July 31, 2025

Roche Molecular Systems, Inc.
Claudia Machay
Regulatory Affairs Project Manager
4300 Hacienda Drive
Pleasanton, California 94588

Re: K243455
Trade/Device Name: cobas Respiratory 4-flex for use on the cobas 5800/6800/8800 Systems
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 5, 2024
Received: November 7, 2024

Dear Claudia Machay:

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 (the 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 available 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.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device" (https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality System (QS) regulation (21 CFR Part 820), which includes, but is not limited to, 21 CFR 820.30, Design controls; 21 CFR 820.90, Nonconforming product; and 21 CFR 820.100, Corrective and preventive action. Please note that regardless of whether a change requires premarket review, the QS regulation requires device manufacturers to review and approve changes to device design and production (21 CFR 820.30 and 21 CFR 820.70) and document changes and approvals in the device master record (21 CFR 820.181).

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 Part 803) for devices or postmarketing safety reporting (21 CFR Part 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 Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

All medical devices, including Class I and unclassified devices and combination product device constituent parts are required to be in compliance with the final Unique Device Identification System rule ("UDI Rule"). The UDI Rule requires, among other things, that a device bear a unique device identifier (UDI) on its label and package (21 CFR 801.20(a)) unless an exception or alternative applies (21 CFR 801.20(b)) and that the dates on the device label be formatted in accordance with 21 CFR 801.18. The UDI Rule (21 CFR 830.300(a) and 830.320(b)) also requires that certain information be submitted to the Global Unique Device Identification Database (GUDID) (21 CFR Part 830 Subpart E). For additional information on these requirements, please see the UDI System webpage at https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/unique-device-identification-system-udi-system.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 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,

ANNA M. MIELECH -S

Anna Mielech, PhD.
Deputy Branch Chief (Acting)
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: 07/31/2026
See PRA Statement below.

510(k) Number *(if known)*
K243455

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

Indications for Use *(Describe)*
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.

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.

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cobas® Respiratory 4-flex
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	Claudia Machay Phone: +41 79 563 9112 Email: claudia.machay@roche.com
Date Prepared	June, 24th, 2025
Proprietary Name	**cobas**® Respiratory 4-flex for use on the **cobas**® 5800/6800/8800 Systems
Common Name	**cobas**® Respiratory 4-flex
Classification Name	Multi-Target Respiratory Specimen Nucleic Acid Test Including Sars-CoV-2 And Other Microbial Agents
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
Regulation Class	Class II (special controls)
Product Codes	QOF
Predicate Devices	BioFire Respiratory Panel 2.1 (RP2.1) (DEN200031)
Establishment Registration	Roche Molecular Systems, Inc. (2243471)

1. DEVICE DESCRIPTION

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 PCR amplification and detection. 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 analytic module. Automated data management is performed by the **cobas**® 5800 System or **cobas**® 6800/8800 Systems software(s), which assigns results for all tests. Results can be reviewed directly on the system screen and printed as a report.

Nucleic acid from patient samples and added Internal Control RNA (RNA IC) molecules are simultaneously extracted. Nucleic acid is released by addition of proteinase and lysis reagent to the sample. 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 sample is achieved by the use of target-specific forward and reverse primers detecting conserved viral genome regions as shown in Table 1

Table 1: cobas® Respiratory 4-flex target regions

Targeted organism	Target gene (symbol)
Influenza A	Matrix protein 1 (M1)
Influenza B	Non-structural protein NS-1/2 (NS1/NEP)
Respiratory Syncytial Virus	Matrix protein (M)
SARS-CoV-2	ORF1 ab polyprotein (ORF1ab) and ORF 1a polyprotein (ORF1a)

Selective amplification of RNA IC is achieved by the use of 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. Roche's temperature assisted generation of signal (TAGS) technology, short TAGS technology, is introduced to differentiate up to three targets per fluorescence channel, enabling the detection

of up to14 targets, and the Internal Control, per 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 reporter 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 TAGS technology captures fluorescence in five available fluorescence channels in combination with three thermal channels (detection of fluorescence at three defined temperatures T1, T2 and T3).

The **cobas**® Respiratory 4-flex master mix contains detection probes which are specific for influenza A virus, influenza B virus, RSV, SARS-CoV-2 and the RNA Internal Control (RNA IC) nucleic acid, which enables simultaneous detection and differentiation of influenza A virus, influenza B virus, RSV, and SARS-CoV-2 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.

The RESP-4FLEX ASAP enables the system to differentiate and report the qualitative results of the four targets influenza A virus, influenza B virus, RSV and SARS-CoV-2. For each specimen the customer can test for any combination of the four enabled virus targets. Also, additional target calculation (digital reflex) can be ordered for the four enabled virus targets (influenza A virus, influenza B virus, RSV and SARS-CoV-2) on the **cobas**® 5800 System.

2. INTENDED 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.

3. TECHNOLOGICAL CHARACTERISTICS

The primary technological characteristics and intended use of the **cobas**® Respiratory 4-flex for use on the **cobas**® 5800/6800/8800 Systems are substantially equivalent to other legally marketed nucleic acid amplification test intended for the qualitative detection and differentiation of influenza A virus, influenza B virus, respiratory syncytial virus (RSV), and SARS-CoV-2 virus.

As indicated in Table 2, the **cobas**® Respiratory 4-flex for use on the **cobas**® 5800/6800/8800 Systems is substantially equivalent to significant characteristics of the identified predicate device, BioFire Respiratory Panel 2.1 (RP2.1) (DEN200031)

Table 2: Comparison of cobas® Respiratory 4-flex with the Predicate Device (BioFire® Respiratory Panel 2.1 (RP2.1))

Comparator	Candidate Device: cobas® Respiratory 4-flex for use on the cobas® 5800/6800/8800 Systems	Predicate Device: BioFire® Respiratory Panel 2.1 (DEN200031)
Regulation Number	21 CFR 866.3981	Same
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.	Same
Regulatory Class	Class II (special controls)	Same
Device Classification Name	Multi-target respiratory specimen nucleic acid test including sars-cov-2 and other microbial agents	Same
Product Code	QOF	Same

Intended 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.	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 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

Comparator	Candidate Device: cobas® Respiratory 4-flex for use on the cobas® 5800/6800/8800 Systems	Predicate Device: BioFire® Respiratory Panel 2.1 (DEN200031)
		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.
Conditions for use	For prescription use	same
Sample Types	Nasopharyngeal swab specimen	same
Analyte Targets	Influenza A virus Influenza B virus Respiratory Syncytial Virus (RSV) 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 the **cobas®** 5800/6800/8800 Systems	Automated by BioFire FilmArray 2.0 or BioFire FilmArray Torch systems

Comparator	Candidate Device: cobas® Respiratory 4-flex for use on the cobas® 5800/6800/8800 Systems	Predicate Device: BioFire® Respiratory Panel 2.1 (DEN200031)
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 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)
Result Analysis	Based on PCR cycle threshold analysis	Endpoint melting curve data to detect target specific amplicons

4. NON-CLINICAL PERFORMANCE EVALUATION

4.1. Analytical Sensitivity (Limit of Detection)

The Limit of Detection (LoD) study determines the lowest detectable concentration of RSV, influenza A (H3N2 and H1N1), SARS-CoV-2, influenza B (Victoria and Yamagata lineage), at which greater or equal to 95% of all (true positive) replicates test positive.

The LoD of **cobas**® Respiratory 4-flex was determined by analysis of serial dilutions co-formulated with cultured RSV, influenza A, influenza B, and inactivated SARS-CoV-2 diluted in nasopharyngeal matrix. Panels of at least five concentration levels plus a blank were tested over three lots of reagents, multiple runs, days, operators, and instruments. The results as well as the materials used are shown in Table 3.

Table 3: Limit of Detection by hit rate ≥ 95%

Target	Strain / Isolate	LoD by Hit Rate ≥ 95%	Hit Rate	Concentration Unit
Influenza A (H1N1)	Brisbane/02/2018	1.00E+02	95.2% (60/63)	cp/mL
Influenza A (H3N2)	A/Darwin/6/2021	5.00E+01	95.2% (60/63)	cp/mL
Influenza B (Victoria)	B/Austria/1359417/2021	2.50E+02	100.0% (63/63)	cp/mL
Influenza B (Yamagata)	Phuket/3073/13	8.00E+02	98.4% (62/63)	cp/mL
RSV A	Respiratory Syncytial Virus A2	4.00E+03	98.4% (62/63)	cp/mL
SARS-CoV-2	1st WHO International Standard NIBSC code 20/146*	8.00E+01	95.2% (60/63)	IU/mL

*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.

4.2. Precision – within laboratory

Precision of **cobas**® Respiratory 4-flex was determined by analysis of panels consisting of different cell culture strains in negative simulated clinical matrix stabilized in UTM™. Two dilution levels were tested in 216 replicates for each level across three lots of reagents using six instruments and five operators over twelve testing days. Each sample was carried through the entire **cobas**® Respiratory 4-flex procedure on fully automated **cobas**® 5800/6800/8800 Systems. Therefore, the precision reported here represents all aspects of the test procedure. The results are shown in Table 4 and Table 5. The results of this study revealed that **cobas**® Respiratory 4-flex for use on the **cobas**® 5800/6800/8800 Systems consistently detects the presence of all targets by achieving ≥99% hit rates around LoD (~1x LoD) and 100% hit rates above LoD (~3x LoD).

Table 4: Precision – Summary of hit rates and confidence intervals

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
Influenza A (H3N2)	~1x LoD	216	216	100	98.31	100
Influenza B (Victoria)	~3x LoD	216	216	100	98.31	100
Influenza B (Victoria)	~1x LoD	215	216	99.54	97.45	99.99
RSV A	~3x LoD	216	216	100	98.31	100
RSV A	~1x LoD	214	216	99.07	96.70	99.89
SARS-CoV-2	~3x LoD	216	216	100	98.31	100
SARS-CoV-2	~1x LoD	216	216	100	98.31	100
N/A	Blank	0	216	0	0.00	3.36

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	100.00%	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
Influenza A (H3N2)	~1x LoD	100.00%	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	100.00%	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
Influenza B (Victoria)	~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	100.00%	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
RSV A	~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	100.00%	35.62	0.03	0.09	0.00	0.00	0.03	0.09	0.00	0.00	0.32	0.89	0.32	0.90
SARS-CoV-2	~1x LoD	100.00%	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

4.3. Reproducibility

The reproducibility of **cobas**® Respiratory 4-flex was evaluated across multiple variables that theoretically could affect reported results, including: reagent lot, testing site/instrument, day, and run. Reproducibility was determined by analysis of panels consisting of different cell culture strains in negative simulated clinical matrix stabilized in UTM™ as well as one sample negative for all viruses (solely negative simulated clinical matrix stabilized in UTM™). Panels were tested in triplicate in each run. Two dilution levels per viral target were tested in 504 replicates distributed across two runs per day at three sites, using three lots of reagents, six days of testing per reagent lot using nine **cobas**® 5800/6800/8800 systems. The results are summarized in Table 6.

The system showed a 99.6% negative percent agreement with the Exact CI of 98.6-100.0%. The test results showed adequate lot-to-lot, instrument-to-instrument (site), day-to-day, and between run variation for the ~1x LoD, and ~3x LoD panel members (Table 6).

Table 6: Overall percentage agreement, mean estimate, standard deviations, and coefficients of variation (%) for cycle threshold values by viral target and expected viral concentration

Target Virus	Viral Concentration	(n/N) [a]	Percent Agreement (%) [b]	Mean Ct	Total SD	Total %CV	Site SD	Site %CV	Lot SD	Lot %CV	Day SD	Day %CV	Run SD	Run %CV	Within Run SD	Within Run %CV
Negative	0	502/504	99.6	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc
Influenza A	~1× LoD	504/504	100	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
Influenza A	~3× LoD	504/504	100	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	99.4	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
Influenza B	~3× LoD	503/503[c]	100	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	99.4	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
RSV	~3× LoD	504/504	100	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	100	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
SARS-CoV-2	~3× LoD	504/504	100	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 positive tests which contributed Ct values to the analysis. N is the total number of valid tests for the panel member.

[b] Percent agreement with expected results.

[c] A single invalid result was obtained for influenza B, resulting in 503 samples with valid results.

4.4. Inclusivity

The inclusivity for the detection of different strains of influenza A, influenza B, RSV and SARS-CoV-2 was assessed by testing relevant strains of each viral target. Each strain was tested with 3 replicates near LoD starting at ~3x LoD. The concentration which showed a 100% hit rate is shown in Table 7 through Table 10. In silico analysis on June 13th, 2025, of the dual target SARS-CoV-2 primer and probe binding regions indicates 99.99% detection of all available sequences in NCBI (>8.32M sequences, no predicted failed detections) and GISAID (>15.96M sequences, <10 predicted failed detections) databases. In silico analysis indicated inclusivity to all known SARS-CoV-2 variants.

Table 7: SARS-CoV-2 Inclusivity strains

Virus type	Strain	Concentration Tested (cp/mL)	100% hit rate at
SARS-CoV-2 Lineage B.1.1.7	England/204820464/2020	312	~3x LoD
SARS-CoV-2 Lineage B.1.351	South Africa/KRISP-K005325/2020	312	~3x LoD
SARS-CoV-2 Lineage P.1	Japan/TY7-503/2021	312	~3x LoD
SARS-CoV-2 B.1.617.2	USA/PHC658/2021	312	~3x LoD
SARS-CoV-2 Lineage B.1.1.529	USA/MD-HP20874/2021	312	~3x LoD
SARS-CoV-2	USA-WA1/2020	312	~3x LoD

Table 8 Influenza A Inclusivity strains

Virus type	Strain	Concentration Tested (cp/mL)	100% hit rate at
Influenza A H1N1	New Caledonia/20/99	165	~3x LoD
Influenza A H1N1	Brisbane/59/07	165	~3x LoD
Influenza A H1N1	California/07/09	165	~3x LoD
Influenza A H1N1	NY/03/09	165	~3x LoD
Influenza A H1N1	A/Victoria/2570/2019	165	~3x LoD
Influenza A H1N1	A/Wisconsin/588/2019	165	~3x LoD
Influenza A H1N1	A/Victoria/4897/2022	165	~3x LoD
Influenza A H1N1	A/Wisconsin/67/2022	330	~6x LoD
Influenza A H1N1	England/73/22	375	~6.8x LoD
Influenza A H1N1	England/55/22	375	~6.8x LoD
Influenza A H3N2	A/Port Chalmers/1/73	165	~3x LoD
Influenza A H3N2	Texas/50/12	242.7	~4.4x LoD
Influenza A H3N2	A/Victoria/3/75	165	~3x LoD

Virus type	Strain	Concentration Tested (cp/mL)	100% hit rate at
Influenza A H3N2	Wisconsin/67/05	165	~3x LoD
Influenza A H3N2	A/Darwin/9/2021	165	~3x LoD
Influenza A H3N2	Hong Kong/4801/14	165	~3x LoD
Influenza A H3N2	Hong Kong/8/68	165	~3x LoD
Influenza A H3N2	A/Perth/16/09	165	~3x LoD
Influenza A H3N2	Kansas/14/17	165	~3x LoD
Influenza A H3N2	Switzerland/9715293/13	165	~3x LoD
Influenza A H5N1	A/mallard/Wisconsin/2576/2009	165	~3x LoD
Influenza A H5N2	A/ruddy turnstone/New Jersey/828212/2001	165	~3x LoD
Influenza A H5N3	A/duck/Singapore/645/1997	165	~3x LoD
Influenza A H7N2	A/northern pintail/Illinois/10OS3959/2010	165	~3x LoD
Influenza A H7N8	A/mallard/Ohio/11OS2033/2011	165	~3x LoD
Influenza A H7N9	A/northern shoveler/Mississippi/11OS145/2011	165	~3x LoD
Influenza A H9N7	A/shorebird/Delaware Bay/31/1996	165	~3x LoD

Table 9: Influenza B Inclusivity strains

Virus type	Strain	Concentration Tested (cp/mL)	100% hit rate at
Influenza B – Victoria	Colorado/6/17	1779	~3x LoD
Influenza B – Victoria	B/Hong Kong/5/72	1779	~3x LoD
Influenza B - Victoria	Brisbane/60/08	1779	~3x LoD
Influenza B - Victoria	Florida/02/06	1779	~3x LoD
Influenza B – Yamagata	B/Massachusetts/2/2012	1779	~3x LoD
Influenza B – Yamagata	B/Wisconsin/1/2010	1779	~3x LoD
Influenza B – Yamagata	B/Florida/4/2006	1779	~3x LoD
Influenza B – Yamagata	Texas/6/11	1779	~3x LoD
Influenza B – Yamagata	Florida/07/04	1779	~3x LoD
Influenza B – Unknown	B/Taiwan/2/62	1779	~3x LoD
Influenza B – Unknown	B/Allen/45	1779	~3x LoD
Influenza B – Unknown	B/Lee/40	1779	~3x LoD

Table 10: Respiratory Syncytial Virus Inclusivity strains

Virus type	Strain	Concentration Tested (cp/mL)	100% hit rate at
RSV Type A	2006 Isolate	18510	~3x LoD
RSV Type A	02/2015	18510	~3x LoD
RSV Type A2	A2	18510	~3x LoD
RSV Type B	CH93(18)-18	18510	~3x LoD
RSV Type B	9320	18510	~3x LoD
RSV Type B	B WV/14617/85	18510	~3x LoD
RSV Type B	18537	18510	~3x LoD

4.5. Matrix equivalency

Equivalency between nasopharyngeal swabs and simulated clinical matrix stabilized in UTM-RT® was evaluated. Pooled negative individual clinical specimens (nasopharyngeal) and simulated clinical matrix stabilized in UTM™ were spiked with two co-formulated panels containing RSV, influenza A & SARS-CoV-2 and influenza B, at a concentration level of ~2x LoD. Forty-two replicates per concentration were tested for each sample type. All replicates tested with the 2x LoD panel were positive for the respective viral target for both matrices with 100% hit rate.

4.6. Analytical specificity (cross-reactivity and microbial interference)

The analytical specificity of **cobas**® Respiratory 4-flex was evaluated by testing a panel of microorganisms including those commonly found in the respiratory tract plus pooled human nasal wash.

The organisms listed in Table 11 were spiked at 1.00E+06 units/mL for bacteria and fungi and at 1.00E+05 units/mL for viruses unless otherwise noted. Testing was performed with each potential interfering organism in the absence and the presence of RSV, influenza A, influenza B and SARS-CoV-2, target spiked at ~3x LoD.

Negative results were obtained with **cobas**® Respiratory 4-flex for all microorganism samples without viral target and positive results were obtained for all microorganism samples with viral target spiked at ~3x LoD.

Table 11: Microorganisms tested for analytical specificity/cross reactivity

Microorganism	Concentration
Aspergillus flavus	1.00E+06 CFU/mL
Bordetella parapertussis	1.00E+06 CFU/mL
Bordetella pertussis	1.00E+06 CFU/mL
Candida albicans	1.00E+06 CFU/mL
Chlamydia pneumoniae	1.00E+06 IFU/mL
Corynebacterium diphtheriae	1.00E+06 CFU/mL
Cytomegalovirus	1.00E+05 $TCID_{50}$/mL
Epstein Barr virus	1.00E+05 cp/mL
Escherichia coli	1.00E+06 CFU/vial
Fusobacterium necrophorum	1.00E+06 CFU/mL
Haemophilus influenzae	1.00E+06 CFU/mL
Lactobacillus acidophilus	1.00E+06 CFU/vial
Legionella pneumophila	1.00E+06 CFU/mL
Measles virus	1.00E+05 $TCID_{50}$/mL
MERS-coronavirus*	1.00E+05 cp/mL
Moraxella catarrhalis	1.00E+06 CFU/mL
Mumps virus	1.00E+05 $TCID_{50}$/mL
Mycobacterium bovis	1.00E+06 CFU/mL
Mycoplasma genitalium	1.00E+06 CFU/vial
Mycoplasma pneumoniae	1.00E+06 CCU/mL
Neisseria elongata	1.00E+06 CFU/mL
Neisseria meningitidis	1.00E+06 CFU/mL
Pneumocystis jirovecii	5.00E+03 organisms/mL
Pseudomonas aeruginosa	1.00E+06 CFU/mL
SARS-coronavirus (SARS-CoV)*	1.00E+05 cp/mL
Staphylococcus aureus	1.00E+06 CFU/mL
Staphylococcus epidermidis	1.00E+06 CFU/mL
Streptococcus pneumoniae	1.00E+06 CFU/mL
Streptococcus pyogenes	1.00E+06 CFU/mL
Streptococcus salivarius	1.00E+06 CFU/mL

*Inactivated virus was used for testing

4.7. Analytical specificity – Interfering substances

Elevated levels of mucin (0.3 – 0.5% w/v) and whole blood (1.5 – 3.0% v/v) were tested in the absence and in the presence of RSV, influenza A, influenza B and SARS-CoV-2target spiked at ~3x LoD. The tested endogenous interferences were shown not to interfere with the test performance of **cobas**® Respiratory 4-flex.

Additionally, negative clinical nasopharyngeal swab specimens collected in Remel media (M4RT, M5 and M6) as well as Greiner tubes (VACUETTE® 3 mL Virus Stabilization Tube) were tested as equivalent collection media. The alternative collection media were tested unspiked and spiked at ~3x LoD. None of the alternative collection media showed interference with the test performance of **cobas**® Respiratory 4-flex.

In addition, drug compounds listed in Table 12 were tested in the presence and absence of all viral targets.

All potentially interfering substances, with the exception of FluMist® and Snuff Tobacco, have been shown to not interfere with the test performance. Negative results were obtained with **cobas**® Respiratory 4-flex for all samples without viral target and positive results were obtained for all samples with viral target.

As expected, FluMist® Quadrivalent, a live quadrivalent vaccine for administration by intranasal spray, consisting of two influenza A and two influenza B vaccine virus strains generated positive results for influenza A and influenza B and negative results for all other targets when solely testing FluMist®.

Furthermore, Snuff Tobacco was identified as a potential interferent of **cobas**® Respiratory 4-flex as invalid results were generated when testing Snuff Tobacco at 0.1% (w/v) without viral targets.

Table 12: Drug compounds tested for interference with cobas® Respiratory 4-flex

Generic drug name	Active Ingredient	Concentration
AXOTIDE Diskus Multidose 250 mcg	Fluticasone propionate	0.167 mg/mL
BACTROBAN Nasal Ointment	Mupirocin	0.20 mg/mL
BUDESONID Sandoz Nasal Spray 64 mcg	Budesonide	0.039 mg/mL
CEPACOL Extra Strength Sore Throat	Benzocaine	5 mg/mL
Chloraseptic max	Phenol	0.47 mg/mL

Generic drug name	Active Ingredient	Concentration
FLUMIST® Quadrivalent	live attenuated influenza A and B viruses	50000000 FFU/mL
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
NASIVIN Pur Spray 0.05%	Oxymetazoline	0.011 mg/mL
OBRACIN Inj Solution 40 mg/mL	Tobramycin	0.018 mg/mL
RELENZA Disk 5 mg	Zanamivir	0.0015 mg/mL
TAMIFLU Kaps 75 mg	Oseltamivir	0.0073 mg/mL
Snuff Tobacco	Nicotine	0.1% w/v
Vaseline	Petroleum Jelly	1% w/v
VICKS VapoRub	Eucalyptus Oil and Menthol	1% w/v
XYLOCAIN Spray 10%	Lidocaine	2.68 mg/mL

4.8. Co-infection (competitive interference)

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 were tested (Table 13). Twelve replicates were tested with one viral target at ~3x LoD which was mixed with a target at high concentration (1.0E+06 units/mL). None of the targets present at very high concentration interfered with the detection of other viral targets at low concentration levels.

Table 13: Combinations tested for potential competitive inhibition

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

Combination	Target 1 (high) ≥ 1.00E+06 unit/mL	Target 2 (low) ~3x LoD
12	SARS-CoV-2	RSV

4.9. Cross contamination

The cross-contamination rate for **cobas**® Respiratory 4-flex was determined by testing 480 replicates of negative simulated clinical matrix and 430 replicates of a high titer SARS-CoV-2 panel at approximately 6.50E+08 particles/mL. In total, five runs were performed on **cobas**® 6800/8800 Systems and 25 runs were performed on **cobas**® 5800 Systems with positive and negative samples in a checkerboard configuration. All 480 replicates of the negative sample were negative, resulting in a cross-contamination rate of 0% (upper one-sided 95% confidence interval 0.62%).

5. CLINICAL PERFORMANCE EVALUATION

5.1. Performance with prospective clinical specimens

The clinical performance of **cobas**® Respiratory 4-flex on the **cobas**® 5800/6800/8800 Systems was evaluated versus an FDA 510(k) cleared comparator in nasopharyngeal swab (NPS) specimens from patients experiencing signs and symptoms of respiratory viral infection. The sample set consisted of a combination of prospective specimens that were either freshly tested (Fresh prospective) or were frozen prior to testing (Frozen prospective) on **cobas**® Respiratory 4-flex. Fresh specimens were collected at eleven collection sites during the 2023-2024 respiratory viral season and tested with the **cobas**® Respiratory 4-flex using the **cobas**® 6800/8800 System at four (4) testing sites. Frozen specimens were prospectively collected during parts of the 2022-2023 respiratory viral season at seven (7) sites and the 2023-2024 respiratory viral season from 14 collection sites and tested with the **cobas**® Respiratory 4-flex assay at three (3) testing sites using the **cobas**® 6800/8800 Systems.

A total of 4,475 NPS specimens (1,869 fresh and 2,606 frozen) were enrolled for the prospective clinical study, of which 4,378 could be tested (1,832 fresh and 2,546 frozen) with **cobas**® Respiratory 4-flex on the **cobas**® 6800/8800 System and the comparator method. Thirty-five fresh specimens could not be tested due to instrument error or protocol deviations, and two (2) fresh specimens were unable to be tested due to sample processing error. Sixty frozen 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 fresh and 2,514 frozen). Five (5) fresh specimens and 32 frozen 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) fresh and one (1) frozen) were excluded from analysis due to inconclusive results obtained from the comparator test. **cobas**® Respiratory 4-flex demonstrated adequate clinical performance, the Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) point estimates between **cobas**® Respiratory 4-flex and the comparator for the different target pathogens are summarized in Table 14.

Table 14: Clinical performance between cobas® Respiratory 4-flex and comparator in the prospective studies

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

Analyte	Positive Percent Agreement (PPA) TP/(TP+FN)	Positive Percent Agreement (PPA) %	Positive Percent Agreement (PPA) 95% CI	Negative Percent Agreement (NPA) TN/(TN+FP)	Negative Percent Agreement (NPA) %	Negative Percent Agreement (NPA) 95% CI

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.

Note: TP (True positives) refers to the number of samples where both the **cobas**® Respiratory 4-flex and the comparator tests are positive; (FP) False positives refers to the number of samples where the **cobas**® Respiratory 4-flex is positive and the comparator is negative; FN (False Negatives) refers to the number of samples where the **cobas**® Respiratory 4-flex is negative and the comparator is positive; TN (True Negatives) refers to the number of samples where both the **cobas**® Respiratory 4-flex and the comparator tests are negative.

5.2. Performance with retrospective clinical specimens

In addition to the prospective clinical data for influenza A, influenza B, and RSV frozen archived NPS specimens in UTM or UVT from individuals with signs and symptoms of respiratory viral infection were tested (Retrospective). The retrospective samples were collected between 2014 and 2022 and were selected for inclusion in the study based on the historical result. Retrospective specimens were tested with **cobas**® Respiratory 4-flex at three (3) U.S testing sites using the **cobas**® 6800/8800 Systems. The comparator method utilized to demonstrate agreement was a U.S. FDA-cleared molecular assay.

A total of 770 NPS specimens were enrolled for the retrospective clinical study, and fifteen specimens were excluded from analysis due to obtaining invalid results on the **cobas**® Respiratory 4-flex. For the influenza A target, 98 additional specimens were excluded from analysis due to failed or invalid comparator test results, leaving 657 evaluable specimens for influenza A. For the influenza B target, 108 additional specimens were excluded from analysis due to failed or invalid comparator test results, leaving 647 evaluable specimens for influenza B. For the RSV target, 96 additional specimens were excluded from analysis due to failed or invalid comparator test results, leaving 659 evaluable specimens for RSV. A summary of the **cobas**® Respiratory 4-flex retrospective clinical study agreement with the expected results is provided in Table 15.

Table 15: Agreement of the cobas® Respiratory 4-flex and comparator test in retrospective samples

Analyte	Positive Percent Agreement (PPA) TP/(TP+FN)	Positive Percent Agreement (PPA) %	Positive Percent Agreement (PPA) 95% CI	Negative Percent Agreement (NPA) TN/(TN+FP)	Negative Percent Agreement (NPA) %	Negative Percent Agreement (NPA) 95% CI
Influenza A	61/61	100.0	(94.1%, 100.0%)	589/596	98.8	(97.6%, 99.4%)
Influenza B	39/40	97.5	(87.1%, 99.6%)	603/607	99.3	(98.3%, 99.7%)
RSV	104/104	100.0	(96.4%, 100.0%)	551/555	99.3	(98.2%, 99.7%)

CI: confidence interval; FN: false negative; FP: false positive; RSV: respiratory syncytial virus; TN: true negative; TP: true positive.

Note: TP (True positives) refers to the number of samples where both the **cobas**® Respiratory 4-flex and the comparator tests are positive; (FP) False positives refers to the number of samples where the **cobas**® Respiratory 4-flex is positive and the comparator is negative; FN (False Negatives) refers to the number of samples where the **cobas**® Respiratory 4-flex is negative and the comparator is positive; TN (True Negatives) refers to the number of samples where both the **cobas**® Respiratory 4-flex and the comparator tests are negative.

5.3. Clinical performance equivalency between the cobas® 6800/8800 and cobas® 5800 Systems

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(s), and the PPA and NPA were directly compared between the two systems. The results were equivalent between the instrument systems.

6. CONCLUSIONS

A comparison of the intended use, technological characteristics, and the results of analytical (non-clinical) and clinical performance studies demonstrates that the **cobas**® Respiratory 4-flex for use on the **cobas**® 5800/6800/8000 Systems is **substantially equivalent** to the predicate device.