Date: July 27, 2023

Roche Molecular Systems, Inc.
Khushvanreep Singh
Regulatory Affairs Specialist
4300 Hacienda Drive
Pleasanton, California 94588-2722

Re: K223591
Trade/Device Name: cobas SARS-CoV-2 & Influenza A/B for use on the cobas Liat System
Regulation Number: 21 CFR 866.3981
Regulation Name: 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
Regulatory Class: Class II
Product Code: QOF
Dated: November 30, 2022
Received: December 1, 2022

Dear Khushvanreep Singh:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Joseph Briggs -S

Joseph Briggs, Ph.D.
Deputy Branch Chief
Viral Respiratory and HPV Branch
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2023
See PRA Statement below.

510(k) Number *(if known)*
K223591

Device Name
cobas® SARS-CoV-2 & Influenza A/B for use on the cobas® Liat® System

Indications for Use *(Describe)*
The cobas® SARS-CoV-2 & Influenza A/B nucleic acid test for use on the cobas® Liat® System (cobas® SARS-CoV-2 & Influenza A/B) is an automated rapid multiplex real-time, reverse transcriptase 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, and influenza B virus nucleic acid in nasopharyngeal swab (NPS) and anterior nasal swab (ANS) specimens from individuals with signs and symptoms of respiratory tract infection. Clinical signs and symptoms of respiratory tract infection due to SARS-CoV-2 and influenza can be similar.

cobas® SARS-CoV-2 & Influenza A/B is intended for use as an aid in the differential diagnosis of SARS-CoV-2, influenza A, and/or influenza B infection if used in conjunction with other clinical and epidemiological information, and laboratory findings. SARS-CoV-2, influenza A and influenza B viral nucleic acid are generally detectable in NPS and ANS specimens during the acute phase of infection.

Positive results do not rule out co-infection with other organisms. The agent(s) detected by the cobas SARS-CoV-2 & Influenza A/B may not be the definite cause of disease.

Negative results do not preclude SARS-CoV-2, influenza A, and/or influenza B infection. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

cobas® SARS-CoV-2 & Influenza A/B for use on the **cobas**® Liat® System 510(k) Summary

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of 21 CFR 807.92.

Submitter Name	Roche Molecular Systems, Inc.
Address	4300 Hacienda Drive Pleasanton, CA 94588-2722
Contact	Khushvanreep Singh Phone: (908) 253-7864 FAX: (925) 225-0207 Email: Khushvanreep.singh@roche.com
Date Prepared	July 24, 2023
Proprietary Name	**cobas**® SARS-CoV-2 & Influenza A/B for use on the **cobas**® Liat System
Common Name	**cobas**® SARS-CoV-2 & Influenza A/B
Classification Name	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
Regulation Number	21 CFR 866.3981
Product Codes	QOF
Predicate Devices	BioFire® RP2.1 Panel (DEN200031)
Establishment Registration	Roche Molecular Systems, Inc. (2243471)

1. DEVICE DESCRIPTION

cobas® SARS-CoV-2 & Influenza A/B assay uses real-time reverse transcriptase polymerase chain reaction (RT-PCR) technology to rapidly (approximately 20 minutes) detect and differentiate between SARS-CoV-2, influenza A, and influenza B viruses from nasopharyngeal and nasal swabs. The automation, small footprint, and easy-to-use interface of the **cobas**® Liat® System enable performance of this test to occur at the POC or in a clinical laboratory setting.

1.1. Principles of the Procedure

The **cobas®** SARS-CoV-2 & Influenza A/B nucleic acid test for use on the **cobas®** Liat® System (**cobas®** SARS-CoV-2 & Influenza A/B) is an automated rapid multiplex real-time, reverse transcriptase 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, and influenza B virus nucleic acid in nasopharyngeal swab (NPS) and anterior nasal swab (ANS) specimens from individuals with signs and symptoms of respiratory tract infection. Clinical signs and symptoms of respiratory tract infection due to SARS-CoV-2 and influenza can be similar.

cobas® SARS-CoV-2 & Influenza A/B is intended for use as an aid in the differential diagnosis of SARS-CoV-2, influenza A, and/or influenza B infection if used in conjunction with other clinical and epidemiological information, and laboratory findings. SARS-CoV-2, influenza A and influenza B viral nucleic acid are generally detectable in NPS and ANS specimens during the acute phase of infection.

Positive results do not rule out co-infection with other organisms. The agent(s) detected by the cobas SARS-CoV-2 & Influenza A/B may not be the definite cause of disease.

Negative results do not preclude SARS-CoV-2, influenza A, and/or influenza B infection. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

2. TECHNOLOGICAL CHARACTERISTICS

The primary technological characteristics and intended use of the RMS **cobas®** SARS CoV-2 & Influenza A/B Nucleic acid test for use on the **cobas®** Liat® System are substantially equivalent to other legally marketed nucleic acid amplification tests intended for the qualitative detection of SARS-CoV-2 & Influenza A/B.

As indicated in Table 1, the RMS **cobas®** SARS-CoV-2 & Influenza A/B test for use on the **cobas®** Liat® System is substantially equivalent to significant characteristics of the identified predicate device, the currently cleared BioFire® RP2.1 Panel (DEN200031).

Table 1: Comparison of the cobas® SARS-CoV-2 & Influenza A/B for use on the cobas® Liat® System and the Predicate Device

	Submitted Device: cobas® SARS-CoV-2 & Influenza A/B for use on the cobas® Liat® System	**Predicate Device: BioFire® RP2.1 Panel (DEN200031)**
Regulation Name	21 CFR 866.3981	Same
Product Code	QOF	QOF
Intended Use	The **cobas®** SARS-CoV-2 & Influenza A/B nucleic acid test for use on the **cobas®** Liat® System (**cobas®** SARS-CoV-2 & Influenza A/B) is an automated rapid multiplex real-time reverse transcriptase 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, and influenza B virus nucleic acid in nasopharyngeal swab (NPS) and anterior nasal swab (ANS) specimens from individuals with signs and symptoms of respiratory tract infection. Clinical signs and symptoms of respiratory tract infection due to SARS-CoV-2 and influenza can be similar. **cobas®** SARS-CoV-2 & Influenza A/B is intended for use as an aid in the differential diagnosis of SARS-CoV-2, influenza A, and/or influenza B infection if used in conjunction with other clinical and epidemiological information, and laboratory findings. SARS-CoV-2, influenza A and influenza B viral nucleic acid are generally detectable in NPS and ANS specimens during the acute phase of infection. Positive results do not rule out co-infection with other organisms. The agent(s) detected by the **cobas®** SARS-CoV-2 & Influenza A/B may not be the definite cause of disease. Negative results do not preclude SARS-CoV-2, influenza A, and/or influenza B infection. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.	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, • 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 pneumoniae Nucleic acids from the respiratory viral and bacterial organisms identified by this test are generally detectable in NPS specimens

	Submitted Device: cobas® SARS-CoV-2 & Influenza A/B for use on the cobas® Liat® System	Predicate Device: BioFire® RP2.1 Panel (DEN200031)
		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.
Sample Types	Nasopharyngeal and nasal swabs	Nasopharyngeal swabs
Analyte Targets	• SARS-CoV-2 ORF1 a/b non-structural region • SARS-CoV-2 nucleocapsid protein gene • Influenza A matrix gene • Influenza B nonstructural protein gene	For SARS-CoV-2 organisms • spike protein (S) gene and • membrane protein (M) gene
Ancillary Collection Kits	• Copan FLOQSwabs™ with UTM™, UVT and other swabs with other viral transport media (VTM) – e.g., M4RT, M4, M5 and M6 • 0.9% Saline	• Viral Transport Media (VTM) • Saline (0.9%)
Sample Preparation	Automated	Same
Amplification Technology	Real-time PCR	2 stage PCR
Detection Chemistry	Multiplex assay using different reporter dyes for target and control	Two Step Nested multiplex PCR: • Reverse transcription, followed by a multiplexed first stage PCR reaction (PCR1).

	Submitted Device: cobas® SARS-CoV-2 & Influenza A/B for use on the cobas® Liat® System	Predicate Device: BioFire® RP2.1 Panel (DEN200031)
		• 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 Used	Sample processing control (IC) Positive and negative control	Two process controls: • RNA Process Control (IC) • PCR2 Control (A positive result indicates that PCR2 was successful)
Results Analysis	PCR Cycle threshold analysis	Endpoint melting curve data to detect target-specific amplicons

3. SPECIAL CONTROLS/STANDARDS/GUIDANCE REFERENCED

Class II Special Controls as per 21 CFR 866.3981.

4. NON-CLINICAL PERFORMANCE EVALUATION

4.1. Non-clinical performance for SARS-CoV-2

4.1.1. Analytical Sensitivity (Limit of Detection)

Limit of detection (LoD) studies determine the lowest detectable concentration of SARS-CoV-2 at which greater than or equal to 95% of all (true positive) replicates give a result of SARS-CoV-2 Detected.

4.1.1.1. WHO International Standard

The LoD using WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146) was determined by reconstituting the WHO Standard to 0.5 mL according to the WHO NIBSC code: 20/146 Instructions for use (Version 1.0, Dated 14-Dec-2020). Following reconstitution, the WHO Standard was diluted to an intermediate stock (IS) concentration in UTM.

WHO Standard IS was serially diluted in pooled negative nasopharyngeal swabs matrix. Five concentration levels were tested with 24 replicates at each level across three lots of assay tubes (8 replicates per lot). Three independent dilution series were used in the study with an

approximately equal numbers of replicates per dilution series. The LoD was determined by 95% hit rate to be 62.5 IU/mL.

The results of the hit rate are shown in Table 2 below.

Table 2: Hit rate and mean Ct results of SARS-CoV-2 LoD determination

Strain - WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146)

Concentration [IU/mL]	Valid positive results	Total valid results	Hit rate [%]	Mean Ct*
125	24	24	100	32.1
62.5	24	24	100	33.2
31.25	17	24	71	34.5
15.625	12	24	50	35.4
7.8125	10	24	42	35.2

*Calculations only include positive results.

4.1.1.2. SARS-CoV-2 viral culture

To determine the LoD for SARS-CoV-2, a heat inactivated cultured virus of an isolate from a US patient (USA-WA1/2020, lot number 324047, 3.16E+06 TCID50/mL, ZeptoMetrix, NY, USA) was serially diluted in pooled negative nasopharyngeal swab matrix. Five concentration levels were tested with 20 replicates except for the highest concentration level, which was tested with 10 replicates. Three lots of assay tubes (approximately equal numbers of replicates per lot), and two independent dilution series (equal numbers of replicates per dilution series) were used in the study.

As shown in Table 3, the concentration level with observed hit rates greater than or equal to 95% was 0.012 TCID50/mL (12 copies/mL) for SARS-CoV-2.

Table 3: LoD determination Using USA-WA1/2020 strain

Strain - USA-WA1/2020 (stock concentration 3.16E+06 TCID50/mL)

Concentration [$TCID_{50}$/mL]	Concentration [copies/mL]	Total valid results	Hit rate [%]	Mean Ct*
0.048	49	10	100	32.6
0.024	24	20	100	33.5

Concentration [$TCID_{50}$/mL]	Concentration [copies/mL]	Total valid results	Hit rate [%]	Mean Ct*
0.012	12	20	100	35.2
0.006	6	20	70	35.7
0.003	3	20	25	36.7

4.1.2. Reactivity/inclusivity

The inclusivity study evaluates the ability of the assay to detect SARS-CoV-2 isolates/variants. The reactivity/inclusivity was evaluated with 16 SARS-CoV-2 isolates/variants. The isolates/variants were tested as inactivated viruses diluted into pooled clinical negative nasopharyngeal swab matrix. The isolates/variants tested in the study and the concentrations that they can be detected are listed in Table 4. *In silico* analysis of additional SARS-CoV-2 sequences indicates that >99.9% of sequences for SARS-CoV-2 have no changes in primer/probe binding sites at both target regions simultaneously. All known sequences are predicted to be detected by at least one of the two target regions.

Table 4: Results of Testing SARS-CoV-2 Isolate/Variant

Isolate/Variant Name	Pango Lineage	WHO Label	Test Concentration (copies/mL)	SARS-CoV-2	Influenza A	Influenza B
SARS-CoV-2 Italy-INMI1	not listed	N/A	2.0E+01	+	-	-
SARS-CoV-2 Hong Kong/VM20001061/2020	A	N/A	2.0E+01	+	-	-
SARS-CoV-2 England/204820464/2020	B.1.1.7	Alpha	5.0E+00	+	-	-
SARS-CoV-2 South Africa/KRISP-K005325/2020	B.1.351	Beta	2.0E+01	+	-	-
USA/COR-22-063113/2022	BA5.5	Omicron	6.00E+00	+	-	-
USA/GA-EHC-2811C/2021	BA.1	Omicron	1.50E+00	+	-	-
hCoV-19/USA/MD-HP40900/2022	B.1.1.529, XBB.1.5	Omicron	6.00E+00	+	-	-
hCoV-19/USA/MD-HP38861/2022	B.1.1.529, BQ.1.1	Omicron	1.20E+01	+	-	-
hCoV-19/USA/MD-HP38288/2022	B.1.1.529, BF.7	Omicron	1.20E+01	+	-	-

Isolate/Variant Name	Pango Lineage	WHO Label	Test Concentration (copies/mL)	SARS-CoV-2	Influenza A	Influenza B
hCoV-19/USA/MD-HP30386/2022	B.1.1.529, BA.4	Omicron	6.00E+00	+	-	-
USA/MD-HP24556/2022	BA.2.3	Omicron	1.20E+01	+	-	-
USA/MD-HP20874/2021	B.1.1.529	Omicron	6.00E+00	+	-	-
hCoV-19/USA/CA-Stanford-15_S02/2021	B.1.617.1	Kappa	1.20E+01	+	-	-
USA/NY-Wadsworth-21025952/2021	B.1.526	Iota	3.60E+01	+	-	-
hCoV-19/USA/PHC658/2021	B.1.617.2	Delta	1.20E+01	+	-	-
hCoV-19/Japan/TY7-503/2021	P.1	Gamma	1.20E+01	+	-	-

4.1.3. Cross Reactivity (Exclusivity)

Cross-reactivity of **cobas**® SARS-CoV-2 & Influenza A/B was evaluated by testing a panel of multiple unique sub-species of microorganisms. High titer stocks of the potentially cross-reacting microorganisms were spiked into pooled negative nasopharyngeal swab clinical matrix to a concentration level of 1.00E+05 units/mL for viruses and 1.00E+06 units/mL for other microorganisms, unless otherwise noted.

None of the organisms tested interfered with **cobas**® SARS-CoV-2 performance by generating false positive results.

Table 5: Cross-reactivity

Microorganisms	Testing conc.*	SARS-CoV-2 result	Influenza A result	Influenza B result
Adenovirus	1.00E+05	Not Detected	Not Detected	Not Detected
Cytomegalovirus	1.00E+05	Not Detected	Not Detected	Not Detected
Epstein-Barr virus	1.00E+05	Not Detected	Not Detected	Not Detected
Human Enterovirus D	1.00E+05	Not Detected	Not Detected	Not Detected
Human Coronavirus 229E	1.00E+05	Not Detected	Not Detected	Not Detected
Human Coronavirus HKU1	1.00E+05	Not Detected	Not Detected	Not Detected
Human Coronavirus NL63	1.00E+05	Not Detected	Not Detected	Not Detected
Human Coronavirus OC43	1.00E+05	Not Detected	Not Detected	Not Detected
MERS-Coronavirus	1.00E+05	Not Detected	Not Detected	Not Detected
SARS Coronavirus	1.00E+05	Not Detected	Not Detected	Not Detected
Human Rhinovirus B	1.00E+05	Not Detected	Not Detected	Not Detected

Microorganisms	Testing conc.*	SARS-CoV-2 result	Influenza A result	Influenza B result
Human Metapneumovirus 27	1.00E+05	Not Detected	Not Detected	Not Detected
Measles	1.00E+05	Not Detected	Not Detected	Not Detected
Mumps	1.00E+05	Not Detected	Not Detected	Not Detected
Parainfluenzavirus Type 1	1.00E+05	Not Detected	Not Detected	Not Detected
Parainfluenzavirus Type 2	1.00E+05	Not Detected	Not Detected	Not Detected
Parainfluenzavirus Type 3	1.00E+05	Not Detected	Not Detected	Not Detected
Parainfluenzavirus Type 4A	1.00E+05	Not Detected	Not Detected	Not Detected
Respiratory Syncytial Virus A2	1.00E+05	Not Detected	Not Detected	Not Detected
Aspergillus Flavus var. flavus	1.00E+06	Not Detected	Not Detected	Not Detected
Bordetella pertussis	1.00E+06	Not Detected	Not Detected	Not Detected
Bordetella parapertussis	1.00E+06	Not Detected	Not Detected	Not Detected
Candida albicans	1.00E+06	Not Detected	Not Detected	Not Detected
Chlamydia pneumoniae	1.00E+06	Not Detected	Not Detected	Not Detected
Corynebacterium flavescens	1.00E+06	Not Detected	Not Detected	Not Detected
Escherichia coli	1.00E+06	Not Detected	Not Detected	Not Detected
Fusobacterium necrophorum subsp. Necrophorum	1.00E+06	Not Detected	Not Detected	Not Detected
Haemophilus influenzae	1.00E+06	Not Detected	Not Detected	Not Detected
Lactobacillus crispatus	1.00E+06	Not Detected	Not Detected	Not Detected
Legionella pneumophila	1.00E+06	Not Detected	Not Detected	Not Detected
Moraxella catarrhalis	1.00E+06	Not Detected	Not Detected	Not Detected
Mycoplasma genitalium	1.00E+06	Not Detected	Not Detected	Not Detected
Mycoplasma pneumoniae	1.00E+06	Not Detected	Not Detected	Not Detected
Mycobacterium tuberculosis	1.00E+06	Not Detected	Not Detected	Not Detected
Neisseria flava	1.00E+06	Not Detected	Not Detected	Not Detected
Neisseria meningitidis	1.00E+06	Not Detected	Not Detected	Not Detected
Pneumocystis jirovecii	5.00E+03	Not Detected	Not Detected	Not Detected
Pneumocystis jirovecii clinical Sample	1:10 diluted	Not Detected	Not Detected	Not Detected
Pseudomonas aeruginosa	1.00E+06	Not Detected	Not Detected	Not Detected
Staphylococcus epidermis	1.00E+06	Not Detected	Not Detected	Not Detected
Staphylococcus aureus	1.00E+06	Not Detected	Not Detected	Not Detected
Streptococcus pneumoniae	1.00E+06	Not Detected	Not Detected	Not Detected
Streptococcus pyogenes	1.00E+06	Not Detected	Not Detected	Not Detected
Streptococcus salivarius	1.00E+06	Not Detected	Not Detected	Not Detected
Nasal wash	1:10 diluted	Not Detected	Not Detected	Not Detected

*EB/mL, CFU/mL, IU/mL, $TCID_{50}$/mL, particles/mL, copies/mL, or PFU/mL

4.1.3.1. Microbial interference

Microbial Interference of **cobas**® SARS-CoV-2 & Influenza A/B was evaluated by testing a panel of multiple unique sub-species of microorganisms (Table 6) in the presence of 3x LoD concentrations of SARS-CoV-2, influenza A and influenza B viruses. High titer stocks of the potentially interfering microorganisms were spiked into pooled negative nasopharyngeal swab clinical matrix with spiked 3x LoD concentrations of SARS-CoV-2, influenza A and influenza B viruses.

Results show that the presence of the microorganisms at the concentrations tested did not interfere with the detection of SARS-CoV-2, influenza A or influenza B by generating false negative results. Please note that in the presence of SARS-coronavirus (SARS-CoV-1) at 1.00E+05 pfu/mL, a 3x LoD concentration of SARS-CoV-2 was not detected but influenza A and influenza B were detected at 3x LoD, when SARS-CoV-1 was at 1.00E+04 pfu/mL, 3x LoD of SARS-CoV-2 can be detected indicating SARS CoV-1 at 1e5 PFU/mL or higher may interfere with SARS-CoV-2 detection. However the likelihood of a co-infection with SARS CoV-1 is remote as the last confirmed case of SARS-CoV-1 was reported in 2004.

Table 6: Microbial interference

Microorganisms	Testing conc.*	SARS-CoV-2 result	Influenza A result	Influenza B result
Adenovirus	1.00E+05	Detected	Detected	Detected
Cytomegalovirus	1.00E+05	Detected	Detected	Detected
Epstein-Barr virus	1.00E+05	Detected	Detected	Detected
Human Enterovirus D	1.00E+05	Detected	Detected	Detected
Human Coronavirus 229E	1.00E+05	Detected	Detected	Detected
Human Coronavirus HKU1	1.00E+05	Detected	Detected	Detected
Human Coronavirus NL63	1.00E+05	Detected	Detected	Detected
Human Coronavirus OC43	1.00E+05	Detected	Detected	Detected
MERS-Coronavirus	1.00E+05	Detected	Detected	Detected
SARS Coronavirus	1.00E+05	Not Detected	Detected	Detected
	1.00E+04	Detected	Detected	Detected
Human Rhinovirus B	1.00E+05	Detected	Detected	Detected
Human Metapneumovirus 27	1.00E+05	Detected	Detected	Detected
Measles	1.00E+05	Detected	Detected	Detected

Microorganisms	Testing conc.*	SARS-CoV-2 result	Influenza A result	Influenza B result
Mumps	1.00E+05	Detected	Detected	Detected
Parainfluenzavirus Type 1	1.00E+05	Detected	Detected	Detected
Parainfluenzavirus Type 2	1.00E+05	Detected	Detected	Detected
Parainfluenzavirus Type 3	1.00E+05	Detected	Detected	Detected
Parainfluenzavirus Type 4A	1.00E+05	Detected	Detected	Detected
Respiratory Syncytial Virus A2	1.00E+05	Detected	Detected	Detected
Aspergillus Flavus var. flavus	1.00E+06	Detected	Detected	Detected
Bordetella pertussis	1.00E+06	Detected	Detected	Detected
Bordetella parapertussis	1.00E+06	Detected	Detected	Detected
Candida albicans	1.00E+06	Detected	Detected	Detected
Chlamydia pneumoniae	1.00E+06	Detected	Detected	Detected
Corynebacterium flavescens	1.00E+06	Detected	Detected	Detected
Escherichia coli	1.00E+06	Detected	Detected	Detected
Fusobacterium necrophorum subsp. Necrophorum	1.00E+06	Detected	Detected	Detected
Haemophilus influenzae	1.00E+06	Detected	Detected	Detected
Lactobacillus crispatus	1.00E+06	Detected	Detected	Detected
Legionella pneumophila	1.00E+06	Detected	Detected	Detected
Moraxella catarrhalis	1.00E+06	Detected	Detected	Detected
Mycoplasma genitalium	1.00E+06	Detected	Detected	Detected
Mycoplasma pneumoniae	1.00E+06	Detected	Detected	Detected
Mycobacterium tuberculosis	1.00E+06	Detected	Detected	Detected
Neisseria flava	1.00E+06	Detected	Detected	Detected
Neisseria meningitidis	1.00E+06	Detected	Detected	Detected
Pneumocystis jirovecii	5.00E+03	Detected	Detected	Detected
Pneumocystis jirovecii clinical Sample	1:10 diluted	Detected	Detected	Detected
Pseudomonas aeruginosa	1.00E+06	Detected	Detected	Detected
Staphylococcus epidermis	1.00E+06	Detected	Detected	Detected
Staphylococcus aureus	1.00E+06	Detected	Detected	Detected
Streptococcus pneumoniae	1.00E+06	Detected	Detected	Detected
Streptococcus pyogenes	1.00E+06	Detected	Detected	Detected
Streptococcus salivarius	1.00E+06	Detected	Detected	Detected
Nasal wash	1:10 diluted	Detected	Detected	Detected

*EB/mL, CFU/mL, IU/mL, $TCID_{50}$/mL, particles/mL, copies/mL, or PFU/mL

4.1.4. Endogenous and exogenous interference

Potentially interfering substances that may be commonly encountered in respiratory specimens were evaluated. Medically and/or physiologically relevant concentrations of potential interferents were tested with 1 influenza A strain, 1 influenza B strain and 1 SARS-CoV-2 strain at ~3x LoD. Each substance was tested, by introducing interferents into pooled negative nasopharyngeal swab specimens (NNPS) in UTM and tested with and without target strains. As shown in Table 7, substances at the concentrations tested did not interfere in the detection of SARS-CoV-2, influenza A and influenza B.

Table 7: Interference testing results

Potential Interferent	Active Ingredient	Concentration Tested
Mucin: bovine submaxillary gland, type I-S	Purified mucin protein	5 mg/mL
Blood	-	5% (v/v)
Peripheral blood mononuclear cell (PBMC)	-	1.0E+06 cells/mL
Nasal spray - Afrin / Anefrin	Oxymetazoline	5% (v/v)
Nasal corticosteroids - Flonase	Fluticasone	5% (v/v)
Nasal gel - Zicam	Galphimia glauca, Histaminum hydrochloricum, Luffa operculata, Sulphur	5% (v/v)
Throat lozenges, oral anesthetic and analgesic - Cepacol	Benzocaine, Menthol	5 mg/mL
Antibiotic, nasal ointment - Bactroban	Mupirocin	5 mg/mL
Antiviral drug - Relenza	Zanamivir	5 mg/mL
Antiviral drug - Tamiflu	Oseltamivir	7.5 mg/mL
Antimicrobial, systemic	Tobramycin	4 µg/mL

4.1.5. Competitive Inhibition

Competitive inhibition for the **cobas**® SARS-CoV-2 & Influenza A/B was evaluated by performing a series of dilution experiments using co-infected samples which contained one panel target at high concentration and one or more additional panel targets at low concentrations. Low concentrations were defined as ~3x LoD. High concentration targets were defined as either high (Ct 20-24) or very high (Ct 12-16) titers. Samples were tested in a series of dilutions until the low concentration targets were detected at 100% hit rate

The results showed that 1) 3x LoD of SARS-CoV-2 can be detected in presence of 8.3E+08 copies/mL of influenza A and 8.1E+05 copies/mL of influenza B; 2) 3x LoD of influenza A can

be detected in presence of 6.5E+06 copies/mL of influenza B and 3.6E+04 copies/mL SARS-CoV-2; 3) 3x LoD of influenza B can be detected in presence of 8.3E+08 copies/mL of influenza A and 3.6E+4 copies/mL of SARS-CoV-2. Competitive inhibition study concluded that the assay detects SARS-CoV-2 in the presence of competing targets of influenza A and influenza B at high levels. High SARS-CoV-2 levels (Ct < 16) inhibit influenza A/B detection. The results demonstrate that the **cobas**® SARS-CoV-2 & Influenza A/B will detect coinfection of influenza A, influenza B and SARS-CoV-2 viruses at the determined concentrations.

4.1.6. Matrix Equivalency

Matrix Equivalency was evaluated by spiking cultured viruses (SARS-CoV-2, influenza A and influenza B) at 2x and 5x LoD into nasopharyngeal swabs (NPS) collected in UTM, M4RT and Saline (0.9% NaCl) in addition to negative samples. Pooled negative clinical specimens and contrived positive clinical specimens were tested with the **cobas**® SARS-CoV-2 & Influenza A/B assay.

For each matrix, 10 replicates of negative samples, 30 replicates of positive samples at 2x LoD and 10 replicates of positive samples at 5x LoD were tested. The expected positive hit rate was 0% for negative samples, ≥95% for positive samples at 2x LoD and 100% for positive samples at 5x LoD. The results showed that the assay was able to correctly detect the presence of the viral targets suspended in all matrices (Table 8) demonstrating that UTM, M4RT, and Saline media are acceptable collection and transport media for use with the **cobas**® SARS-CoV-2 & Influenza A/B for use on the **cobas**® Liat® System.

Table 8: Summary of Matrix Equivalency Study Results

Target	Sample Concentration	NPS Collection Media	Hit Rate % (positive/tests)
SARS-CoV-2	Negative	UTM	0% (0/10)
		M4RT	0% (0/10)
		SALINE	0% (0/10)
	2x LoD	UTM	100% (30/30)
		M4RT	100% (30/30)
		SALINE	100% (30/30)
	5x LoD	UTM	100% (10/10)
		M4RT	100% (10/10)
		SALINE	100% (10/10)
Influenza A	Negative	UTM	0% (0/10)

Target	Sample Concentration	NPS Collection Media	Hit Rate % (positive/tests)
		M4RT	0% (0/10)
		SALINE	0% (0/10)
	2x LoD	UTM	100% (30/30)
		M4RT	100% (30/30)
		SALINE	97% (29/30)
	5x LoD	UTM	100% (10/10)
		M4RT	100% (10/10)
		SALINE	100% (10/10)
Influenza B	Negative	UTM	0% (0/10)
		M4RT	0% (0/10)
		SALINE	0% (0/10)
	2x LoD	UTM	100% (30/30)
		M4RT	100% (30/30)
		SALINE	100% (30/30)
	5x LoD	UTM	100% (10/10)
		M4RT	100% (10/10)
		SALINE	100% (10/10)

4.2. Non-clinical performance for Influenza A/B

4.2.1. Analytical sensitivity

The Limit of Detection (LoD) was evaluated using 3 strains of influenza A and 2 strains of influenza B. The LoD was determined by limiting dilution studies using these titered viruses. The viruses were spiked into negative nasopharyngeal swab (NPS) in UTM sample matrix. The LoD was determined to be 2×10^{-3} - 2×10^{-2} $TCID_{50}$/mL for influenza A strains, and 2×10^{-3} - 4×10^{-3} $TCID_{50}$/mL for influenza B strains (Table 9)

Table 9: LoD determination for influenza A and influenza B strains

Virus Strain	LoD ($TCID_{50}$/mL)
A/Brisbane/10/07	2.0×10^{-2}
A/Brisbane/59/07	2.0×10^{-3}
A/NY/01/2009	2.0×10^{-2}
B/Florida/04/06	2.0×10^{-3}
B/Malaysia/2506/04	4.0×10^{-3}

Virus Strain	LoD ($TCID_{50}$/mL)

Note: Analytical sensitivity of the **cobas**® SARS-CoV-2 & Influenza A/B assay was evaluated and shown to be equivalent to the **cobas**® Influenza A/B & RSV assay using cultured A/Brisbane/59/07 and B/Florida/04/06 (data not shown).

4.2.2. Reactivity/inclusivity

The reactivity study evaluates the ability to detect influenza strains representing temporal and geographical diversity. The reactivity/inclusivity was evaluated with 28 influenza A and 15 influenza B strains. Influenza A strains included 14 influenza A/H1 strains (including 3 H1N1 pdm09 strains), 12 influenza A/H3 strains (including 1 H3N2v strain), 1 influenza A/H7N9 strain, and 1 influenza A/H5N1 reassortant strain. Influenza B strains included that from both the Victoria lineage and Yamagata lineage. All strains were detected at the concentrations tested (Table 10). *In silico* analysis of influenza A and influenza B sequences predicted that the **cobas®** SARS-CoV-2 & Influenza A/B Test detects all the recorded circulating strains as of January 2023.

Table 10: Results of testing Influenza A and Influenza B strains

Virus Strain	Type / Subtype	Test Concentration	Inf A Result	Inf B Result
A/Aichi/2/68	Influenza A/H3N2	1.0×10^{2} $CEID_{50}$/mL	+	–
A/Alice	Influenza A/H3N2	5.0×10^{1} $CEID_{50}$/mL	+	–
A/Anhui/1/2013	Influenza A/H7N9 (Eurasian lineage)	1.0×10^{3} $TCID_{50}$/mL	+	–
A/Brisbane/10/07	Influenza A/H3N2	2.0×10^{-2} $TCID_{50}$/mL	+	–
A/Brisbane/59/07	Influenza A/H1N1	2.0×10^{-3} $TCID_{50}$/mL	+	–
A/Cambodia/X0810301/2013(H5N1)-PR8-IDCDC-RG34B	Influenza A/H5N1 reassortant	2.5×10^{1} $CEID_{50}$/mL	+	–
A/Denver/1/57	Influenza A/H1N1	1.0×10^{2} $CEID_{50}$/mL	+	–
A/FM/1/47	Influenza A/H1N1	1.0×10^{2} $CEID_{50}$/mL	+	–
A/H3/Perth/16/09	Influenza A/H3N2	2.5×10^{-1} $TCID_{50}$/mL	+	–
A/Hong Kong/8/68	Influenza A/H3N2	1.0×10^{2} $TCID_{50}$/mL	+	–
A/Indiana/8/2011	Influenza A/H3N2v	5.0×10^{-1} $TCID_{50}$/mL	+	–
A/Mal/302/54	Influenza A/H1N1	4.0×10^{2} $CEID_{50}$/mL	+	–
A/MRC2	Influenza A/H3	1.0×10^{2} $CEID_{50}$/mL	+	–
A/New Caledonia/20/99	Influenza A/H1N1	1.0×10^{2} $TCID_{50}$/mL	+	–
A/New Jersey/8/76	Influenza A/H1N1	1.0×10^{1} $CEID_{50}$/mL	+	–
A/NY/01/2009	Influenza A/H1N1 pdm09	2.0×10^{-2} $TCID_{50}$/mL	+	–
A/NY/02/2009	Influenza A/H1N1 pdm09	2.5×10^{-2} $TCID_{50}$/mL	+	–
A/NY/03/2009	Influenza A/H1N1 pdm09	2.0×10^{-1} $TCID_{50}$/mL	+	–
A/Port Chalmers/1/73	Influenza A/H3N2	1.0×10^{2} $CEID_{50}$/mL	+	–
A/PR/8/34	Influenza A/H1N1	5.0×10^{0} $TCID_{50}$/mL	+	–
A/Solomon Island/3/2006	Influenza A/H1N1	5.0×10^{-2} $TCID_{50}$/mL	+	–
A/Swine/1976/31	Influenza A/H1N1	1.0×10^{1} $CEID_{50}$/mL	+	–
A/Swine/Iowa/15/30	Influenza A/H1N1	1.0×10^{2} $CEID_{50}$/mL	+	–

Virus Strain	Type / Subtype	Test Concentration	Inf A Result	Inf B Result
A/Texas/50/2012	Influenza A/H3N2	1.0×10^{-1} $TCID_{50}$/mL	+	–
A/Victoria/3/75	Influenza A/H3N2	1.0×10^{2} $CEID_{50}$/mL	+	–
A/Victoria/361/2011	Influenza A/H3N2	2.0×10^{-2} $TCID_{50}$/mL	+	–
A/Weiss/43	Influenza A/H1N1	1.0×10^{3} $TCID_{50}$/mL	+	–
A/Wisconsin/67/05	Influenza A/H3N2	5.0×10^{-1} $TCID_{50}$/mL	+	–
B/Allen/45	Influenza B	5.0×10^{-1} $TCID_{50}$/mL	–	+
B/Brisbane/60/2008	Influenza B (Victoria lineage)	1.0×10^{-2} $TCID_{50}$/mL	–	+
B/Florida/04/06	Influenza B (Yamagata lineage)	2.0×10^{-3} $TCID_{50}$/mL	–	+
B/Florida/07/04	Influenza B (Yamagata lineage)	5.0×10^{-2} $TCID_{50}$/mL	–	+
B/GL/1739/54	Influenza B	2.0×10^{0} $TCID_{50}$/mL	–	+
B/HongKong/5/72	Influenza B	2.5×10^{-1} $TCID_{50}$/mL	–	+
B/Lee/40	Influenza B	2.5×10^{-1} $TCID_{50}$/mL	–	+
B/Malaysia/2506/04	Influenza B (Victoria lineage)	4.0×10^{-3} $TCID_{50}$/mL	–	+
B/Maryland/1/59	Influenza B	2.0×10^{-2} $TCID_{50}$/mL	–	+
B/Mass/3/66	Influenza B	1.0×10^{1} $TCID_{50}$/mL	–	+
B/Massachusetts/2/2012	Influenza B (Yamagata lineage)	5.0×10^{-3} $TCID_{50}$/mL	–	+
B/Nevada/03/2011	Influenza B (Victoria lineage)	2.5×10^{-1} $CEID_{50}$/mL	–	+
B/Taiwan/2/62	Influenza B	2.0×10^{-1} $TCID_{50}$/mL	–	+
B/Texas/6/2011	Influenza B (Yamagata lineage)	1.0×10^{-1} $TCID_{50}$/mL	–	+
B/Wisconsin/1/2010	Influenza B (Yamagata lineage)	5.0×10^{-1} $TCID_{50}$/mL	–	+

4.2.3. Cross reactivity

Cross-reactivity study evaluates potential cross reactivity with non-influenza microorganisms that may be present in nasopharyngeal swab samples. The cross reactivity was evaluated against a panel comprising human genomic DNA and 35 microorganisms. Bacteria and *Candida albicans* were tested at $\geq 10^6$ CFU/mL. Viruses were tested at $\geq 10^5$ $TCID_{50}$/mL, or the highest available concentration. No cross reactivity was observed for the human genomic DNA or the microorganisms at the concentrations tested (Table 11).

Table 11: Influenza A/B cross-reactivity testing results

Microorganism	Test Concentration		Inf A Result	Inf B Result
Adenovirus Type 1	9.0×10^{5}	$TCID_{50}$/mL	–	–
Adenovirus Type 7	1.4×10^{5}	$TCID_{50}$/mL	–	–
Cytomegalovirus	4.5×10^{4}	$TCID_{50}$/mL	–	–
Epstein Barr Virus	2.5×10^{5}	$TCID_{50}$/mL	–	–
Herpes Simplex Virus	1.4×10^{5}	$TCID_{50}$/mL	–	–
Human Coronavirus 229E	8.0×10^{3}	$TCID_{50}$/mL	–	–
Human Coronavirus OC43	8.0×10^{4}	$TCID_{50}$/mL	–	–
Human Enterovirus 68	1.0×10^{5}	$TCID_{50}$/mL	–	–
Human Metapneumovirus	7.0×10^{3}	$TCID_{50}$/mL	–	–
Human Parainfluenza Type 1	3.7×10^{5}	$TCID_{50}$/mL	–	–

Microorganism	Test Concentration		Inf A Result	Inf B Result
Human Parainfluenza Type 2	7.5×10^5	$TCID_{50}$/mL	–	–
Human Parainfluenza Type 3	4.5×10^5	$TCID_{50}$/mL	–	–
Human Rhinovirus Type 1A	8.0×10^5	$TCID_{50}$/mL	–	–
Measles	8.0×10^4	$TCID_{50}$/mL	–	–
Mumps Virus	8.0×10^4	$TCID_{50}$/mL	–	–
Varicella-Zoster Virus	4.4×10^3	$TCID_{50}$/mL	–	–
Bordetella pertussis	2.2×10^6	CFU/mL	–	–
Candida albicans	4.2×10^6	CFU/mL	–	–
Chlamydia pneumoniae	8.0×104	$TCID_{50}$/mL	–	–
Corynebacterium sp	3.6×10^6	CFU/mL	–	–
Escherichia coli	1.9×10^6	CFU/mL	–	–
Haemophilus influenzae	2.3×10^6	CFU/mL	–	–
Lactobacillus sp	1.9×10^6	CFU/mL	–	–
Legionella pneumophila	6.7×10^6	CFU/mL	–	–
Moraxella catarrhalis	2.5×10^6	CFU/mL	–	–
Mycobacterium tuberculosis	2.8×10^6	copies/mL†	–	–
Mycoplasma pneumoniae	2.9×10^6	copies/mL†	–	–
Neisseria elongate	2.0×10^6	CFU/mL	–	–
Neisseria meningitidis	2.2×10^6	CFU/mL	–	–
Pseudomonas aeruginosa	2.3×10^6	CFU/mL	–	–
Staphylococcus aureus	2.4×10^6	CFU/mL	–	–
Staphylococcus epidermidis	1.9×10^6	CFU/mL	–	–
Streptococcus pneumoniae	1.8×10^6	CFU/mL	–	–
Streptococcus pyogenes	2.5×10^6	CFU/mL	–	–
Streptococcus salivarius	4.3×10^6	CFU/mL	–	–
Human genomic DNA	1.0×10^4	copies/mL	–	–

† Testing was performed with genomic DNA due to difficulties in propagation of these bacteria.

4.2.4. Interfering microorganisms

Interfering microorganism study evaluates whether non-influenza microorganisms that may be present in nasopharyngeal swab samples can interfere in the detection of influenza A or influenza B. The panel comprising human genomic DNA and 35 microorganisms tested in the cross-reactivity study was tested for potential interference. Bacteria and *Candida albicans* were tested at $\geq 10^6$ CFU/mL and viruses were tested at $\geq 10^5$ $TCID_{50}$/mL or the highest available concentration, in the presence of 1 influenza A strain and 1 influenza B strain at ~3x LoD

concentration in negative NPS in UTM matrix. Results show that the presence of human genomic DNA or the microorganisms at the concentrations tested did not interfere with the detection of influenza A or influenza B (Table 12).

Table 12: Influenza A/B interfering microorganisms study results

Microorganism	Test Concentration		1 Influenza A & 1 Influenza B strain at ~3x LoD	
			Inf A Result	Inf B Result
Adenovirus Type 1	9.0×10^5	$TCID_{50}$/mL	+	+
Adenovirus Type 7	1.4×10^5	$TCID_{50}$/mL	+	+
Cytomegalovirus	4.5×10^4	$TCID_{50}$/mL	+	+
Epstein Barr Virus	2.5×10^5	$TCID_{50}$/mL	+	+
Herpes Simplex Virus	1.4×10^5	$TCID_{50}$/mL	+	+
Human Coronavirus 229E	8.0×10^3	$TCID_{50}$/mL	+	+
Human Coronavirus OC43	8.0×10^4	$TCID_{50}$/mL	+	+
Human Enterovirus 68	1.0×10^5	$TCID_{50}$/mL	+	+
Human Metapneumovirus	7.0×10^3	$TCID_{50}$/mL	+	+
Human Parainfluenza Type 1	3.7×10^5	$TCID_{50}$/mL	+	+
Human Parainfluenza Type 2	7.5×10^5	$TCID_{50}$/mL	+	+
Human Parainfluenza Type 3	4.5×10^5	$TCID_{50}$/mL	+	+
Human Rhinovirus Type 1A	8.0×10^5	$TCID_{50}$/mL	+	+
Measles	8.0×10^4	$TCID_{50}$/mL	+	+
Mumps Virus	8.0×10^4	$TCID_{50}$/mL	+	+
Varicella-Zoster Virus	4.4×10^3	$TCID_{50}$/mL	+	+
Bordetella pertussis	2.2×10^6	CFU/mL	+	+
Candida albicans	4.2×10^6	CFU/mL	+	+
Chlamydia pneumoniae	8.0×10^4	$TCID_{50}$/mL	+	+
Corynebacterium sp	3.6×10^6	CFU/mL	+	+
Escherichia coli	1.9×10^6	CFU/mL	+	+
Haemophilus influenzae	2.3×10^6	CFU/mL	+	+
Lactobacillus sp	1.9×10^6	CFU/mL	+	+
Legionella pneumophila	6.7×10^6	CFU/mL	+	+
Moraxella catarrhalis	2.5×10^6	CFU/mL	+	+
Mycobacterium tuberculosis	2.8×10^6	copies/mL†	+	+
Mycoplasma pneumoniae	2.9×10^6	copies/mL†	+	+
Neisseria elongata	2.0×10^6	CFU/mL	+	+
Neisseria meningitidis	2.2×10^6	CFU/mL	+	+
Pseudomonas aeruginosa	2.3×10^6	CFU/mL	+	+

Microorganism	Test Concentration		1 Influenza A & 1 Influenza B strain at ~3x LoD	
			Inf A Result	Inf B Result
Staphylococcus aureus	2.4×10^6	CFU/mL	+	+
Staphylococcus epidermidis	1.9×10^6	CFU/mL	+	+
Streptococcus pneumoniae	1.8×10^6	CFU/mL	+	+
Streptococcus pyogenes	2.5×10^6	CFU/mL	+	+
Streptococcus salivarius	4.3×10^6	CFU/mL	+	+
Human Genomic DNA	1.0×10^4	copies/mL	+	+

† Testing was performed with genomic DNA due to difficulties in propagation of these bacteria.

5. CLINICAL PERFORMANCE EVALUATION

The clinical performance of the **cobas®** SARS-CoV-2 & Influenza A/B test for the detection of SARS-CoV-2, influenza A, and influenza B was separately evaluated using unpaired retrospective and paired prospective clinical nasopharyngeal swab (NPS) and nasal swab (NS) specimens collected from individuals with signs and symptoms of respiratory viral infection. Testing of clinical samples was performed with the **cobas®** SARS-CoV-2 & Influenza A/B test at 10 point-of-care healthcare facilities (e.g., emergency rooms, outpatient clinics, and physician offices). For the SARS-CoV-2 target, results from **cobas®** SARS-CoV-2 & Influenza A/B were compared to results from three highly sensitive FDA-authorized laboratory-based RT-PCR EUA assays (composite comparator method). For influenza A/B targets, results from **cobas®** SARS-CoV-2 & Influenza A/B were compared to results from an acceptable molecular comparator for influenza (comparator method).

Prospective clinical specimens were collected and tested February–June 2022. In total, prospectively collected specimens from 640 evaluable symptomatic individuals were included in the analysis population for the evaluation of **cobas®** SARS-CoV-2 & Influenza A/B. No coinfections with SARS-CoV-2 and influenza A/B were detected by the comparator method. No prospective fresh specimens tested in this performance evaluation were influenza B positive by the comparator method.

Additionally, to supplement the prospective data for influenza A and influenza B, retrospective frozen positive and negative NPS (n=178) and NS (n=190) specimens prospectively obtained during the 2013-2014, 2014-2015, and 2019-2020 flu seasons and during the COVID-19

pandemic (March–June 2021) were distributed to 4 of the 10 sites and worked into the daily workflow of sites for testing.

The clinical performance of the **cobas®** SARS-CoV-2 & Influenza A/B test for the detection of SARS-CoV-2, influenza A, and influenza B from healthcare-provider collected prospective nasopharyngeal (NPS) specimens collected in UTM/UVT was evaluated from a total of 640 symptomatic subjects. Of these, 13 NPS specimens had no comparator results due to incidents (11) or missing/not tested (2); 11 NPS specimen results from **cobas®** SARS-CoV-2 & Influenza A/B were non-evaluable due to protocol deviation (8), not tested (1), or invalids (2). In addition, 178 retrospective NPS specimens (44 influenza A-positive, 22 influenza B-positive, and 112 negative) were tested at sites. Of these, two retrospective NPS samples were non-evaluable due to obtaining invalid results from the comparator device, and three obtained invalid results for influenza B with the candidate device, leaving 176 evaluable retrospective NPS samples for influenza A and 173 for influenza B. In total, the remaining 616 NPS specimens for SARS-CoV-2, 792 NPS specimens for influenza A, and 789 NPS specimens for influenza B were evaluable and included in the clinical performance evaluation of **cobas®** SARS-CoV-2 & Influenza A/B.

As shown in Table 13 for prospective symptomatic subjects, 101 NPS specimens tested positive for SARS-CoV-2 with both the **cobas®** SARS-CoV-2 & Influenza A/B test on **cobas®** Liat System and the composite comparator; five SARS-CoV-2-positive specimens tested negative for SARS-CoV-2 with the **cobas®** SARS-CoV-2 & Influenza A/B test. A total of 507 NPS specimens tested negative for SARS-CoV-2 with both the **cobas®** SARS-CoV-2 & Influenza A/B test and the composite comparator; three SARS-CoV-2-negative specimens tested positive for SARS-CoV-2 with the **cobas®** SARS-CoV-2 & Influenza A/B test. All discordant SARS-CoV-2 results showed late Ct values, which are indicative of NPS specimens from individuals with viral loads near or below the limit of detection of both **cobas®** SARS-CoV-2 & Influenza A/B and the composite comparator methods.

For SARS-CoV-2, the results of the clinical performance evaluation using NPS specimens from prospective symptomatic subjects demonstrated 95.3% positive percent agreement (PPA) (101/106; 95% score CI: 89.4% - 98.0%) and 99.4% negative percent agreement (NPA) (507/510; 95% score CI: 98.3% - 99.8%) as compared to the composite comparator method.

Table 13: Clinical performance comparison – SARS-CoV-2 for prospective NPS specimens

		Composite Comparator Method SARS-CoV-2 Result	
		Positive	Negative
cobas® SARS-CoV-2 & Influenza A/B on **cobas**® Liat® System **Nasopharyngeal Swab (NPS)**	Positive	101	3
	Negative	5	507
	PPA	95.3% (95% CI: 89.4% - 98.0%)	
	NPA	99.4% (95% CI: 98.3% - 99.8%)	

As shown in Table 14 for prospective symptomatic subjects, 18 NPS specimens tested positive for influenza A with both the **cobas**® SARS-CoV-2 & Influenza A/B test on **cobas**® Liat System and the comparator assay; one influenza A-positive specimen tested negative for influenza A with the **cobas**® SARS-CoV-2 & Influenza A/B test. A total of 595 NPS specimens tested negative for influenza A with both the **cobas**® SARS-CoV-2 & Influenza A/B test and the comparator assay; two influenza A-negative specimens tested positive for influenza A with the **cobas**® SARS-CoV-2 & Influenza A/B test.

For influenza A, the results of the clinical performance evaluation using NPS specimens from prospective symptomatic subjects demonstrated 94.7% PPA (18/19; 95% score CI: 75.4% - 99.1%) and 99.7% NPA (595/597; 95% score CI: 98.8% – 99.9%) as compared to the comparator method.

Table 14: Clinical performance comparison – Influenza A for prospective NPS specimens

		Comparator Method Influenza A Result	
		Positive	Negative
cobas® SARS-CoV-2 & Influenza A/B on **cobas**® Liat® System **Nasopharyngeal Swab (NPS)**	Positive	18	2
	Negative	1	595
PPA		94.7% (95% CI: 75.4% - 99.1%)	
NPA		99.7% (95% CI: 98.8% - 99.9%)	

As shown in Table 15 for retrospective NPS specimens, the results of the clinical performance evaluation for influenza A demonstrated 97.7% PPA (43/44; 95% score CI: 88.2% - 99.6%) and 99.2% NPA (131/132; 95% score CI: 95.8% – 99.9%) as compared to the comparator method.

Table 15: Clinical performance comparison – Influenza A for retrospective NPS specimens

		Comparator Method Influenza A Result	
		Positive	Negative
cobas® SARS-CoV-2 & Influenza A/B on **cobas**® Liat® System **Nasopharyngeal Swab (NPS)**	Positive	43	1
	Negative	1	131

PPA	97.7% (95% CI: 88.2% - 99.6%)
NPA	99.2% (95% CI: 95.8% - 99.9%)

As shown in Table 16 for retrospective NPS specimens, the results of the clinical performance evaluation for influenza B demonstrated 100.0% PPA (22/22; 95% score CI: 85.1% - 100.0%) and 100.0% NPA (151/151; 95% score CI: 97.5% - 100.0%) as compared to the comparator method.

For prospective symptomatic subjects, PPA was not calculable because no fresh specimens were influenza B-positive by the comparator method. For influenza B, the results of the clinical performance evaluation using NPS specimens from prospective symptomatic subjects demonstrated 100.0% NPA (616/616; 95% score CI: 99.4% – 100.0%) as compared to the comparator method.

Table 16: Clinical performance comparison – Influenza B for retrospective NPS specimens

		Comparator Method Influenza B Result	
		Positive	Negative
cobas® SARS-CoV-2 & Influenza A/B on **cobas**® Liat® System **Nasopharyngeal Swab (NPS)**	Positive	22	0
	Negative	0	151

PPA	100.0% (95% CI: 85.1% - 100.0%)
NPA	100.0% (95% CI: 97.5% - 100.0%)

5.1. Clinical performance evaluation using NS clinical specimens

The clinical performance of the **cobas®** SARS-CoV-2 & Influenza A/B test for the detection of SARS-CoV-2, influenza A, and influenza B from prospective nasal (NS) specimens collected in UTM/UVT was evaluated from a total of 640 symptomatic subjects; prospective NS specimens were comprised of either healthcare provider-collected (n=325, 50.8%) or self-collected swabs (n=315, 49.2%). Of these, 11 NS specimens had no comparator results due to incidents (9) or missing/not tested (2); 13 NS specimen results from **cobas®** SARS-CoV-2 & Influenza A/B were non-evaluable due to protocol deviation (8) or invalids (5). In addition, 190 retrospective NS specimens (37 influenza A-positive, 35 influenza B-positive, and 118 negative) were tested at sites. Of these, three retrospective NS samples were non-evaluable due to obtaining invalid results from the comparator device, and one was aborted by the candidate device, leaving 186 evaluable retrospective NS samples for influenza A and influenza B. In total, the remaining 616 NS specimens for SARS-CoV-2, 802 NS specimens for influenza A, and 802 NS specimens for influenza B were evaluable and included in the clinical performance evaluation of **cobas®** SARS-CoV-2 & Influenza A/B.

As shown in Table 17 for prospective symptomatic subjects, 105 NS specimens tested positive for SARS-CoV-2 with both the **cobas®** SARS-CoV-2 & Influenza A/B test on **cobas®** Liat System and the composite comparator; four SARS-CoV-2-positive specimens tested negative for SARS-CoV-2 with the **cobas®** SARS-CoV-2 & Influenza A/B test. A total of 503 NS specimens tested negative for SARS-CoV-2 with both the **cobas®** SARS-CoV-2 & Influenza A/B test and the composite comparator; four SARS-CoV-2-negative specimens tested positive for SARS-CoV-2 with the **cobas®** SARS-CoV-2 & Influenza A/B test. All eight of the discordant SARS-CoV-2 results showed late Ct values, which are indicative of NS specimens from individuals with viral loads near or below the limit of detection of both **cobas®** SARS-CoV-2 & Influenza A/B and the composite comparator methods.

For SARS-CoV-2, the results of the clinical performance evaluation using NS specimens from prospective symptomatic subjects demonstrated 96.3% PPA (105/109; 95% score CI: 90.9% - 98.6%) and 99.2% NPA (503/507; 95% score CI: 98.0% - 99.7%) as compared to the composite comparator method.

Table 17: Clinical performance comparison – SARS-CoV-2 for prospective NS specimens

		Composite Comparator Method SARS-CoV-2 Result	
		Positive	Negative
cobas® SARS-CoV-2 & Influenza A/B on **cobas**® Liat® System **Nasal Swab (NS)**	Positive	105	4
	Negative	4	503

PPA	96.3% (95% CI: 90.9% - 98.6%)
NPA	99.2% (95% CI: 98.0% - 99.7%)

Note: The nasal swabs were comprised of healthcare provider-collected nasal swab specimens and nasal swab specimens self-collected on-site with healthcare provider instructions.

As shown in Table 18 for prospective symptomatic subjects, all 20 NS specimens tested positive for influenza A with both the **cobas**® SARS-CoV-2 & Influenza A/B test on **cobas**® Liat System and the comparator assay. A total of 595 NS specimens tested negative for influenza A with both the **cobas**® SARS-CoV-2 & Influenza A/B test and the comparator assay; one influenza A-negative specimens tested positive for influenza A with the **cobas**® SARS-CoV-2 & Influenza A/B test.

For influenza A, the results of the clinical performance evaluation using NS specimens from prospective symptomatic subjects demonstrated 100.0% PPA (20/20; 95% score CI: 83.9% - 100.0%) and 99.8% NPA (595/596; 95% score CI: 99.1% - 100.0%) as compared to the comparator method.

Table 18: Clinical performance comparison – Influenza A for prospective NS specimens

		Comparator Method Influenza A Result	
		Positive	Negative
cobas® SARS-CoV-2 & Influenza A/B on **cobas**® Liat® System **Nasal Swab (NS)**	Positive	20	1
	Negative	0	595

PPA	100.0% (95% CI: 83.9% - 100.0%)
NPA	99.8% (95% CI: 99.1% - 100.0%)

Note: The nasal swabs were comprised of healthcare provider-collected nasal swab specimens and nasal swab specimens self-collected on-site with healthcare provider instructions.

As shown in Table 19 for retrospective NS specimens, the results of the clinical performance evaluation for influenza A demonstrated 97.2% PPA (35/36; 95% score CI: 85.8% - 99.5%) and 100.0% NPA (150/150; 95% score CI: 97.5% - 100.0%) as compared to the comparator method.

Table 19: Clinical performance comparison – Influenza A for retrospective NS specimens

		Comparator Method Influenza A Result	
		Positive	Negative
cobas® SARS-CoV-2 & Influenza A/B on **cobas**® Liat® System **Nasal Swab (NS)**	Positive	35	0
	Negative	1	150

PPA	97.2% (95% CI: 85.8% - 99.5%)
NPA	100.0% (95% CI: 97.5% - 100.0%)

As shown in Table 20 for retrospective NS specimens, the results of the clinical performance evaluation for influenza B demonstrated 100.0% PPA (32/32; 95% score CI: 89.3% - 100.0%) and 100.0% NPA (154/154; 95% score CI: 97.6% - 100.0%) as compared to the comparator method.

For prospective symptomatic subjects, PPA was not calculable because no fresh specimens were influenza B-positive by the comparator method. For influenza B, the results of the clinical performance evaluation using NS specimens from prospective symptomatic subjects demonstrated 100.0% NPA (616/616; 95% score CI: 99.4% - 100.0%) as compared to the comparator method.

Table 20: Clinical performance comparison – Influenza B for retrospective NS specimens

		Comparator Method Influenza B Result	
		Positive	Negative
cobas® SARS-CoV-2 & Influenza A/B on **cobas**® Liat® System **Nasal Swab (NS)**	Positive	32	0
	Negative	0	154

PPA	100.0% (95% CI: 89.3% - 100.0%)
NPA	100.0% (95% CI: 97.6% - 100.0%)

5.1.1. Expected Values

For the prospective clinical performance evaluation of **cobas**® SARS-CoV-2 & Influenza A/B, paired NPS and NS specimens from 640 evaluable subjects, including 616 evaluable results, were freshly collected and tested at 10 point-of-care clinical sites in the United States during February–June 2022. Expected value (as determined by **cobas**® SARS-CoV-2 & Influenza A/B) summaries for prospective specimens, stratified by specimen collection/testing site are presented for SARS-CoV-2 and influenza A targets in Table 21 and Table 22, respectively. No prospective fresh specimens tested in this performance evaluation were influenza B positive by either **cobas**® SARS-CoV-2 & Influenza A/B or comparator test methods.

Table 21 Expected value summary by clinical site for prospective clinical evaluation for SARS-CoV-2 (as determined by cobas® SARS-CoV-2 & Influenza A/B)

Clinical Site ID	Site location	NPS Specimens			NS Specimens		
		Total No.	No. Positive for SARS-CoV-2	Expected Value	Total No.	No. Positive for SARS-CoV-2	Expected Value
Overall		616	104	16.9%	616	109	17.7%
1	Albuquerque, NM	23	0	0.0%	22	1	4.5%
2	Vienna, VA	241	30	12.4%	240	34	14.2%
3	Northridge, CA	6	0	0.0%	6	0	0.0%
4	Savannah, GA	46	12	26.1%	46	12	26.1%
5	North Miami, FL	52	12	23.1%	52	11	21.2%
6	Indianapolis, IN	9	1	11.1%	8	1	12.5%
7	Las Vegas, NV	20	0	0.0%	20	0	0.0%
8	Evanston, IL	89	27	30.3%	89	27	30.3%
9	Seneca, SC	25	1	4.0%	28	2	7.1%
10	Rochester, NY	105	21	20.0%	105	21	20.0%

Table 22 Expected value summary by clinical site for prospective clinical evaluation for influenza A (as determined by cobas® SARS-CoV-2 & Influenza A/B)

Clinical Site ID	Site location	NPS Specimens			NS Specimens		
		Total No.	No. Positive for Influenza A	Expected Value	Total No.	No. Positive for Influenza A	Expected Value
Overall		616	20	3.2%	616	21	3.4%
1	Albuquerque, NM	23	1	4.3%	22	1	4.5%
2	Vienna, VA	241	6	2.5%	240	7	2.9%
3	Northridge, CA	6	0	0.0%	6	0	0.0%
4	Savannah, GA	46	2	4.3%	46	2	4.3%
5	North Miami, FL	52	0	0.0%	52	0	0.0%
6	Indianapolis, IN	9	0	0.0%	8	0	0.0%
7	Las Vegas, NV	20	0	0.0%	20	0	0.0%
8	Evanston, IL	89	2	2.2%	89	2	2.2%
9	Seneca, SC	25	2	8.0%	28	2	7.1%
10	Rochester, NY	105	7	6.7%	105	7	6.7%

5.2. Reproducibility

Reproducibility study assesses the total variability of the assay in detecting SARS-CoV-2, influenza A, and influenza B across operators, study sites, testing days, Analyzers, and assay tube lots. The reproducibility was evaluated at 3 study sites. Two operators at each of the 3 sites tested a 3-member reproducibility panel in triplicate on 5 different days, for a total of ~270 runs (3 panel members x 3 replicates x 2 operators x 5 days x 3 sites). Nine Analyzers and 3 assay tube lots were used. The reproducibility panel comprises a low positive and a moderate positive for each of SARS-CoV-2, influenza A, and influenza B, in addition to a negative sample. The expected result for the true negative panel member is “Not Detected,” while the expected result for the low positive and moderate positive panel member is “Detected.” Percent agreement with expected result, mean Ct, Ct SD, and Ct %CV are shown in Table 23–Table 25

Table 23: SARS-CoV-2 reproducibility

Number of Valid Test Runs		Negative	SARS-CoV-2 Low Positive	SARS-CoV-2 Moderate Positive
		266	263	268
Ct	Mean	-	33.3	32.1
Ct	SD	-	1.18	0.97
Ct	CV (%)	-	3.5	3.0
Site	1	100.0% (89/89)	100.0% (90/90)	98.9% (88/89)
Site	2	100.0% (90/90)	98.9% (89/90)	100.0% (89/89)
Site	3	100.0% (87/87)	97.6% (81/83)	100.0% (90/90)
Overall Hit Rate	Agreement (n/N)	100.0% (266/266)	98.9% (260/263)	99.6% (267/268)
Overall Hit Rate	95% CI	98.6% - 100.0%	96.7% - 99.6%	97.9% - 99.9%

Table 24: Influenza A reproducibility

Number of Valid Test Runs		Negative	Influenza A Low Positive	Influenza A Moderate Positive
		266	263	268
Ct	Mean	-	33.0	31.9
Ct	SD	-	0.97	0.79
Ct	CV (%)	-	2.9	2.5
Site	1	100.0% (89/89)	100.0% (90/90)	100.0% (89/89)
Site	2	100.0% (90/90)	95.6% (86/90)	100.0% (89/89)
Site	3	100.0% (87/87)	100.0% (83/83)	100.0% (90/90)
Overall Hit Rate	Agreement (n/N)	100.0% (266/266)	98.5% (259/263)	100.0% (268/268)
Overall Hit Rate	95% CI	98.6% - 100.0%	96.2% - 99.4%	98.6% - 100.0%

Table 25: Influenza B reproducibility

Number of Valid Test Runs		Negative	Influenza B Low Positive	Influenza B Moderate Positive
		266	263	268
Ct	Mean	-	30.2	29.3
Ct	SD	-	0.92	1.05
Ct	CV (%)	-	3.1	3.6

Number of Valid Test Runs		Negative	Influenza B Low Positive	Influenza B Moderate Positive
		266	**263**	**268**
Site	**1**	100.0% (89/89)	100.0% (90/90)	98.9% (88/89)
Site	**2**	100.0% (90/90)	100.0% (90/90)	100.0% (89/89)
Site	**3**	100.0% (87/87)	100.0% (83/83)	100.0% (90/90)
Overall Hit Rate	**Agreement (n/N)**	**100.0% (266/266)**	**100.0% (263/263)**	**99.6% (267/268)**
Overall Hit Rate	**95% CI**	98.6% - 100.0%	98.6% - 100.0%	97.9% - 99.9%

6. CONCLUSIONS

A comparison of the intended use, technological characteristics, and the results of non-clinical analytical and clinical performance studies demonstrate that **cobas**® SARS-CoV-2 & Influenza A/B for use on the **cobas**® Liat® System is **substantially equivalent** to the predicate device.