN Nasal Ointment	Mupirocin	0.20 mg/mL	10/10	10/10
BUDESONID Sandoz Nasal Spray 64 mcg	Budesonide	0.039 mg/mL	10/10	10/10
CEPACOL Extra Strength Sore Throat	Benzocaine	5 mg/mL	10/10	10/10
Chloraseptic max	Phenol	0.47 mg/mL	10/10	10/10
FLUMIST Quadrivalent	live attenuated influenza A and B viruses	50000000 FFU/mL	10/10[a]	10/10
Heel Luffeel Nasal Spray	Luffa operculata Thryallis glauca Histaminum Sulphur	2.99 mg/mL 2.99 mg/mL 1.5 mg/mL 1.5 mg/mL	10/10	10/10
NASIVIN Pur Spray 0.05%	Oxymetazoline	0.011 mg/mL	10/10	10/10
OBRACIN Inj Solution 40 mg/mL	Tobramycin	0.018 mg/mL	10/10	10/10
RELENZA Disk 5 mg	Zanamivir	0.0015 mg/mL	10/10	10/10
TAMIFLU Kaps 75 mg	Oseltamivir	0.0073 mg/mL	10/10	10/10
Snuff Tobacco	Nicotine	0.1% w/v	0/0[b]	10/10
Vaseline	Petroleum Jelly	1% w/v	10/10	10/10
VICKS VapoRub	Eucalyptus Oil and Menthol	1% w/v	10/10	10/10
XYLOCAIN Spray 10%	Lidocaine	2.68 mg/mL	10/10	10/10

NA-Not Applicable

[a] FluMist is expected to give positive (detected) results for influenza A and influenza B targets. 10/10 represents SARS-CoV-2 and RSV targets.

[b] All replicates had a negative RNA-IC result and were therefore invalid.

Competitive Interference (Co-Infection)

To assess potential competitive interference between the viral targets, a total of 12 panels composed of various combinations of the cobas Respiratory 4-flex targets (**Table 3**) were tested. Samples were prepared with one viral target at ~3x LoD mixed with another target at a high concentration (1.0E+06 units/mL). Twelve replicates per condition were tested. As shown in **Table 14**, none of the targets present at very high concentration interfered with the detection of other viral targets at low concentration levels.

Table 14. Competitive Interference (Co-infection) Study Results

Combination	Target 1 (high) ≥ 1.00E+06 unit/mL	Target 2 (low) ~3x LoD	% Detected (# Detected / # Tested)		
			Target 1	Target 2	Target 3
1	Influenza A	SARS-CoV-2	100% (12/12)	100% (12/12)	100% (12/12)
2	Influenza B	SARS-CoV-2	100% (12/12)	100% (12/12)	100% (12/12)
3	RSV	SARS-CoV-2	100% (12/12)	100% (12/12)	100% (12/12)
4	SARS-CoV-2	Influenza A	100% (12/12)	100% (12/12)	100% (12/12)
5	Influenza B	Influenza A	100% (12/12)	100% (12/12)	100% (12/12)
6	RSV	Influenza A	100% (12/12)	100% (12/12)	100% (12/12)
7	Influenza A	Influenza B	100% (12/12)	100% (12/12)	100% (12/12)
8	SARS-CoV-2	Influenza B	100% (12/12)	100% (12/12)	100% (12/12)
9	RSV	Influenza B	100% (12/12)	100% (12/12)	100% (12/12)
10	Influenza A	RSV	100% (12/12)	100% (12/12)	100% (12/12)
11	Influenza B	RSV	100% (12/12)	100% (12/12)	100% (12/12)
12	SARS-CoV-2	RSV	100% (12/12)	100% (12/12)	100% (12/12)

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 (RNA-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 sample stability claims:

      Primary Sample (NP swab in UTM)

- Up to 12-hours at 2-25℃, followed by up to 3-days at 2-8℃, followed by up to 30-days at ≤ -20℃. Frozen samples may undergo up to three freeze/thaw cycles.

6. Detection Limit:
Limit of detection (LoD) studies determine the lowest detectable concentration of SARS-CoV-2, influenza A, influenza B, and RSV at which equal to or greater than 95% of all replicates test positive. To determine the LoD for each target, co-spiked panels were formulated using cultured viral material or inactivated virus diluted in nasopharyngeal (NP) matrix. Twenty-one replicates per reagent lot per dilution were tested for five (5) 2-fold dilutions using three reagent lots distributed across six (6) cobas 5800 Systems, three (3) cobas 6800 Systems and one (1) cobas 8800 System. The strains evaluated, as well as their corresponding LoD values are shown in **Table 15.**

Table 15. LoD Study Results

Target	Subtype/Strain/Isolate	LoD Concentration (By Hit Rate)	n Detected/ N Tested (% Positive)
Influenza A	H3N2, A/Darwin/6/2021	50 cp/mL	60/63 (95.24%)
	H1N1 pdm09, Brisbane/02/2018	100 cp/mL	60/63 (95.24%)
Influenza B	Yamagata, Phuket/3073/13	800 cp/mL	62/63 (98.41%)
	Victoria, B/Austria/1359417/2021	250 cp/mL	63/63 (100%)
RSV	Respiratory Syncytial Virus A2	4000 cp/mL	62/63 (95.41%)
SARS-CoV-2*	England/02/2020 NIBSC code: 20/146	80 IU/mL	60/63 (95.24%)

*Inactivated SARS-CoV-2 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:
Data from several analytical studies were used to analyze the Ct distribution and set cutoffs for all organisms to maximize sensitivity and specificity.

8. Accuracy (Instrument):
Not applicable.

9. Carry-Over:
A total of 430 SARS-CoV-2 high positive samples prepared at approximately 6.50E+08 particles/mL in negative simulated NPS matrix and 480 negative samples consisting of negative simulated NPS matrix were tested in 25 checkerboard configuration runs, using both cobas 5800 and 6800/8800 Systems. The cross-contamination rate calculated per system (cobas 5800 and cobas 6800/8800) and for all systems combined was in both cases 0.0%.

B Comparison Studies:

1. Method Comparison with Predicate Device:
Not applicable.

2. Matrix Comparison:
A subset of analytical studies were conducted using simulated matrix, therefore equivalency between clinical NPS matrix and simulated NPS matrix was evaluated. For this study, pooled clinical NPS matrix and simulated matrix were spiked with co-formulated panels containing RSV, influenza A, influenza B and SARS-CoV-2 at a concentration level of ~2x LoD. Forty-two replicates were tested per co-formulated panel and matrix type combination. All replicates tested with the ~2x LoD panels were positive for the respective viral targets. The results demonstrate equivalent performance of the cobas Respiratory 4-flex when testing samples contrived in clinical NPS matrix and simulated matrix and support the use of simulated matrix in a subset of analytical studies.

3. Media Equivalency Study
An analytical study was performed to assess the performance of cobas Respiratory 4-flex with common transport medias that may be utilized to collect NPS specimens for testing. Negative clinical NPS matrix in each of the media types illustrated in **Table 16** were evaluated in the presence and absence of target organisms (**Table 3**) co-formulated at ~3x LoD. Testing for each condition was performed in replicates of ten. None of the media types evaluated interfered with detection of the candidate assay targets at the concentration tested.

Table 16. Media Equivalency Study Results

Media Type	SARS-CoV-2, Influenza A, Influenza B, RSV Absent	SARS-CoV-2, Influenza A, Influenza B, RSV Present
	Valid Negative Results/ Total # of Valid Results	Valid Positive Results/ Total # of Valid Results
Remel M4RT (Transport Media)	10/10	10/10
Remel M5 (Transport Media)	10/10	10/10
Remel M6 (Transport Media)	10/10	10/10
Greiner VACUETTE Virus Stabilization Tube (Transport Media)	10/10	10/10

C Clinical Studies:

1. Clinical Sensitivity:

Prospective Clinical Studies
The clinical performance of the cobas Respiratory 4-flex was established in multi-center prospective studies conducted with nasopharyngeal swab (NPS) specimens in VTM or UVT from individuals exhibiting signs and symptoms of respiratory viral infection. Prospective NPS specimens were collected at eleven collection sites (eight (8) in the U.S. and three (3) OUS) during the 2023-2024 respiratory viral season and tested fresh (Category I specimens).

Category I specimens were tested with the cobas Respiratory 4-flex using the cobas 6800/8800 System at four (4) testing sites (three (3) in the U.S and one (1) OUS). Additionally, residual (leftover) and de-identified NPS specimens (Category II) were prospectively collected during parts of the 2022-2023 respiratory viral seasons at seven (7) U.S. sites and the 2023-2024 respiratory viral season from 14 U.S. collection sites. Category II specimens were tested with the cobas Respiratory 4-flex at three (3) U.S testing sites using the cobas 6800/8800 Systems. The comparator method utilized to establish performance was a U.S. FDA-cleared molecular assay. Comparator testing was performed in accordance with the package insert.

A total of 4,475 NPS specimens (1,869 Category I and 2,606 Category II) were enrolled for the prospective clinical study, of which 4,378 could be tested (1,832 Category I and 2,546 Category II) with cobas Respiratory 4-flex on the cobas 6800/8800 System and the comparator method. Thirty-five Category I specimens could not be tested due to instrument error or protocol deviations, and two (2) Category I specimens were unable to be tested due to sample processing error. Sixty Category II samples were not tested due to insufficient specimen volume, instrument error, or specimens lost in transit by the courier.

Of the 4,378 prospective NPS specimens tested, 4,341 specimens were evaluable (1,827 Category I and 2,514 Category II). Five (5) Category I specimens and 32 Category II specimens were non-evaluable due to obtaining invalid results with cobas Respiratory 4-flex. For the influenza A target, three (3) additional specimens (two (2) Category I and one (1) Category II) were excluded from analysis due to inconclusive results obtained from the comparator test. The initial prospective clinical study invalid rate was 1.3% (57/4,378), and 0.9% (40/4,378) following retesting. **Table 17** below provides a summary of demographic information for the 4,341 specimens included in the prospective clinical study.

Table 17. Demographics of Subjects from Prospective Population that were Included in the Performance Analysis

Age group (years)	Total Number (%)		
	Category I	**Category II**	**Overall**
Total	1,827	2,514	4,341
<6	49 (2.7%)	183 (7.3%)	232 (5.3%)
6 - <14	215 (11.8%)	225 (8.9%)	440 (10.1%)
14 - <18	84 (4.6%)	85 (3.4%)	169 (3.9%)
18 - <65	1,249 (68.4%)	1,721 (68.5%)	2,970 (68.4%)
>=65	230 (12.6%)	300 (11.9%)	530 (12.2%)
Missing	0 (0.0%)	0 (0.0%)	0 (0.0%)

A summary of the cobas Respiratory 4-flex prospective clinical study performance is provided in **Table 18**.

Positive Percent Agreement (PPA) for the NPS specimen tested on the candidate and comparator device was calculated as 100% × (TP/(TP+FN)). True positive (TP) indicates that both the cobas Respiratory 4-flex and the comparator method had a positive result for the specific analyte, and false negative (FN) indicates that the cobas Respiratory 4-flex was

negative while the comparator result was positive. Negative Percent Agreement (NPA) for the NPS specimen tested on the candidate and comparator device was calculated as 100% × (TN / (TN+FP)). True negative (TN) indicates that both the cobas Respiratory 4-flex and the comparator method had negative results, and false positive (FP) indicates that the cobas Respiratory 4-flex was positive while the comparator result was negative.

Table 18. Clinical Performance of the cobas Respiratory 4-flex in the Prospective Studies

Analyte		Positive Percent Agreement			Negative Percent Agreement		
		TP/ (TP+FN)	%	95% CI	TN/ (TN+FP)	%	95% CI
Influenza A	Cat I	100/104	96.2	90.5-98.5	1716/1721	99.7	99.3-99.9
	Cat II	60/62	96.8	89.0-99.1	2443/2451	99.7	99.4-99.8
	All	***160/166***	***96.4***	***92.3-98.3***	***4159/4172***	***99.7***	***99.5-99.8***
Influenza B	Cat I	66/66	100	94.5-100	1759/1761	99.9	99.6-100
	Cat II	23/24	95.8	79.8-99.3	2489/2490	100.0	99.8-100
	All	***89/90***	***98.9***	***94.0-99.8***	***4248/4251***	***99.9***	***99.8-100***
RSV	Cat I	47/53	88.7	77.4-94.7	1774/1774	100	99.8-100
	Cat II	66/73	90.4	81.5-95.3	2441/2441	100.0	99.8-100
	All	***113/126***	***89.7***	***83.1-93.9***	***4215/4215***	***100.0***	***99.9-100***
SARS-CoV-2	Cat I	145/150	96.7	92.4-98.6	1654/1677	98.6	98.0-99.1
	Cat II	295/302	97.7	95.3-98.9	2176/2212	98.4	97.8-98.8
	All	***440/452***	***97.3***	***95.4-98.5***	***3830/3889***	***98.5***	***98.0-98.8***

CI: confidence interval; FN: false negative; FP: false positive; RSV: respiratory syncytial virus; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; TN: true negative; TP: true positive.

Number of samples with positive results for more than one target observed in the prospective cohort as detected by the cobas Respiratory 4-flex and comparator method are listed in **Table 19**.

Table 19. Multiple Detection Combination by cobas Respiratory 4-flex in the Prospective Studies

Sample Category	Analyte 1	Analyte 2	Total Multiple Detections	Number of Specimen with False Positive Detections	False Positive Analyte(s)
Category I	Influenza A	SARS-CoV-2	5	3	SARS-CoV-2 (3)
Category I	Influenza B	RSV	1	1	Influenza B (1)
Category II	Influenza A	SARS-CoV-2	3	0	None
Category II	Influenza B	RSV	1	0	None
Category II	Influenza B	SARS-CoV-2	1	1	SARS-CoV-2 (1)
Category II	RSV	SARS-CoV-2	4	4	SARS-CoV-2 (4)

Note: False positive is when a sample is detected by cobas Respiratory 4-flex but not detected by comparator method.

2. Clinical Specificity:

See section **VII. Performance Characteristics. C. Clinical Studies 1. Clinical Sensitivity**, above.

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

Clinical Equivalency Study

A clinical performance equivalency study was conducted to demonstrate equivalent performance between the cobas 6800/8800 and cobas 5800 Systems. All specimens in the clinical study were tested with the candidate assay on the cobas 6800/8800 System and then a subset of samples, chosen at random, were tested with the cobas 5800 System. The results from each system were evaluated against the respective comparator assay, and the PPA and NPA were directly compared between the two systems. The results were equivalent between the instrument systems.

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

The cobas Respiratory 4-flex prospective clinical studies included a total of 4,378 prospectively collected NPS specimens, of which 4,341 NPS specimens were evaluable. Of these evaluable specimens, 1,827 NPS specimens were collected during the 2023-2024 respiratory viral season and tested fresh (Category I) with the cobas Respiratory 4-flex, 785 specimens were collected during the 2022-2023 respiratory viral season and frozen prior to testing (Category II) and 1,729 were collected during the 2023-2024 respiratory viral season and frozen prior to testing (Category II). The number and percentage of cases positive for SARS-CoV-2, influenza A, influenza B, and RSV, as determined by the cobas Respiratory 4-flex, are presented in **Tables 20-22**, stratified by collection site. **Tables 23** below shows the expected values, as determined by the cobas Respiratory 4-flex, stratified by the patient's age.

Table 20. cobas Respiratory 4-flex – Expected Values by Specimen Collection Site for Category I NPS Specimens Collected in 2023-2024 in the Prospective Study

Sample Category	Collection Period	Collection site	Target Virus			
			Influenza A	Influenza B	Respiratory Syncytial Virus	SARS-CoV-2
Category I	2023-2024	1	0.0% (0/139)	2.9% (4/139)	0.0% (0/139)	5.0% (7/139)
		2	0.6% (1/170)	0.0% (0/171)	2.3% (4/171)	2.9% (5/171)
		3	0.8% (3/389)	0.5% (2/389)	0.5% (2/389)	2.3% (9/389)
		4	0.0% (0/61)	1.6% (1/61)	1.6% (1/61)	8.2% (5/61)
		5	17.5% (25/143)	5.6% (8/144)	9.0% (13/144)	13.2% (19/144)
		6	14.7% (11/75)	4.0% (3/75)	4.0% (3/75)	12.0% (9/75)
		7	0.0% (0/6)	0.0% (0/6)	0.0% (0/6)	16.7% (1/6)
		8	6.3% (7/112)	1.8% (2/112)	0.9% (1/112)	15.2% (17/112)
		9	2.7% (7/258)	2.3% (6/258)	1.9% (5/258)	4.7% (12/258)
		10	0.0% (0/21)	0.0% (0/21)	0.0% (0/21)	0.0% (0/21)
		11	11.3% (51/451)	9.3% (42/451)	4.0% (18/451)	18.6% (84/451)

Sample Category	Collection Period	Collection site	Target Virus			
			Influenza A	Influenza B	Respiratory Syncytial Virus	SARS-CoV-2
		Overall	5.8% (105/1825)	3.7% (68/1827)	2.6% (47/1827)	9.2% (168/1827)

Table 21. cobas Respiratory 4-flex – Expected Values by Specimen Collection Site for Category II NPS Specimens Collected in 2022-2023 in the Prospective Study

Sample Category	Collection Period	Collection site	Target Virus			
			Influenza A	Influenza B	Respiratory Syncytial Virus	SARS-CoV-2
Category II	2022-2023	1	0.4% (1/223)	0.0% (0/223)	0.0% (0/223)	2.7% (6/223)
		2	0.0% (0/18)	0.0% (0/18)	0.0% (0/18)	0.0% (0/18)
		3	0.0% (0/51)	0.0% (0/51)	0.0% (0/51)	62.7% (32/51)
		4	2.7% (1/37)	0.0% (0/38)	0.0% (0/38)	5.3% (2/38)
		5	0.0% (0/36)	2.8% (1/36)	0.0% (0/36)	5.6% (2/36)
		6	0.0% (0/12)	0.0% (0/12)	0.0% (0/12)	16.7% (2/12)
		7	2.5% (10/407)	0.0% (0/407)	0.2% (1/407)	11.1% (45/407)
		Overall	1.5% (12/784)	0.1% (1/785)	0.1% (1/785)	11.3% (89/785)

Table 22. cobas Respiratory 4-flex – Expected Values by Specimen Collection Site for Category II NPS Specimens Collected in 2023-2024 in the Prospective Study

Sample Category	Collection Period	Collection site	Target Virus			
			Influenza A	Influenza B	Respiratory Syncytial Virus	SARS-CoV-2
Category I	2023-2024	1	4.5% (3/67)	0.0% (0/67)	0.0% (0/67)	6.0% (4/67)
		2	2.4% (1/41)	2.4% (1/41)	4.9% (2/41)	12.2% (5/41)
		3	3.8% (7/185)	5.9% (11/185)	13.0% (24/185)	7.6% (14/185)
		4	0.0% (0/33)	0.0% (0/33)	12.1% (4/33)	6.1% (2/33)
		5	7.1% (3/42)	0.0% (0/42)	16.7% (7/42)	0.0% (0/42)
		6	0.0% (0/326)	0.6% (2/326)	1.2% (4/326)	17.8% (58/326)
		7	9.2% (16/174)	0.0% (0/174)	3.4% (6/174)	17.8% (31/174)
		8	3.1% (5/161)	0.6% (1/161)	3.1% (5/161)	23.6% (38/161)
		9	0.0% (0/66)	1.5% (1/66)	1.5% (1/66)	4.5% (3/66)
		10	0.6% (1/168)	0.0% (0/168)	1.2% (2/168)	17.3% (29/168)
		11	1.0% (2/201)	0.0% (0/201)	0.0% (0/201)	12.9% (26/201)
		12	7.7% (12/155)	1.3% (2/155)	5.8% (9/155)	6.5% (10/155)
		13	0.0% (0/56)	8.9% (5/56)	1.8% (1/56)	14.3% (8/56)

Sample Category	Collection Period	Collection site	Target Virus			
			Influenza A	Influenza B	Respiratory Syncytial Virus	SARS-CoV-2
		14	11.1% (6/54)	0.0% (0/54)	0.0% (0/54)	25.9% (14/54)
		Overall	3.2% (56/1729)	1.3% (23/1729)	3.8% (65/1729)	14.0% (242/1729)

Table 23. cobas Respiratory 4-flex – Expected Values by Patient Age for the Prospective Studies

Study Phase	Analyte	Positive Results Stratified by Age (Years) % Positive (n Detected/ N Tested)				
		All Ages	≤5	>5 - 21	>21 - 65	>65
Category I: 2023-2024	Influenza A	5.8% (105/1825)	15.4% (6/39)	9.1% (32/352)	4.8% (58/1205)	3.9% (9/229)
	Influenza B	3.7% (68/1827)	7.7% (3/39)	7.9% (28/353)	3.0% (36/1206)	0.4% (1/229)
	RSV	2.6% (47/1827)	25.6% (10/39)	3.4% (12/353)	1.7% (21/1206)	1.7% (4/229)
	SARS-CoV-2	9.2% (168/1827)	7.7% (3/39)	7.9% (28/353)	9.7% (117/1206)	8.7% (20/229)
Category II: 2022-2023	Influenza A	1.5% (12/784)	NC (0/0)	0.0% (0/47)	1.8% (11/598)	0.7% (1/139)
	Influenza B	0.1% (1/785)	NC (0/0)	0.0% (0/47)	0.2% (1/599)	0.0% (0/139)
	RSV	0.1% (1/785)	NC (0/0)	0.0% (0/47)	0.2% (1/599)	0.0% (0/139)
	SARS-CoV-2	11.3% (89/785)	NC (0/0)	2.1% (1/47)	11.0% (66/599)	15.8% (22/139)
Category II: 2023-2024	Influenza A	3.2% (56/1729)	4.9% (9/183)	4.1% (18/434)	2.9% (28/967)	0.7% (1/145)
	Influenza B	1.3% (23/1729)	1.1% (2/183)	4.4% (19/434)	0.2% (2/967)	0.0% (0/145)
	RSV	3.8% (65/1729)	17.5% (32/183)	3.7% (16/434)	1.3% (13/967)	2.8% (4/145)
	SARS-CoV-2	14.0% (242/1729)	5.5% (10/183)	6.7% (29/434)	15.0% (145/967)	40.0% (58/145)

Note: NC = Not Calculable due to no samples from the age group.

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.