cobas® Respiratory flex

Qualitative nucleic acid test for use on the cobas® 5800/6800/8800 Systems

For in vitro diagnostic use

cobas® Respiratory flex	P/N: 09623701190
For use on the cobas® 5800 System	
cobas® Respiratory flex Control Kit	P/N: 09623728190
cobas® Buffer Negative Control Kit	P/N: 09051953190
For use on the cobas® 6800/8800 Systems	
cobas® Respiratory flex Control Kit	P/N: 09623728190
cobas® Buffer Negative Control Kit	P/N: 09051953190 or P/N: 07002238190

Table of contents

Intended use

cobas® Respiratory flex for use on the **cobas**® 5800/6800/8800 Systems (**cobas**® Respiratory flex) is an automated, multiplex, nucleic acid test that utilizes real-time polymerase chain reaction (PCR) technology for simultaneous in vitro qualitative detection and differentiation of adenovirus (species B, C and E), common human coronaviruses (229E, HKU1, NL63, OC43), human metapneumovirus, human rhinovirus/enterovirus, influenza A virus, influenza B virus, parainfluenza viruses 1, 2, 3, and 4, respiratory syncytial virus (RSV), and SARS-CoV-2 in nasopharyngeal swab specimens obtained from individuals with signs and symptoms of respiratory tract infections in conjunction with clinical and epidemiological risk factors.

The detection and identification of specific viral nucleic acids from individuals presenting with signs and symptoms of a respiratory infection aids in the diagnosis of respiratory infection if used in conjunction with other clinical and epidemiological information. Negative results do not preclude a respiratory infection and should not be used as the sole basis for treatment or other patient management decisions. Conversely, positive results do not rule out coinfection with other organisms, and the agent detected may not be the definite cause of disease.

Due to the genetic similarity between human rhinovirus and enterovirus, the **cobas**® Respiratory flex test cannot reliably differentiate them.

Summary and explanation of the test

Background

Acute respiratory tract infections are significant causes of worldwide morbidity and mortality.[1-5] Acute upper respiratory tract infections (URTI), while less severe than lower respiratory tract infections (LRTI), are more common and a leading cause of physician visits and absenteeism.[6] URTIs most frequently manifest as the "Common Cold," a self-limiting infection characterized by common symptoms such as a runny nose, nasal congestion, sneezing, cough, tiredness, sore throat, or fever. Common Colds are rarely bacterial and most are caused by viruses such as rhinovirus, metapneumovirus, coronavirus, parainfluenza, adenovirus, RSV, and influenza.[6,7]

In higher-risk populations, such as infants, young children, elderly, or those with compromised immunity, transplants or chronic disease, URTIs can more often progress to serious LRTIs, such as pneumonia, bronchitis or bronchiolitis.[8,9] However, the infectious signs and symptoms of URTIs are insufficient, especially in the early infectious phase, to definitively diagnose the causative pathogen or clinically distinguish them from LRTIs that can be caused by an even wider range of pathogens that includes viruses, bacteria and fungi.[10] With nucleic acid amplification test (NAAT) technology, viral URTIs can be reliably detected using nasopharyngeal swab specimens in the outpatient setting, where a majority of the patients are presenting and where viral respiratory infections are more common.[11,12] The detection of LRTIs often requires more invasive sampling (such as tracheal aspirates, induced sputum or bronchoalveolar lavage) that is typically performed in the inpatient or interventional setting. The value of more easily diagnosing the most common viral causes of URTIs is that it enables a more informed assessment, including the need for empiric antibiotic therapy, infection control measures, additional testing or hospitalization.[13]

Influenza, RSV, and severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) cause URTIs and frequently progress to LRTIs, which deserve particular attention due to their higher (relative to other URTIs) worldwide incidence, morbidity, and mortality.[1,3,14] Effective diagnosis and differentiation of influenza, SARS-CoV-2, and RSV infection from other respiratory pathogens in select patients provides valuable diagnostic information. The global seasonality and clinical presentations of influenza and RSV overlap, with peaks of infectious activity occurring in the respective winter months for temperate climates in the Northern and Southern hemispheres.[15] Reliable and accurate detection of influenza and RSV infections can help to target the use of antivirals and implementation of infection control measures, avoid inappropriate antibiotic use, reduce ancillary testing and hospitalizations, and identify local outbreaks of disease sooner.

COVID-19 cases first appeared in late 2019, as an outbreak of this novel coronavirus spread worldwide, prompting the World Health Organization (WHO) to declare a public health emergency of international concern in early 2020.[16,17] Globally, as of January 2023, there have been more than 750 million confirmed cases of COVID-19 including 6.8 million deaths reported to WHO, although actual case numbers are estimated to be higher.[18] The implicated pathogen, SARS-CoV-2, is an enveloped ribonucleic acid virus of zoonotic origin.[19] Coronaviruses (CoVs) are a large family of viruses that are common in many different animal species, including some that are common in humans (e.g., 229E, NL63, OC43, and HKU1).[19,20]

Influenza virus is globally estimated to cause over one billion infections and 500,000 deaths each year, with the highest burdens in infants, young children, the elderly, and those with underlying medical conditions, such as chronic lung disease.[21] Influenza types A and B can cause human epidemics, however in the case of most human pandemics, novel strain emergence and a greater overall disease burden is attributed to type A. [2,22] RSV is a leading cause of LRTIs and hospitalizations in infants and children, with most children having an RSV infection by two years of age. [4,23] In children five years of age or younger, there are over three million hospitalizations and over 100,000 globally estimated deaths from lower respiratory RSV infections each year.[4] More recently, due in part to diagnostic improvements, RSV has also been associated with a substantial disease and health economic burden in older adults as well.[14]

Explanation of the test

cobas® Respiratory flex is a qualitative nucleic acid test for the use on the **cobas**® 5800 System, **cobas**® 6800 System or **cobas**® 8800 System for the detection and differentiation of adenovirus (species B,C and E), common human coronaviruses (229E, HKU1, NL63, OC43), human metapneumovirus, human rhinovirus/enterovirus, influenza A virus, influenza B virus, parainfluenza viruses 1, 2, 3, and 4, RSV, and SARS-CoV-2 RNA in nasopharyngeal swab samples collected in Copan Universal Transport Medium System (UTM-RT®) or BD™ Universal Viral Transport System (UVT) or equivalent. The RNA IC, used to monitor the entire sample preparation and PCR amplification process, is introduced into each specimen during sample processing. In addition, the test utilizes external controls (a low titer positive control and a negative control).

Principles of the procedure

cobas® Respiratory 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 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)
Adenovirus B/E	Terminal protein precursor (E2B)
Adenovirus C	Capsid protein precursor (L3)
Human Metapneumovirus	Matrix protein (M)
Enterovirus/Rhinovirus	5' untranslated region (5'UTR)
Coronavirus OC43	Replicase polyprotein 1 a/b (ORF1ab)
Coronavirus HKU1	Replicase polyprotein 1 a/b (ORF1ab)
Coronavirus 229E	Replicase polyprotein 1 a/b (ORF1ab)
Coronavirus NL63	Replicase polyprotein 1 a/b (ORF1ab)
Human Parainfluenza Virus 1	L polymerase protein (L)
Human Parainfluenza Virus 2	Large protein (L)
Human Parainfluenza Virus 3	Nucleocapsid protein (N)
Human Parainfluenza Virus 4	Large protein (L)

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 12 targets, and the Internal Control, per well. A thermostable DNA polymerase enzyme is used for amplification.

The **cobas**® Respiratory flex master mix contains detection probes which are specific for adenovirus (species B,C and E), common human coronaviruses (229E, HKU1, NL63, OC43), human metapneumovirus, human rhinovirus/enterovirus, influenza A virus, influenza B virus, parainfluenza viruses 1, 2, 3, and 4, RSV, and SARS-CoV-2, and the RNA Internal Control nucleic acid. 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 test captures fluorescence in five available fluorescence channels in combination with three thermal channels (detection of fluorescence at three defined temperatures T1 T2 and T3), which enables simultaneous detection and differentiation of the amplified adenovirus (species B,C and E), common human coronaviruses (229E, HKU1, NL63, OC43), human metapneumovirus, human rhinovirus/enterovirus, influenza A virus, influenza B virus, parainfluenza viruses 1, 2, 3, and 4, 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.

Reagents and materials

The materials provided for the **cobas**® Respiratory flex kit can be found in Table 2. Materials required, but not provided, can be found in Table 3 through Table 5 and Table 9 through Table 11.

Refer to the **Reagents and materials** section and **Precautions and handling requirements** section for the hazard information for the product.

cobas® Respiratory flex reagents and controls

All unopened reagents and controls shall be stored as recommended in Table 2 to Table 6.

Table 2 cobas® Respiratory flex

(RESP FLEX)

Store at 2-8°C

192 test cassette (P/N 09623701190)

Kit components	Reagent ingredients	Quantity per kit 192 tests
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% proteinase, glycerol EUH210: Safety data sheet available on request. EUH208: Contains Subtilisin from Bacillus subtilis. May produce an allergic reaction.	22.3 mL
RNA Internal Control (RNA IC)	Tris buffer, < 0.05% EDTA, < 0.001% non-target related armored RNA construct containing primer and probe specific sequence regions (non-infectious RNA in MS2 bacteriophage), < 0.1% sodium azide	21.2 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxybenzoate	21.2 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	7.5 mL
Respiratory flex Master Mix Reagent 2 (RESP FLEX MMX-R2)	Tricine buffer, potassium acetate, < 18% dimethyl sulfoxide, glycerol, < 0.1% Tween 20, EDTA, < 0.12% dATP, dCTP, dGTP, dUTPs, < 0.01% upstream and downstream primers, < 0.01% Internal Control forward and reverse primers, < 0.01% fluorescent-labeled oligonucleotide probes and the RNA Internal Control, < 0.01% oligonucleotide aptamer, < 0.1% Z05D DNA polymerase, < 0.10% AmpErase (uracil-N-glycosylase) enzyme (microbial), < 0.1% sodium azide	9.7 mL

Table 3 cobas® Respiratory flex Control Kit

(RESP FLEX CTL)

Store at 2–8°C

(P/N 09623728190)

Kit components	Reagent ingredients	Quantity per kit
Respiratory flex Positive Control (RESP FLEX CTL)	Tris buffer, < 0.05% Sodium azide, < 0.005% EDTA, 0.003% Poly rA, < 0.01% Non-infectious plasmid DNA (microbial) containing adenovirus sequence, < 0.01% Non-infectious plasmid DNA (microbial) containing coronavirus (229E) sequence, < 0.01% Non-infectious plasmid DNA (microbial) containing human metapneumovirus sequence, < 0.01% Non-infectious plasmid DNA (microbial) containing rhinovirus sequence, < 0.01% Non-infectious plasmid DNA (microbial) containing influenza A sequence, < 0.01% Non-infectious plasmid DNA (microbial) containing influenza B sequence, < 0.01% Non-infectious plasmid DNA (microbial) containing human parainfluenza virus 1 sequence, < 0.01% Non-infectious plasmid DNA (microbial) containing human parainfluenza virus 2 sequence, < 0.01% Non-infectious plasmid DNA (microbial) containing human parainfluenza virus 3 sequence, < 0.01% Non-infectious plasmid DNA (microbial) containing human parainfluenza virus 4 sequence, < 0.01% Non-infectious plasmid DNA (microbial) containing respiratory syncytial virus sequence, < 0.01% Non-infectious plasmid DNA (microbial) containing SARS-CoV-2 sequence	6.4 mL (16 x 0.4 mL)

Table 4 cobas® Buffer Negative Control Kit

(BUF (-) C)

Store at 2-8°C

For use on the **cobas**® 5800 System (P/N 09051953190)
For use on the **cobas**® 6800/8800 Systems (P/N 07002238190 or P/N 09051953190)

Kit components	Reagent ingredients	Quantity per kit
cobas® Buffer Negative Control (BUF (-) C)	Tris buffer, < 0.1% sodium azide, EDTA, 0.002% Poly rA RNA (synthetic)	16 mL (16 x 1 mL)

cobas® omni reagents for sample preparation

Table 5 cobas® omni reagents for sample preparation*

Reagents	Reagent ingredients	Quantity per kit	Safety symbol and warning**
cobas® omni MGP Reagent (MGP) Store at 2–8°C (P/N 06997546190)	Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	480 tests	Not applicable
cobas® omni Specimen Diluent (SPEC DIL) Store at 2–8°C (P/N 06997511190)	Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	4 x 875 mL	Not applicable
cobas® omni Lysis Reagent (LYS) Store at 2–8°C (P/N 06997538190)	43% (w/w) guanidine thiocyanate***, 5% (w/v) polydocanol***, 2% (w/v) dithiothreitol***, dihydro sodium citrate	4 x 875 mL	 **DANGER** H302: Harmful if swallowed H314: Causes severe skin burns and eye damage. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. EUH071: Corrosive to the respiratory tract. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection. P301 + P330 + P331: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol
cobas® omni Wash Reagent (WASH) Store at 15–30°C (P/N 06997503190)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2 L	Not applicable

* These reagents are not included in the **cobas**® Respiratory flex test kit. See listing of additional materials required (Table 9 through Table 11).

** Product safety labeling primarily follows EU GHS guidance.

***Hazardous substance

Reagent storage requirements

Reagents shall be stored and will be handled as specified in Table 6, Table 7 and Table 8.

When reagents are not loaded on the **cobas**® 5800 or **cobas**® 6800/8800 Systems, store them at the corresponding temperature specified in Table 6.

Table 6 Reagent storage (when reagent is not on the system)

Reagent	Storage temperature
cobas® Respiratory flex – 192T	2–8°C
cobas® Respiratory flex Control Kit	2–8°C
cobas® Buffer Negative Control Kit	2–8°C
cobas® **omni** Lysis Reagent	2–8°C
cobas® **omni** MGP Reagent	2–8°C
cobas® **omni** Specimen Diluent	2–8°C
cobas® **omni** Wash Reagent	15–30°C

Reagent handling requirements for cobas® 5800 System

Reagents loaded onto the **cobas**® 5800 System are stored at appropriate temperatures and their expiration is monitored by the system. The system allows reagents to be used only if all of the conditions shown in Table 7 are met. The system automatically prevents use of expired reagents. Table 7 allows the user to understand the reagent handling conditions enforced by the **cobas**® 5800 System.

Table 7 Reagent expiry conditions enforced by the **cobas**® 5800 System

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	On-board stability
cobas® Respiratory flex – 192T	Date not passed	90 days from first usage	Max 40 runs	Max 36 days[b]
cobas® Respiratory flex Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 36 days[b]
cobas® Buffer Negative Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 36 days[b]
cobas® **omni** Lysis Reagent	Date not passed	30 days from loading[b]	Not applicable	Not applicable
cobas® **omni** MGP Reagent	Date not passed	30 days from loading[b]	Not applicable	Not applicable
cobas® **omni** Specimen Diluent	Date not passed	30 days from loading[b]	Not applicable	Not applicable
cobas® **omni** Wash Reagent	Date not passed	30 days from loading[b]	Not applicable	Not applicable

[a]Kit consists of single use control vials.

[b]Time is measured from the first time that reagent is loaded onto the **cobas**® 5800 System.

Reagent handling requirements for cobas® 6800/8800 Systems

Reagents loaded onto the **cobas**® 6800/8800 Systems are stored at appropriate temperatures and their expiration is monitored by the system. The **cobas**® 6800/8800 Systems allow reagents to be used only if all of the conditions shown in Table 8 are met. The system automatically prevents use of expired reagents. Table 8 allows the user to understand the reagent handling conditions enforced by the **cobas**® 6800/8800 Systems.

Table 8 Reagent expiry conditions enforced by the **cobas**® 6800/8800 Systems

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	On-board stability (cumulative time on board outside refrigerator)
cobas® Respiratory flex – 192T	Date not passed	90 days from first usage	Max 40 runs	Max 40 hours
cobas® Respiratory flex Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 10 hours
cobas® Buffer Negative Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 10 hours
cobas® **omni** Lysis Reagent	Date not passed	30 days from loading[b]	Not applicable	Not applicable
cobas® **omni** MGP Reagent	Date not passed	30 days from loading[b]	Not applicable	Not applicable
cobas® **omni** Specimen Diluent	Date not passed	30 days from loading[b]	Not applicable	Not applicable
cobas® **omni** Wash Reagent	Date not passed	30 days from loading[b]	Not applicable	Not applicable

[a]Kit consists of single use control vials.

[b]Time is measured from the first time that reagent is loaded onto the **cobas**® 6800/8800 Systems.

Additional materials required for cobas® 5800 System

Table 9 Material and consumables for use on the **cobas**® 5800 System

Material	P/N
cobas® **omni** Processing Plate 24	08413975001
cobas® **omni** Amplification Plate 24	08499853001
cobas® **omni** Liquid Waste Plate 24	08413983001
Tip CORE TIPS with Filter, 1 mL	04639642001
Tip CORE TIPS with Filter, 300 µL	07345607001
cobas® **omni** Liquid Waste Container	07094388001
cobas® **omni** Lysis Reagent	06997538190
cobas® **omni** MGP Reagent	06997546190
cobas® **omni** Specimen Diluent	06997511190
cobas® **omni** Wash Reagent	06997503190
Solid Waste Bag or Solid Waste Bag With Insert	07435967001 or 08030073001
cobas® **omni** Secondary Tubes 13x75 (optional)	06438776001
MPA RACK 13 or 16 MM[a]	N/A
RD5 RACK – RD Standard rack[a]	N/A
16-position tube carrier[a]	09224319001

Material	P/N
5-position rack carrier[b]	09224475001

[a] Please contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack carriers accepted on the instruments and compatible with the assay.

[b] RD5 or MPA racks are required in combination with the 5-position Rack Carrier on the **cobas**® 5800 System.

Additional materials required for cobas® 6800/8800 Systems

Table 10 Materials and consumables for use on **cobas**® 6800/8800 Systems

Material	P/N
cobas® **omni** Processing Plate	05534917001
cobas® **omni** Amplification Plate	05534941001
cobas® **omni** Pipette Tips	05534925001
cobas® **omni** Liquid Waste Container	07094388001
cobas® **omni** Lysis Reagent	06997538190
cobas® **omni** MGP Reagent	06997546190
cobas® **omni** Specimen Diluent	06997511190
cobas® **omni** Wash Reagent	06997503190
Solid Waste Bag and Solid Waste Container or Solid Waste Bag With Insert and Kit Drawer	07435967001 and 07094361001 or 08030073001 and 08387281001
cobas® **omni** Secondary Tubes 13x75 (optional)	06438776001
MPA RACK 13 or 16 MM[a]	N/A
RD5 RACK – RD Standard rack[a]	N/A

[a] Please contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack carriers accepted on the instruments and compatible with the assay.

Additional materials required for pre-analytic workflow

Table 11 Other materials required for pre-analytic workflow

Material	P/N
cobas® Microbial Inactivation Solution	08185476001

Instrumentation and software required

The **cobas**® 5800 software and **cobas**® Respiratory flex analysis package for the **cobas**® 5800 System must be installed on the **cobas**® 5800 instrument. The Data Manager software and computer unit for the **cobas**® 5800 System will be provided with the system.

The **cobas**® 6800/8800 Systems software and **cobas**® Respiratory flex analysis package(s) for the **cobas**® 6800/8800 Systems must be installed on the instrument(s). The Instrument Gateway (IG) server will be provided with the system.

Table 12 Instrumentation

Equipment	P/N
cobas® 5800 System	08707464001
cobas® 6800 System (Option Moveable)	05524245001 and 06379672001
cobas® 6800 System (Fix)	05524245001 and 06379664001
cobas® 8800 System	05412722001
Sample Supply Module (**cobas**® 6800/8800 Systems only)	06301037001
Instrument Gateway	06349595001

Refer to the **cobas**® 5800 System or **cobas**® 6800/8800 Systems – User Assistance and/or User Guides for additional information.

Precautions and handling requirements

Warnings and precautions

As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

- For in vitro diagnostic use.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.[24,25] Only personnel proficient in handling infectious materials and the use of **cobas**® Respiratory flex and the **cobas**® 5800/6800/8800 Systems should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect following appropriate site procedures.
- If spillage of samples in MIS (which contains guanidine thiocyanate) occurs, do not allow it to come in contact with sodium hypochlorite containing disinfectants such as bleach. This mixture can produce a highly toxic gas.
- If spillage of samples in MIS occurs, FIRST clean with a suitable laboratory detergent and water, and then with 70% ethanol.
- MIS is light-sensitive and shipped in light-protective bottles. MIS must be stored upright.
- The use of sterile disposable pipettes and nuclease-free pipette tips is recommended. Use only supplied or specified required consumables to ensure optimal test performance.
- Safety Data Sheets (SDS) are available on request from your local Roche representative.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.
- Inform your local competent authority and manufacturer about any serious incidents which may occur when using this assay.

Reagent handling

- Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples or controls.
- Before use, visually inspect each reagent cassette, diluent, lysis reagent, and wash reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- **cobas**® **omni** Lysis Reagent and MIS contain guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water, otherwise, burns can occur.
- **cobas**® Respiratory flex test kit, **cobas**® Respiratory flex Control kit, **cobas**® Buffer Negative Control kit, **cobas**® **omni** MGP Reagent, and **cobas**® **omni** Specimen Diluent contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water, otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
- Do not allow **cobas**® **omni** Lysis Reagent or MIS, which contain guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.

- Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink, or smoke in designated work areas.
- Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples and **cobas**® Respiratory flex kits, **cobas**® Respiratory flex Control kit, **cobas**® Buffer Negative Control kit and **cobas**® **omni** reagents to prevent contamination. Avoid contaminating gloves when handling samples and controls.
- Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water. Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas**® 5800 or **cobas**® 6800/8800 instrument, follow the instructions in the **cobas**® 5800 or **cobas**® 6800/8800 Systems – User Assistance and/or User Guides to properly clean and decontaminate the surface of instrument(s).

Sample collection, transport, and storage

Note: Handle all samples and controls as if they are capable of transmitting infectious agents.

Store all samples at specified temperatures.
Sample stability is affected by elevated temperatures.
Always use caution when transferring specimens from a primary collection tube to a secondary tube.
Use pipettes with aerosol-barrier or positive-displacement tips to handle specimens.
Always use a new pipette tip for each specimen.
Ensure samples are equilibrated to room temperature prior to transfer into a **cobas**® **omni** Secondary Tube.

Sample collection

- Collect nasopharyngeal specimens according to standard collection technique using flocked or polyester-tipped swabs and immediately place in 3 mL of UTM-RT® or UVT or equivalent.
- Refer to the Instructions for Use of the Collection Devices for hazard information.

Transport and storage

- Transportation of collected specimens must comply with all applicable regulations for the transport of etiologic agents.
- Samples collected in UTM-RT® or UVT or equivalent:
- After collection, specimens can be stored for up to 12 hours at 2-25°C followed by up to 3 days at 2-8°C and at ≤ -18C for up to 30 days.
- Specimens are stable for up to three freeze/thaw cycles when frozen at ≤ -18°C.

Instructions for use

Procedural notes

- Do not use **cobas**® Respiratory flex reagents, **cobas**® Respiratory flex Control Kit, **cobas**® Buffer Negative Control Kit, or **cobas**® **omni** reagents after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- Ensure that specimen barcode labels on sample tubes are visible through the openings on the side of the sample racks. Refer to the **cobas**® 5800 System or **cobas**® 6800/8800 Systems User Guides for proper barcode specifications and additional information on loading sample tubes.
- Refer to the **cobas**® 5800 System or **cobas**® 6800/8800 Systems – User Assistance and/or User Guides for proper maintenance of instruments.

Processing of nasopharyngeal specimens

Prior to running **cobas**® Respiratory flex on the **cobas**® 5800/6800/8800 Systems, specimens have to be processed using a secondary tube with a maximum dead volume of 350µl. The **cobas**® **omni** Secondary Tube is the preferred option. Additional secondary tubes for testing **cobas**® Respiratory flex are available. Contact your local Roche representative for detailed testing instructions and an order of secondary tubes compatible with the instruments.

Note: Always use caution when transferring specimens from a primary collection tube to a secondary tube.
Use pipettes with aerosol-barrier or positive-displacement tips to handle specimens.
Always use a new pipette tip for each specimen.
Ensure samples are equilibrated to room temperature prior to transfer into a **cobas**® **omni** Secondary Tube.

Follow the steps below to transfer and dilute patient sample from a primary collection tube into a **cobas**® **omni** Secondary Tube:

- Confirm that the nasopharyngeal sample tube is properly labeled and contains a minimum of 0.4 mL of specimen. If stored frozen, thaw and equilibrate the sample to ambient temperature.
- Invert the MIS bottles two to four times before use.
- Transfer 0.8 mL of MIS into the prepared barcoded secondary tube. Prepared secondary tubes containing 0.8 mL of MIS can be stored closed for up to 7 days at 2-8°C.
- Unscrew the primary sample tube cap.
- Lift the cap and any attached swab to allow a pipette to be inserted into the sample tube.
- Transfer 0.4 mL into the prepared barcoded secondary tube containing MIS. No further mixing is required.
- Transfer the secondary tube to a rack.
- Close the primary sample tube cap.

Note: Nasopharyngeal specimens diluted in MIS may be stored for up to 8 hours at 37°C or 20 hours at 25°C.

Running cobas® Respiratory flex

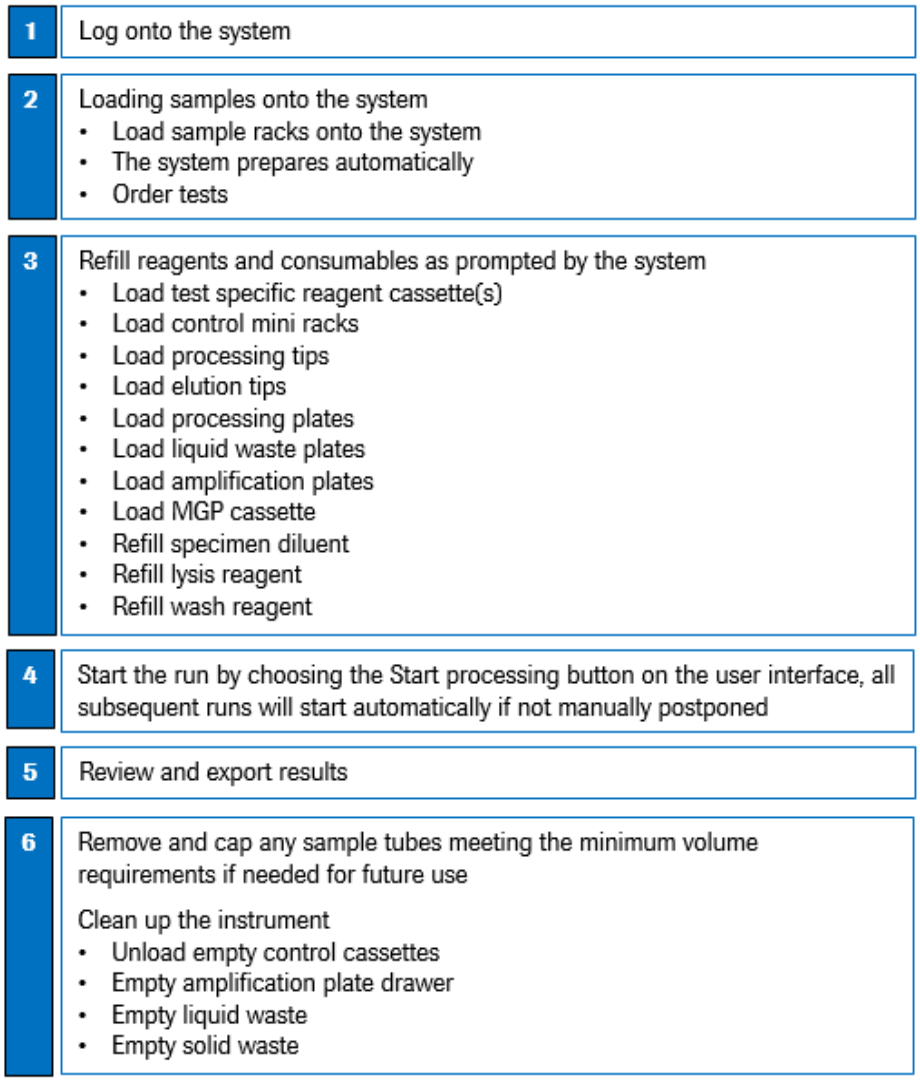
cobas® Respiratory flex can be run with a sample volume of 1.2 mL (0.4 mL of sample and 0.8 mL of MIS) of which 850 µL is processed. The sample type "Diluted in **cobas**® MIS" has to be selected to run the **cobas**® Respiratory flex.

Running cobas® Respiratory flex on cobas® 5800 System

The test procedure is described in detail in the **cobas**® 5800 Systems User Assistance and/or User Guide. Figure 1 below summarizes the procedure. **cobas**® Respiratory flex enables flexible ordering options:

- Configuring target groups: Each specimen can be tested for any combinations of viral targets (adenovirus, pan-coronavirus (229E, HKU1, NL63, OC43), human metapneumovirus, human rhinovirus/enterovirus, influenza A virus, influenza B virus, parainfluenza viruses 1, 2, 3, and 4, RSV, and SARS-CoV-2.
- Additional target calculation (digital reflex): Based on the initial test results additional targets out of the multiplex test panel can be ordered in the predefined time period. The additional ordered targets will then be calculated based on the already measured raw data. The sample does not need to be processed again to get the additional test results.

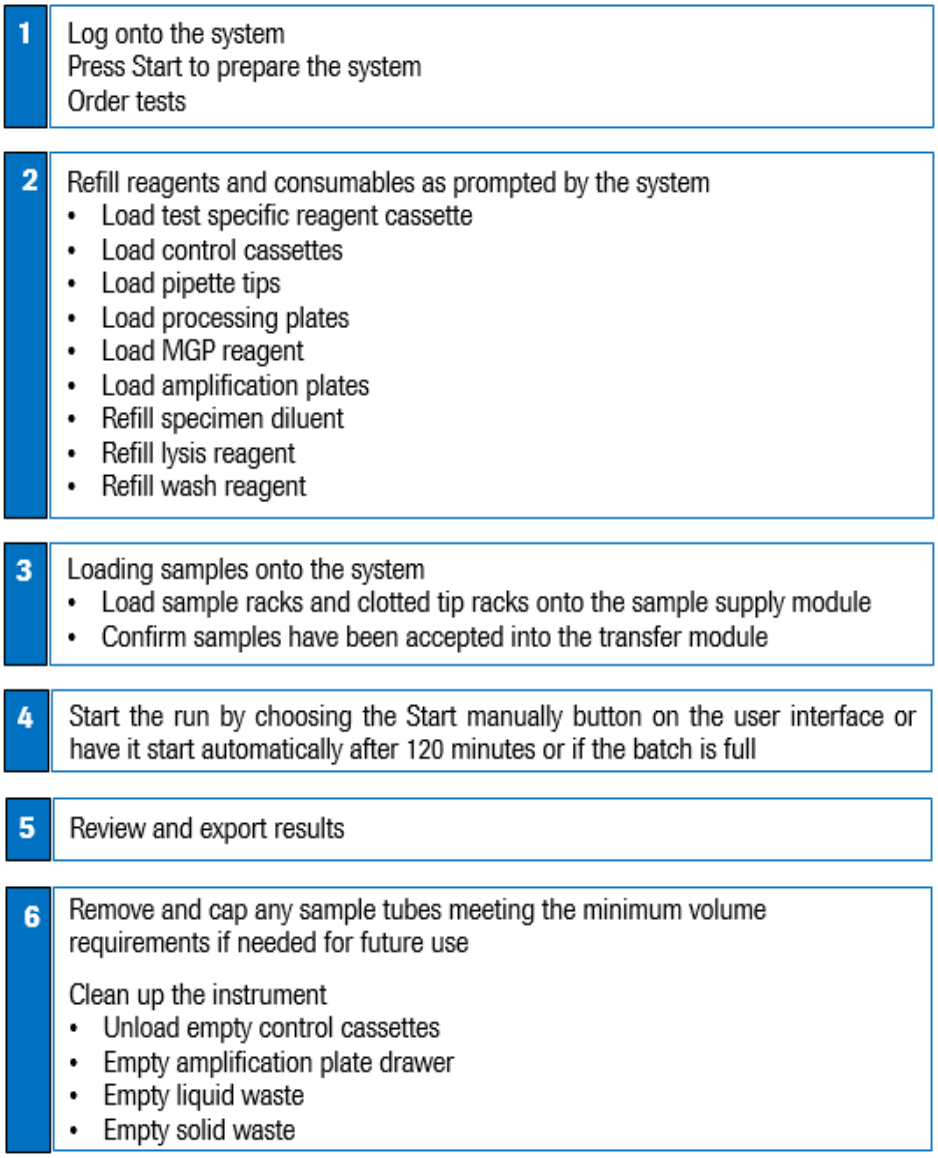
Figure 1 **cobas**® Respiratory flex test procedure on **cobas**® 5800 System



Running cobas® Respiratory flex on cobas® 6800/8800 Systems

The test procedure is described in detail in the **cobas**® 6800/8800 Systems User Assistance and/or User Guide. Figure 2 below summarizes the procedure. **cobas**® Respiratory flex enables flexible ordering options. Each specimen can be tested with the available Assay Specific Analysis Packages (ASAPs) enabling full panel testing, predefined subgroups or a digital reflex option. The automated digital reflex ASAP first calculates and displays a pre-defined set of targets (eg. FluA, FluB, RSV, SARS-CoV-2). If the sample is negative for these targets, the test will then automatically calculate and display the results of the remaining targets in the panel (automated digital reflex). If a sample is positive or invalid for one of the initial targets, the additional targets will not be calculated. Each sample is handled independently of the other samples. That means that some samples will only have results for the most common targets while other samples will display results for a larger number of targets.

Figure 2 **cobas**® Respiratory flex procedure on the **cobas**® 6800/8800 Systems



Results

The **cobas**® 5800 System and **cobas**® 6800/8800 Systems automatically detects adenovirus (species B,C and E), common human coronaviruses (229E, HKU1, NL63, OC43), human metapneumovirus, human rhinovirus/enterovirus, influenza A virus, influenza B virus, parainfluenza viruses 1, 2, 3, and 4, RSV, and SARS-CoV-2 for each individually processed sample and control, displaying individual target results for samples as well as test validity and overall results for controls.

Quality control and validity of results on the cobas® 5800 System

- One **cobas**® Buffer Negative Control [(-) Ctrl] and one positive control [RESP-FLEX (+) C] are processed at least every 72 hours or with every new kit lot. Positive and/or negative controls can be scheduled more frequently based on laboratory procedures and/or local regulations.
- In the **cobas**® 5800 System software and/or report, check for flags and their associated results to ensure the result validity.

Invalidation of results is performed automatically by the **cobas**® 5800 software based on negative or positive control failures.

NOTE: The **cobas**® 5800 System will be delivered with the standard setting of running a set of controls (positive and negative) with every run, but can be configured to a less frequent scheduling up to every 72 hours based on laboratory procedures and/or local regulations. Please contact your Roche service engineer and/or Roche customer technical support for more information.

Interpretation of results on the cobas® 5800 System

The results of the samples are shown in the **cobas**® 5800 software in the "Results" app.

For a valid control batch, check each individual sample for flags in the **cobas**® 5800 System software and/or report. The result interpretation should be as follows:

- Samples associated with a valid control batch are shown as "Valid" in the "Control result" column if all Control Target Results reported valid. Samples associated with a failed control batch are shown as "Invalid" in the "Control result" column if Control Results are reported invalid.
- If the associated controls of a sample result are invalid, a specific flag will be added to the sample result as follows:
- Q05D: Result validation failure because of an invalid positive control.
- Q06D: Result validation failure because of an invalid negative control.
- The values in "Results" column for individual sample target result should be interpreted as shown in Table 13.

If one or more sample targets are marked with "Invalid" the **cobas**® 5800 software shows a flag in the "Flag" column. More information on why the sample target(s) is reported invalid including flag information is shown in the detail view.

Invalid results for one or more target combinations are possible and are reported out specifically for each target. If any individual target result is invalid, the presence or absence of that individual target cannot be determined.

Table 13 Example of **cobas**® Respiratory flex results display on **cobas**® 5800 System

Sample ID	Test	Control Result	Flags*	Status	Result	Creation date/time
Sample_01	RESP-FLEX	Valid		Released	Negative (12)	7/7/2021 8:27:39 AM
Sample_C1	RESP-FLEX	Valid		Released	Invalid (12)	7/7/2021 8:27:39 AM
Sample_B1	RESP-FLEX	Valid		Released	Positive (1), Negative (11)	7/7/2021 8:27:39 AM
Sample_B2	RESP-FLEX	Valid		Released	Negative (12)	7/7/2021 8:27:39 AM
Sample_D1	RESP-FLEX	Valid		Released	Positive (2), Negative (10)	7/7/2021 8:27:39 AM
Sample_A6	RESP-FLEX	Valid		Released	Negative (12)	7/7/2021 8:27:39 AM
Sample_A2	RESP-FLEX	Invalid		Released	Invalid (12)	7/7/2021 8:27:39 AM

*The result overview shows a flag symbol in case of invalid results. Detailed flag descriptions are available in the result details.

Quality control and validity of results on the cobas® 6800/8800 Systems

- One **cobas**® Buffer Negative Control [(-) Ctrl] and one positive control [RESP-FLEX (+) C] are processed with each batch.
- In the **cobas**® 6800/8800 Systems software and/or report, check for flags and their associated results to ensure the batch validity.
- The batch is valid if no flags appear for any controls. If the batch is invalid, repeat testing of the entire batch.
- All flags are described in the **cobas**® 6800/8800 Systems User Guide.

Validation of results is performed automatically by the **cobas**® 6800/8800 Systems software based on negative and positive control performance.

Interpretation of results on the cobas® 6800/8800 Systems

For a valid batch, check each individual sample for flags in the **cobas**® 6800/8800 Systems software and/or report. The result interpretation should be as follows:

- A valid batch may include both valid and invalid sample results.
- Invalid results for one or more target combinations are possible and are reported out specifically for each target. If any individual target result is invalid, the presence or absence of that individual target cannot be determined.
- Other initial valid target results can be interpreted as described in the table. Results and their corresponding interpretation are shown in Table 15.

Results display examples for **cobas**® Respiratory flex are shown in Table 14.

Table 14 Example of **cobas**® Respiratory flex results display on **cobas**® 6800/8800 Systems

Sample ID	Test name*	Positive	Negative	Invalid	Status	Creation date/time
Sample_01	RESP-FLEX	0	12	NA	Released	7/7/2021 8:27:39 AM
Sample_C1	RESP-FLEX	0	0	12	Not Released	7/7/2021 8:27:39 AM
Sample_B1	RESP-FLEX	1	11	NA	Released	7/7/2021 8:27:39 AM
Sample_B2	RESP-FLEX	4	8	NA	Released	7/7/2021 8:27:39 AM
Sample_D1	RESP-FLEX	0	12	NA	Released	7/7/2021 8:27:39 AM
Sample_A6	RESP-FLEX	0	12	NA	Released	7/7/2021 8:27:39 AM
Sample_A2	RESP-FLEX	0	0	12	Released	7/7/2021 8:27:39 AM

* Test name might differ depending on the chosen ASAP for **cobas**® Respiratory flex

Interpretation of results

For a valid run/ control batch, check each individual sample for flags in the **cobas**® 5800 System and **cobas**® 6800/8800 Systems software and/or report. The result interpretation should be as follows:

- A valid batch may include both valid and invalid sample results.
- Invalid results for one or more target combinations are possible and are reported out specifically for each channel.
- Results of this test should only be interpreted in conjunction with information available from clinical evaluation of the patient and patient history.

Results display examples for **cobas**® Respiratory flex are shown in Table 13 and Table 14.

Results and their corresponding interpretation for detecting adenovirus (species B, C, and E), common human coronaviruses (229E, HKU1, NL63, OC43), human metapneumovirus, human rhinovirus/enterovirus, influenza A virus, influenza B virus, parainfluenza viruses 1, 2, 3, and 4, RSV, and SARS-CoV-2 are shown below (Table 15).

Table 15 Target results for individual target result interpretation

Target result*	Interpretation
Negative	No target signal detected for the corresponding viral target and IC signal detected.
Positive	Target signal detected for the corresponding viral target and IC signal may or may not be detected.

*Shown for each of the 12 viral targets (influenza A virus (FluA), influenza B virus (FluB), respiratory syncytial virus (RSV), SARS-CoV-2 (SCoV2), adenovirus (AdV), human metapneumovirus (MPV), human rhinovirus/enterovirus (EVRV), common human coronavirus (CoV) and parainfluenza viruses 1 (hPIV1), 2 (hPIV2), 3 (hPIV3), and 4 (hPIV4) individually.

If any individual target result is invalid, the presence or absence of that individual target cannot be determined. Other initial valid target results can be interpreted as described in Table 15.

Procedural limitations

- **cobas**® Respiratory flex has been evaluated only for use in combination with the **cobas**® Respiratory flex Control Kit, **cobas**® Buffer Negative Control Kit, **cobas**® **omni** MGP Reagent, **cobas**® **omni** Lysis Reagent, **cobas**® **omni** Specimen Diluent, and **cobas**® **omni** Wash Reagent for use on the **cobas**® 5800/6800/8800 Systems.
- Patient management decisions should not be made solely on the **cobas**® Respiratory flex test results, but rather with the consideration of clinical observations, patient history, recent exposures, epidemiological information and other diagnostic information.
- Reliable results depend on proper sample collection, storage and handling procedures. Individuals should not eat, drink, smoke, vape, or use snuff tobacco products 30 minutes prior to sample collection.
- FluMist® Quadrivalent, a live quadrivalent intranasal vaccine may result in positive results for influenza A and influenza B. Recent administration of FluMist® within 6 weeks prior to collection was not evaluated to assess the potential impact of interference with other targets on the clinical performance of the assay.
- This test is intended to be used with nasopharyngeal swab samples collected in a UTM-RT® or UVT or equivalent. Testing of other sample types with **cobas**® Respiratory flex may result in inaccurate results.
- Detection of respiratory viruses may be affected by sample collection methods, patient factors (e.g. presence of symptoms), and/or stage of infection.
- As with any molecular test, mutations within the target regions of **cobas**® Respiratory flex could affect primer and/or probe binding, resulting in failure to detect the presence of virus.
- False negative or invalid results may occur due to interference. The Internal Control is included in **cobas**® Respiratory flex to help identify the specimens containing substances that may interfere with nucleic acid isolation and PCR amplification.
- The addition of AmpErase enzyme into the **cobas**® Respiratory flex Master Mix reagent enables selective amplification of target RNA and DNA; however, good laboratory practices and careful adherence to the procedures specified in this Instructions For Use document are necessary to avoid contamination of reagents.

Non-clinical performance evaluation

Key performance characteristics

Analytical sensitivity (Limit of Detection)

The limit of detection (LoD) of **cobas**® Respiratory flex was determined by analysis of serial co-formulated dilutions for human common coronavirus, RSV, influenza A, influenza B, SARS-CoV-2, adenovirus, rhinovirus, human metapneumovirus and human parainfluenza viruses 1, 2, 3 & 4 diluted in negative simulated clinical matrix stabilized in UTM™. Panels of at least five concentration levels plus a blank were tested over three lots of **cobas**® Respiratory flex reagents, multiple runs, days, operators, and instruments. The results as well as the materials used are shown in Table 16.

Table 16 Limit of Detection by hit rate ≥ 95% and 95% Probit, including confidence intervals

Target	Strain / Isolate	LoD by Hit Rate ≥ 95%	95% LoD PROBIT	95% Confidence Interval	Concentration Unit
Influenza A (H1N1)	Brisbane/02/2018	1.00E+02	8.39E+01	6.59E+01 – 1.19E+02	cp/mL
Influenza A (H3N2)	A/Darwin/6/2021	5.00E+01	5.36E+01	4.06E+01 – 8.09E+01	cp/mL
Influenza B (Victoria)	B/Austria/1359417/2021	2.50E+02	2.28E+02	1.82E+02 – 3.16E+02	cp/mL
Influenza B (Yamagata)	Phuket/3073/13	8.00E+02	6.84E+02	5.57E+02 – 9.12E+02	cp/mL
RSV A	Respiratory Syncytial Virus A2	4.00E+03	3.28E+03	2.60E+03 – 4.58E+03	cp/mL
SARS-CoV-2	1st WHO International Standard NIBSC code 20/146	8.00E+01	7.07E+01	5.45E+01 – 1.04E+02	IU/mL
Adenovirus B	Type 3 Isolate 1921/08	5.00E+02	5.00E+02	4.30E+02 – 6.21E+02	cp/mL
Adenovirus C	1st WHO International Standard NIBSC code 16/324	1.20E+02	7.77E+01	5.92E+01 – 1.14E+02	IU/mL
human Metapneumovirus	hMPV-27 Type A2 IA27-2004	1.70E+03	1.96E+03	1.61E+03 – 2.55E+03	cp/mL
Rhinovirus B	B42 Zeptometrix 0810286CF	1.80E+03	9.07E+02	7.29E+02 – 1.21E+03	cp/mL
Coronavirus 229E	229E Zeptometrix 0810229CF	3.50E+02	3.64E+02	2.83E+02 - 5.23E+02	cp/mL
Coronavirus NL63	NL63 Zeptometrix 0810228CF-CL	1.80E+02	1.77E+02	1.34E+02 – 2.72E+02	cp/mL
Coronavirus OC43	OC43 Zeptometrix 0810024CF	1.60E+03	8.53E+02	6.50E+02 – 1.27E+03	cp/mL
Coronavirus HKU1	aRNA	2.40E+02	1.84E+02	1.44E+02 – 2.58E+02	cp/mL
human Parainfluenza 1	Type 1 Zeptometrix 0810014CF-CL	3.00E+03	2.11E+03	1.82E+03 – 2.61E+03	cp/mL
human Parainfluenza 2	Type 2 Zeptometrix 0810015CF-CL	7.00E+02	6.85E+02	5.13E+02 – 1.06E+03	cp/mL
human Parainfluenza 3	Type 3, Zeptometrix 0810016CF-CL	3.80E+03	2.56E+03	2.15E+03 – 3.26E+03	cp/mL
human Parainfluenza 4	Type 4a Zeptometrix 0810060CF-CL	4.80E+04	3.05E+04	2.49E+04 – 4.02E+04	cp/mL

Precision – within laboratory

Precision of **cobas**® Respiratory 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 **cobas**® Respiratory flex reagents using six instruments and five operators over twelve testing days. Each sample was carried through the entire **cobas**® Respiratory 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 17 and Table 18. The results of this study revealed that **cobas**® Respiratory flex for use on the **cobas**® 5800/6800/8800 Systems consistently detects the presence of all targets by achieving ≥95% hit rates around LoD (~1x LoD) and ≥99% hit rates above LoD (~3x LoD).

Table 17 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
Adenovirus B	~3x LoD	216	216	100	98.31	100
Adenovirus B	~1x LoD	216	216	100	98.31	100
human Metapneumovirus	~3x LoD	216	216	100	98.31	100
human Metapneumovirus	~1x LoD	216	216	100	98.31	100
Rhinovirus B	~3x LoD	216	216	100	98.31	100
Rhinovirus B	~1x LoD	216	216	100	98.31	100
Coronavirus 229E	~3x LoD	216	216	100	98.31	100
Coronavirus 229E	~1x LoD	216	216	100	98.31	100
human Parainfluenza 1	~3x LoD	216	216	100	98.31	100
human Parainfluenza 1	~1x LoD	216	216	100	98.31	100
human Parainfluenza 2	~3x LoD	216	216	100	98.31	100
human Parainfluenza 2	~1x LoD	216	216	100	98.31	100
human Parainfluenza 3	~3x LoD	216	216	100	98.31	100
human Parainfluenza 3	~1x LoD	215	216	99.54	97.45	99.99
human Parainfluenza 4	~3x LoD	216	216	100	98.31	100
human Parainfluenza 4	~1x LoD	214	216	99.07	96.70	99.89
N/A	Blank	0	216	0	0.00	3.36

Table 18 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%	37.28	0.09	0.24	0.08	0.21	0.00	0.00	0.07	0.20	0.48	1.28	0.50	1.34
Influenza A (H3N2)	~1x LoD	100.00%	39.00	0.13	0.33	0.13	0.34	0.23	0.60	0.00	0.00	1.02	2.62	1.06	2.73
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
Adenovirus B	~3x LoD	100.00%	30.50	0.18	0.58	0.00	0.00	0.06	0.19	0.00	0.00	0.69	2.28	0.72	2.36
Adenovirus B	~1x LoD	100.00%	31.22	0.07	0.21	0.06	0.18	0.02	0.07	0.00	0.00	0.16	0.52	0.19	0.59
human Metapneumovirus	~3x LoD	100.00%	34.18	0.08	0.24	0.02	0.06	0.05	0.15	0.00	0.00	0.24	0.70	0.26	0.76
human Metapneumovirus	~1x LoD	100.00%	35.15	0.08	0.23	0.02	0.07	0.04	0.11	0.00	0.00	0.30	0.86	0.32	0.90
Rhinovirus B	~3x LoD	100.00%	33.68	0.08	0.24	0.25	0.73	0.02	0.07	0.00	0.00	0.26	0.79	0.37	1.10
Rhinovirus B	~1x LoD	100.00%	34.74	0.04	0.10	0.20	0.56	0.07	0.19	0.00	0.00	0.30	0.87	0.37	1.06
Coronavirus 229E	~3x LoD	100.00%	33.11	0.12	0.36	0.05	0.15	0.00	0.00	0.00	0.00	0.45	1.36	0.47	1.41
Coronavirus 229E	~1x LoD	100.00%	33.63	0.08	0.23	0.03	0.08	0.00	0.00	0.03	0.09	0.32	0.95	0.33	0.98
human Parainfluenza 1	~3x LoD	100.00%	33.62	0.08	0.23	0.00	0.00	0.08	0.24	0.02	0.05	0.22	0.66	0.25	0.74
human Parainfluenza 1	~1x LoD	100.00%	34.46	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.99	0.34	0.99
human Parainfluenza 2	~3x LoD	100.00%	34.83	0.14	0.41	0.07	0.21	0.10	0.28	0.05	0.13	0.59	1.70	0.62	1.79
human Parainfluenza 2	~1x LoD	100.00%	36.45	0.11	0.30	0.06	0.17	0.15	0.41	0.00	0.00	0.80	2.21	0.83	2.27
human Parainfluenza 3	~3x LoD	100.00%	34.82	0.06	0.17	0.04	0.12	0.04	0.11	0.00	0.00	0.21	0.59	0.22	0.64
human Parainfluenza 3	~1x LoD	99.54%	35.72	0.09	0.25	0.02	0.06	0.04	0.11	0.00	0.00	0.26	0.71	0.27	0.77
human Parainfluenza 4	~3x LoD	100.00%	35.00	0.09	0.26	0.00	0.00	0.01	0.02	0.04	0.12	0.26	0.75	0.28	0.80
human Parainfluenza 4	~1x LoD	99.07%	35.65	0.07	0.20	0.02	0.05	0.04	0.12	0.00	0.00	0.31	0.88	0.33	0.91

Inclusivity

The inclusivity for the detection of different strains of influenza A, influenza B, RSV, SARS-CoV-2, adenovirus, human metapneumovirus, enterovirus, rhinovirus, human common coronavirus and human parainfluenza viruses 1, 2, 3 & 4 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 19 through Table 28.

Table 19 Influenza A Inclusivity strains

Virus type	Strain	Vendor ID	100% hit rate at
Influenza A H1N1	New Caledonia/20/99	0810036CF	~3x LoD
Influenza A H1N1	Brisbane/59/07	0810244CF	~3x LoD
Influenza A H1N1	California/07/09	0810165CF	~3x LoD
Influenza A H1N1	NY/03/09	0810249CF	~3x LoD
Influenza A H1N1	A/Victoria/2570/2019	SD-VIC219A-7	~3x LoD
Influenza A H1N1	A/Wisconsin/588/2019	SD-WA519A-8	~3x LoD
Influenza A H1N1	A/Victoria/4897/2022	SD-VIC9722B	~3x LoD
Influenza A H1N1	A/Wisconsin/67/2022	SD-WI6722MS1B	~6x LoD
Influenza A H1N1	England/73/22	GISAID ID EPI_ISL_15803829	~3x LoD
Influenza A H1N1	England/55/22	GISAID ID EPI_ISL_14387941	~3x LoD
Influenza A H3N2	A/Port Chalmers/1/73	VR-810	~3x LoD
Influenza A H3N2	Texas/50/12	0810238CF	~3x LoD
Influenza A H3N2	A/Victoria/3/75	VR-822	~3x LoD
Influenza A H3N2	Wisconsin/67/05	0810252CF	~3x LoD
Influenza A H3N2	A/Darwin/9/2021	SD-DRW921-6	~3x LoD
Influenza A H3N2	Hong Kong/4801/14	0810526CF	~3x LoD
Influenza A H3N2	Hong Kong/8/68	0810250CF	~3x LoD
Influenza A H3N2	A/Perth/16/09	0810251CF	~3x LoD
Influenza A H3N2	Kansas/14/17	0810586CF	~3x LoD
Influenza A H3N2	Switzerland/9715293/13	0810511CF	~3x LoD
Influenza A H5N1	A/mallard/Wisconsin/2576/2009	NR-31131	~3x LoD
Influenza A H5N2	A/ruddy turnstone/New Jersey/828212/2001	NR-44298	~3x LoD
Influenza A H5N3	A/duck/Singapore/645/1997	NR-3558	~3x LoD
Influenza A H7N2	A/northern pintail/Illinois/10OS3959/2010	NR-35979	~3x LoD
Influenza A H7N8	A/mallard/Ohio/11OS2033/2011	NR-36008	~3x LoD
Influenza A H7N9	A/northern shoveler/Mississippi/11OS145/2011	NR-36001	~3x LoD
Influenza A H9N7	A/shorebird/Delaware Bay/31/1996	NR-45171	~3x LoD

Table 20 Influenza B Inclusivity strains

Virus type	Strain	Vendor ID	100% hit rate at
Influenza B – Victoria	Colorado/6/17	0810573CF	~3x LoD
Influenza B – Victoria	B/Hong Kong/5/72	VR-823	~3x LoD
Influenza B - Victoria	Brisbane/60/08	0810254CF	~3x LoD
Influenza B - Victoria	Florida/02/06	0810037CF	~3x LoD
Influenza B – Yamagata	B/Massachusetts/2/2012	VR-1813	~3x LoD
Influenza B – Yamagata	B/Wisconsin/1/2010	VR-1883	~3x LoD
Influenza B – Yamagata	B/Florida/4/2006	VR-1804	~3x LoD
Influenza B – Yamagata	Texas/6/11	0810242CF	~3x LoD
Influenza B – Yamagata	Florida/07/04	0810256CF	~3x LoD
Influenza B – Unknown	B/Taiwan/2/62	VR-295	~3x LoD
Influenza B – Unknown	B/Allen/45	VR-102	~3x LoD
Influenza B – Unknown	B/Lee/40	VR-101	~3x LoD

Table 21 Respiratory Syncytial Virus Inclusivity strains

Virus type	Strain	Vendor ID	100% hit rate at
RSV Type A	2006 Isolate	0810040ACF-CL	~3x LoD
RSV Type A	02/2015	0810475CF	~3x LoD
RSV Type A2	A2	VR-1540	~3x LoD
RSV Type B	CH93(18)-18	0810040CF-CL	~3x LoD
RSV Type B	9320	VPL-030	~3x LoD
RSV Type B	B WV/14617/85	VR-1400	~3x LoD
RSV Type B	18537	VR-1580	~3x LoD

Table 22 SARS-CoV-2 Inclusivity strains

Virus type	Strain	Vendor ID	100% hit rate at
SARS-CoV-2 Lineage B.1.1.7	England/204820464/2020	0810614CFHI-CL	~3x LoD
SARS-CoV-2 Lineage B.1.351	South Africa/KRISP-K005325/2020	0810613CFHI-CL	~3x LoD
SARS-CoV-2 Lineage P.1	Japan/TY7-503/2021	0810616CFHI-CL	~3x LoD
SARS-CoV-2 B.1.617.2	USA/PHC658/2021	0810624CFHI-CL	~3x LoD
SARS-CoV-2 Lineage B.1.1.529	USA/MD-HP20874/2021	0810642CFHI-CL	~3x LoD
SARS-CoV-2	USA-WA1/2020	0810587CFHI	~3x LoD

Table 23 Adenovirus Inclusivity strains

Virus type	Subtype	Vendor ID	100% hit rate at
Human Mastadenovirus B	Type 03	0810062CF	~3x LoD
Human Mastadenovirus B	Type 7A	0810021CF	~3x LoD
Human Mastadenovirus B	Type 11	0810112CF	~3x LoD
Human Mastadenovirus B	Type 14	0810108CF	~3x LoD
Human Mastadenovirus B	Type 16	VR-17	~12x LoD
Human Mastadenovirus B	Type 21	0810116CF	~6x LoD
Human Mastadenovirus B	Type 34	VR-716	~3x LoD
Human Mastadenovirus B	Type 35	VR-718	~3x LoD
Human Mastadenovirus C	Type 1	VR-1	~3x LoD
Human Mastadenovirus C	Type 2	VR-846	~3x LoD
Human Mastadenovirus C	Type 5	0810020CF	~3x LoD
Human Mastadenovirus C	Type 5	0810020CF	~3x LoD
Human Mastadenovirus C	Type 6	VR-6	~3x LoD
Human Mastadenovirus E	Type 4	0810070CF	~3x LoD
Human Mastadenovirus E	Type 4	0810326CF	~3x LoD

Table 24 Human Metapneumovirus Inclusivity strains

Virus type	Type/Strain	Vendor ID	100% hit rate at
Human Metapneumovirus A1	Type 9 – Strain: IA3-2002	0810160CF	~3x LoD
Human Metapneumovirus A1	Type 16 – Strain:IA10-2003	0810161CF-CL	~3x LoD
Human Metapneumovirus A2	Type 27 – Strain: IA27-2004	0810164CF	~3x LoD
Human Metapneumovirus B1	Type 5 – Strain: Peru3-2003	0810158CF-CL	~6x LoD
Human Metapneumovirus B2	Type 8 – Strain: Peru6-2003	0810159CF	~3x LoD
Human Metapneumovirus B2	Type 18 – Strain: IA18-2003	0810162CF	~3x LoD

Table 25 Enterovirus Inclusivity strains

Virus type	Subtype	Vendor ID	100% hit rate at
Enterovirus A	Type A10	VR-168	~3x LoD
Enterovirus A	Type 71	VR-1775	~3x LoD
Enterovirus B	Type A9	0810017CF	~3x LoD
Enterovirus B	Type B3	0810074CF	~3x LoD
Enterovirus B	Type B4	0810075CF	~3x LoD
Enterovirus B	Type 6	0810076CF	~3x LoD
Enterovirus B	Type 9	0810077CF	~3x LoD
Enterovirus B	Type 11	0810023CF	~3x LoD
Enterovirus C	Type A21	VR-850	~3x LoD
Enterovirus C	Type A24	VR-1662	~3x LoD
Enterovirus D	Type 68	VR-1823	~3x LoD

Table 26 Rhinovirus Inclusivity strains

Virus type	Subtype	Vendor ID	100% hit rate at
Human Rhinovirus A	Type 1A	0810012CFN	~3x LoD
Human Rhinovirus A	Type 2	VR-482	~3x LoD
Human Rhinovirus A	Type 7	VR-1601	~35x LoD*
Human Rhinovirus A	Type 16	VR-283	~3x LoD
Human Rhinovirus A	Type 34	VR-1365	~3x LoD
Human Rhinovirus A	Type 57	VR-1600	~3x LoD
Human Rhinovirus A	Type 77	VR-1187	~3x LoD
Human Rhinovirus A	Type 85	VR-1195	~3x LoD
Human Rhinovirus B	Type 3	VR-483	~3x LoD
Human Rhinovirus B	Type 14	VR-284	~3x LoD
Human Rhinovirus B	Type 17	VR-1663	~3x LoD
Human Rhinovirus B	Type: 27	VR-502	~3x LoD
Human Rhinovirus B	Type 83	VR-1193	~3x LoD

*Human Rhinovirus Type 7 (ATCC VR-1601) is a strain that was in vitro derived from NIAID reagent V-127-001-021 (VR-117) by passage at ATCC and is not a clinical isolate with clinical significance. Based on in silico analysis that is representing a broader genetic variability of this subtype, type 7 strains of rhinovirus should be detected by **cobas**® Respiratory flex.

Table 27 Common Coronavirus Inclusivity strains

Virus type	Strain	Vendor ID	100% hit rate at
Coronavirus	229E	0810229CF	~3x LoD
Coronavirus	229E	VR-740	~3x LoD
Coronavirus	NL63	NR-470	~3x LoD
Coronavirus	OC43	VR-1558	~3x LoD

Table 28 Human Parainfluenzavirus Inclusivity strains

Virus type	Strain	Vendor ID	100% hit rate at
Human Parainfluenzavirus 1	N/A	0810014CF	~3x LoD
Human Parainfluenzavirus 1	C35	VR-94	~3x LoD
Human Parainfluenzavirus 2	N/A	0810015CF	~3x LoD
Human Parainfluenzavirus 2	Greer	VR-92	~3x LoD
Human Parainfluenzavirus 3	N/A	0810016CF	~3x LoD
Human Parainfluenzavirus 4A	N/A	0810060CF	~3x LoD
Human Parainfluenzavirus 4B	CH 19503	VR-1377	~3x LoD
Human Parainfluenzavirus 4B	N/A	0810060BCF	~3x LoD

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 three co-formulated panels containing human common coronavirus, RSV, influenza A & SARS-CoV-2 (panel 1), influenza B, adenovirus, rhinovirus & human parainfluenza virus 3 (panel 2) and human metapneumovirus, human parainfluenza viruses 1, 2 & 4 (panel 3) 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.

Analytical specificity (cross-reactivity and microbial interference)

The analytical specificity of **cobas**® Respiratory 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 29 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 human common coronavirus, RSV, influenza A, influenza B, SARS-CoV-2, adenovirus, rhinovirus, human metapneumovirus and human parainfluenza viruses 1, 2, 3 & 4 target spiked at ~3x LoD.

Negative results were obtained with **cobas**® Respiratory 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 29 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 TCID50/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 TCID50/mL
MERS-coronavirus	1.00E+05 cp/mL
Moracella catarrhalis	1.00E+06 CFU/mL
Mumps virus	1.00E+05 TCID50/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)	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

Analytical specificity – interfering substances

Elevated levels of mucin (0.3 – 0.5% w/v) and whole blood (1.5 – 3.0% v/v) in simulated clinical matrix stabilized in UTM-RT® were tested in the absence and in the presence of human common coronavirus, RSV, influenza A, influenza B, SARS-CoV-2, adenovirus, rhinovirus, human metapneumovirus and human parainfluenza viruses 1, 2, 3 & 4 target spiked at ~3x LoD. The tested endogenous interferences were shown not to interfere with the test performance of **cobas**® Respiratory 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 flex.

In addition, drug compounds listed in Table 30 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 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 flex as invalid results were generated when testing Snuff Tobacco at 0.1% (w/v) without viral target and negative/invalid results were observed when testing samples with viral targets.

Table 30 Drug compounds tested for interference with **cobas**® Respiratory 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
FLUMIST® Quadrivalent	live attenuated influenza A and B viruses	50000000 FFU/mL
Heel Lufteel 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%	Lidocain	2.68 mg/mL

Co-infection (competitive interference)

To assess potential competitive interference between the viral targets, a total of 30 panels composed of various combinations of the **cobas**® Respiratory flex targets were tested. This includes combinations of all medically relevant respiratory tract co-infections as listed in Table 31. Twelve replicates were tested with one or two viral targets at ~3x LoD which were 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 31 Combinations tested for potential competitive inhibition

Combination	Target 1 (high) ≥ 1.00E+06 unit/mL	Target 2 (low) ~3x LoD	Target 3 (low) ~3x LoD
1	Influenza A	Adenovirus	SARS-CoV-2
2	Influenza B	Adenovirus	SARS-CoV-2
3	RSV	Adenovirus	SARS-CoV-2
4	Common human coronavirus	Influenza A	SARS-CoV-2
5	Adenovirus	Influenza A	SARS-CoV-2
6	EV/RV	RSV	SARS-CoV-2
7	hMPV	RSV	SARS-CoV-2
8	SARS-CoV-2	EV/RV	Flu A
9	Influenza B	EV/RV	Flu A
10	RSV	EV/RV	Flu A
11	hPIV-1	Influenza B	Flu A
12	hPIV-2	Influenza B	Flu A
13	hPIV-3	SARS-CoV-2	Flu A
14	hPIV-4	SARS-CoV-2	Flu A
15	Influenza A	Common human coronavirus	Flu B
16	SARS-CoV-2	Common human coronavirus	Flu B
17	RSV	Common human coronavirus	Flu B
18	CoV	RSV	Flu B
19	Adenovirus	RSV	Flu B
20	EV/RV	Influenza A	Flu B
21	hMPV	Influenza A	Flu B
22	Influenza A	EV/RV	RSV
23	Influenza B	CoV	RSV
24	SARS-CoV-2	Adenovirus	RSV
25	hPIV-1	SARS-CoV-2	RSV
26	hPIV-2	SARS-CoV-2	RSV
27	hPIV-3	Influenza B	RSV
28	hPIV-4	Influenza B	RSV
29	Adenovirus	EV/RV	-
30	EV/RV	Adenovirus	-

Whole system failure

The whole system failure rate for **cobas**® Respiratory flex was determined by testing 100 replicates of negative simulated clinical matrix spiked with viral target. These samples were tested at a concentration of ~3x LoD. The results of this study determined that all replicates were valid and positive for the corresponding viral targets, resulting in a whole system failure rate of 0% (upper one-sided 95% confidence interval 2.95%).

Cross contamination

The cross-contamination rate for **cobas**® Respiratory 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%).

Clinical performance evaluation

The clinical performance of **cobas**® Respiratory flex on the **cobas**® 5800/6800/ 8800 Systems was evaluated versus FDA 510(k) cleared and CE-marked comparators in nasopharyngeal swab (NPS) specimens from symptomatic patients. The sample set consisted of a combination of prospective specimens that were frozen prior to testing on **cobas**® Respiratory flex (prospective samples) and retrospective archived clinical specimens that were collected in UTM-RT® or UVT.

A total of 1439 NPS specimens were included in the study (884 prospective and 555 archived) of which 1360 could be tested (824 prospective and 536 archived), and eventually 1306 (792 prospective and 514 archived) were evaluable. **cobas**® Respiratory flex demonstrated good clinical performance, the respective overall Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) point estimates between **cobas**® Respiratory flex and the respective comparators for the different target pathogens are summarized in Table 32.

Table 32 Summary of agreement analysis between **cobas**® Respiratory flex and comparators

Target Virus	Sample Category	PPA (a/a+b)	PPA 95% CI	NPA (c/c+d)	NPA 95% CI	OPA (a + d/N)	OPA 95% CI
Influenza A	Prospective	100.0% (8/8)	(67.6%, 100.0%)	99.5% (779/783)	(98.7%, 99.8%)	99.5% (787/791)	(98.7%, 99.8%)
Influenza A	Archived	100.0% (44/44)	(92.0%, 100.0%)	98.2% (331/337)	(96.2%, 99.2%)	98.4% (375/381)	(96.6%, 99.3%)
Influenza A	**Overall**	**100.0% (52/52)**	**(93.1%, 100.0%)**	**99.1% (1110/1120)**	**(98.4%, 99.5%)**	**99.1% (1162/1172)**	**(98.4%, 99.5%)**
Influenza B	Prospective	100.0% (1/1)	(20.7%, 100.0%)	100.0% (791/791)	(99.5%, 100.0%)	100.0% (792/792)	(99.5%, 100.0%)
Influenza B	Archived	100.0% (8/8)	(67.6%, 100.0%)	99.4% (361/363)	(98.0%, 99.8%)	99.5% (369/371)	(98.1%, 99.9%)
Influenza B	**Overall**	**100.0% (9/9)**	**(70.1%, 100.0%)**	**99.8% (1152/1154)**	**(99.4%, 100.0%)**	**99.8% (1161/1163)**	**(99.4%, 100.0%)**
RSV	Prospective	33.3% (1/3)	(6.1%, 79.2%)	100.0% (789/789)	(99.5%, 100.0%)	99.7% (790/792)	(99.1%, 99.9%)
RSV	Archived	100.0% (47/47)	(92.4%, 100.0%)	99.4% (333/335)	(97.8%, 99.8%)	99.5% (380/382)	(98.1%, 99.9%)
RSV	**Overall**	**96.0% (48/50)**	**(86.5%, 98.9%)**	**99.8% (1122/1124)**	**(99.4%, 100.0%)**	**99.7% (1170/1174)**	**(99.1%, 99.9%)**
SARS-CoV-2	Prospective	97.4% (76/78)	(91.1%, 99.3%)	98.2% (701/714)	(96.9%, 98.9%)	98.1% (777/792)	(96.9%, 98.8%)
SARS-CoV-2	Archived	100.0% (47/47)	(92.4%, 100.0%)	0/0	Not Calculable	100.0% (47/47)	(92.4%, 100.0%)
SARS-CoV-2	**Overall**	**98.4% (123/125)**	**(94.4%, 99.6%)**	**98.2% (701/714)**	**(96.9%, 98.9%)**	**98.2% (824/839)**	**(97.1%, 98.9%)**
Adenovirus	Prospective	100.0% (2/2)	(34.2%, 100.0%)	99.6% (785/788)	(98.9%, 99.9%)	99.6% (787/790)	(98.9%, 99.9%)
Adenovirus	Archived	100.0% (37/37)	(90.6%, 100.0%)	95.6% (328/343)	(92.9%, 97.3%)	96.1% (365/380)	(93.6%, 97.6%)
Adenovirus	**Overall**	**100.0% (39/39)**	**(91.0%, 100.0%)**	**98.4% (1113/1131)**	**(97.5%, 99.0%)**	**98.5% (1152/1170)**	**(97.6%, 99.0%)**
Human Metapneumovirus	Prospective	90.9% (10/11)	(62.3%, 98.4%)	99.9% (780/781)	(99.3%, 100.0%)	99.7% (790/792)	(99.1%, 99.9%)
Human Metapneumovirus	Archived	97.7% (42/43)	(87.9%, 99.6%)	99.7% (334/335)	(98.3%, 99.9%)	99.5% (376/378)	(98.1%, 99.9%)

Target Virus	Sample Category	PPA (a/a+b)	PPA 95% CI	NPA (c/c+d)	NPA 95% CI	OPA (a + d/N)	OPA 95% CI
Human Metapneumovirus	**Overall**	**96.3% (52/54)**	**(87.5%, 99.0%)**	**99.8% (1114/1116)**	**(99.3%, 100.0%)**	**99.7% (1166/1170)**	**(99.1%, 99.9%)**
Enterovirus and Rhinovirus	Prospective	77.0% (47/61)	(65.1%, 85.8%)	99.2% (725/731)	(98.2%, 99.6%)	97.5% (772/792)	(96.1%, 98.4%)
Enterovirus and Rhinovirus	Archived	96.9% (31/32)	(84.3%, 99.4%)	96.8% (332/343)	(94.3%, 98.2%)	96.8% (363/375)	(94.5%, 98.2%)
Enterovirus and Rhinovirus	**Overall**	**83.9% (78/93)**	**(75.1%, 90.0%)**	**98.4% (1057/1074)**	**(97.5%, 99.0%)**	**97.3% (1135/1167)**	**(96.2%, 98.1%)**
Common Human Coronaviruses (229E, HKU1, NL63, OC43)	Prospective	90.0% (18/20)	(69.9%, 97.2%)	99.9% (771/772)	(99.3%, 100.0%)	99.6% (789/792)	(98.9%, 99.9%)
Common Human Coronaviruses (229E, HKU1, NL63, OC43)	Archived	98.4% (63/64)	(91.7%, 99.7%)	91.0% (283/311)	(87.3%, 93.7%)	92.3% (346/375)	(89.1%, 94.6%)
Common Human Coronaviruses (229E, HKU1, NL63, OC43)	**Overall**	**96.4% (81/84)**	**(90.0%, 98.8%)**	**97.3% (1054/1083)**	**(96.2%, 98.1%)**	**97.3% (1135/1167)**	**(96.2%, 98.1%)**
Parainfluenza virus 1	Prospective	0/0	Not Calculable	100.0% (792/792)	(99.5%, 100.0%)	100.0% (792/792)	(99.5%, 100.0%)
Parainfluenza virus 1	Archived	100.0% (40/40)	(91.2%, 100.0%)	97.6% (327/335)	(95.4%, 98.8%)	97.9% (367/375)	(95.8%, 98.9%)
Parainfluenza virus 1	**Overall**	**100.0% (40/40)**	**(91.2%, 100.0%)**	**99.3% (1119/1127)**	**(98.6%, 99.6%)**	**99.3% (1159/1167)**	**(98.7%, 99.7%)**
Parainfluenza virus 2	Prospective	100.0% (2/2)	(34.2%, 100.0%)	100.0% (790/790)	(99.5%, 100.0%)	100.0% (792/792)	(99.5%, 100.0%)
Parainfluenza virus 2	Archived	100.0% (44/44)	(92.0%, 100.0%)	98.5% (330/335)	(96.6%, 99.4%)	98.7% (374/379)	(96.9%, 99.4%)
Parainfluenza virus 2	**Overall**	**100.0% (46/46)**	**(92.3%, 100.0%)**	**99.6% (1120/1125)**	**(99.0%, 99.8%)**	**99.6% (1166/1171)**	**(99.0%, 99.8%)**
Parainfluenza virus 3	Prospective	100.0% (5/5)	(56.6%, 100.0%)	100.0% (787/787)	(99.5%, 100.0%)	100.0% (792/792)	(99.5%, 100.0%)
Parainfluenza virus 3	Archived	95.3% (41/43)	(84.5%, 98.7%)	99.7% (336/337)	(98.3%, 99.9%)	99.2% (377/380)	(97.7%, 99.7%)
Parainfluenza virus 3	**Overall**	**95.8% (46/48)**	**(86.0%, 98.8%)**	**99.9% (1123/1124)**	**(99.5%, 100.0%)**	**99.7% (1169/1172)**	**(99.3%, 99.9%)**
Parainfluenza virus 4	Prospective	100.0% (1/1)	(20.7%, 100.0%)	100.0% (791/791)	(99.5%, 100.0%)	100.0% (792/792)	(99.5%, 100.0%)
Parainfluenza virus 4	Archived	97.3% (36/37)	(86.2%, 99.5%)	98.3% (337/343)	(96.2%, 99.2%)	98.2% (373/380)	(96.2%, 99.1%)
Parainfluenza virus 4	**Overall**	**97.4% (37/38)**	**(86.5%, 99.5%)**	**99.5% (1128/1134)**	**(98.9%, 99.8%)**	**99.4% (1165/1172)**	**(98.8%, 99.7%)**

Note: a = number of samples where both **cobas**® Respiratory flex and the comparator tests are positive; b = number of samples where **cobas**® Respiratory flex is negative and the comparator is positive; c = number of sample where **cobas**® Respiratory flex is positive and the comparator is negative; d = number of samples where both **cobas**® Respiratory flex and the comparator are negative; N = Total number of paired samples. PPA: Positive Percent Agreement. NPA: Negative Percent Agreement. OPA: Overall Percent Agreement.

RSV: respiratory syncytial virus, SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

A total of 140 results showed discrepancy between the **cobas**® Respiratory flex test and the respective comparator): of these, 113 results were positive by **cobas**® Respiratory flex and negative by the comparator, while 27 results were negative

by **cobas**® Respiratory flex and positive with the comparator. Analysis of discrepant results of the 113 **cobas**® Respiratory flex positive specimens after additional testing of the specimens by an alternative 510(k) cleared and CE-marked assay and/or DNA sequencing of the amplicons confirmed the presence of the target organisms in 104 specimens. In the remaining 9 samples discrepant analysis was not possible due to limited sample volume. The vast majority (96/113, 85.0%) of these discrepant results on **cobas**® Respiratory flex were presumably low titer samples (Ct >30) (at or around the LoD of the candidate and comparator assays), where differences between analytical LoDs between methods can commonly lead to discrepancies.

Of the 27 **cobas**® Respiratory flex negative specimens, discrepant analysis was not possible for 1 sample due to limited sample volume. Discordant analysis testing was performed on the remaining 26 **cobas**® Respiratory flex negative specimens by an alternative 510(k) cleared and CE-marked NAAT. Discordant testing confirmed the initial **cobas**® Respiratory flex result in 16 samples and confirmed the results of the comparator test in 10 specimens.

Table 33 presents instances of multiple virus detection by **cobas**® Respiratory flex. The most frequently identified combination, found in nine samples, was a combination of Adenovirus and Rhinovirus/Enterovirus. Out of these, six were also detected by a comparator test.

Table 33 Multiple virus detection (≥ 3 instances) by **cobas**® Respiratory flex

Analyte 1	Analyte 2	Total Multiple Detections	Number of Specimen with False Positive Detections	False Positive Analyte(s)
Adenovirus	Rhinovirus/Enterovirus	9	3	Rhinovirus/Enterovirus (1), Adenovirus (2)
Respiratory Syncytial Virus	Rhinovirus/Enterovirus	8	3	Rhinovirus/Enterovirus (2), Respiratory Syncytial Virus (1)
Adenovirus	Respiratory Syncytial Virus	6	5	Adenovirus (5)
Human Parainfluenza 1	Rhinovirus/Enterovirus	6	3	Rhinovirus/Enterovirus (1), Human Parainfluenza 1 (2)
Coronavirus	Influenza A	5	3	Influenza A (1), Coronavirus (2)
Coronavirus	Respiratory Syncytial Virus	5	4	Coronavirus (4), Respiratory Syncytial Virus (1)
Coronavirus	Rhinovirus/Enterovirus	5	4	Coronavirus (4), Rhinovirus/Enterovirus (1)
Adenovirus	Coronavirus	4	2	Adenovirus (2)
Adenovirus	Human Metapneumovirus	3	1	Adenovirus (1)
Coronavirus	Human Metapneumovirus	3	1	Coronavirus (1)
Coronavirus	Human Parainfluenza 3	3	2	Coronavirus (2)
Coronavirus	SARS-CoV-2	3	1	Coronavirus (1)
Human Parainfluenza 1	Influenza A	3	2	Human Parainfluenza 1 (2)

Note: False positive is when a sample is detected by **cobas**® Respiratory flex but not detected by the comparator.

Additional information

Key test features

Sample type	Nasopharyngeal swab samples collected in the Copan UTM-RT® System or the BD™ UVT System or equivalent diluted in **cobas**® MIS
Amount of sample required	1.2 mL (0.4 mL patient sample diluted in 0.8 mL **cobas**® MIS)
Sample processing volume	0.85 mL

Symbols

The following symbols are used in labeling for Roche PCR diagnostic products.

Table 34 Symbols used in labeling for Roche PCR diagnostics products

Age/DOB Age or Date of Birth	Device not for near-patient testing	QS IU/PCR QS IU per PCR reaction, use the QS International Units (IU) per PCR reaction in calculation of the results.
SW Ancillary Software	Device not for self-testing	
Assigned Range [copies/mL] Assigned Range (copies/mL)	Distributor *(Note: The applicable country/region may be designated beneath the symbol)*	SN Serial number
Assigned Range [IU/mL] Assigned Range (IU/mL)	Do not re-use	Site Site
EC REP Authorized representative in the European Community	Female	Procedure Standard Standard Procedure
BARCODE Barcode Data Sheet	For IVD performance evaluation only	STERILE EO Sterilized using ethylene oxide
LOT Batch code	GTIN Global Trade Item Number	Store in dark
Biological risks	Importer	Temperature limit
REF Catalogue number	IVD In vitro diagnostic medical device	TDF Test Definition File
CE CE marking of conformity; this device is in conformity with the applicable requirements for CE marking of an in vitro diagnostic medical device	LLR Lower Limit of Assigned Range	This way up
	Male	Procedure UltraSensitive Ultrasensitive Procedure
Collect Date Collect date	Manufacturer	UDI Unique Device Identifier
Consult instructions for use	CONTROL − Negative control	ULR Upper Limit of Assigned Range
Σ Contains sufficient for <*n*> tests	NON STERILE Non-sterile	Urine Fill Line Urine Fill Line
CONTENT Content of kit	Patient Name	Rx only For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.
CONTROL Control	Patient number	Use-by date
Date of manufacture	Peel here	
Device for near-patient testing	CONTROL + Positive control	
Device for self-testing	QS copies / PCR QS copies per PCR reaction, use the QS copies per PCR reaction in calculation of the results.	

Technical support

For technical support (assistance) please reach out to your local affiliate:
https://www.roche.com/about/business/roche_worldwide.htm

Manufacturer and importer

Table 35 Manufacturer and importer

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Trademarks and patents

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CE 2797

EC REP Roche Diagnostics GmbH
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IVD

References

1. Ferkol T, Schraufnagel D. The global burden of respiratory disease. *Ann Am Thorac Soc*. 2014;11:404-6.
2. Ghebrehewet S, MacPherson P, Ho A. Influenza. *BMJ*. 2016;355:i6258.
3. World Health Organization. The global burden of disease: 2004 update. Published: 2 Mar 2004; Accessed 29 Jan 2024. https://iris.who.int/bitstream/handle/10665/43942/9789241563710_eng.pdf?sequence=1.
4. Shi T, McAllister DA, O'Brien KL, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: A systematic review and modelling study. *Lancet*. 2017;390:946-58.
5. Mokdad AH, Marks JS, Stroup DF, Gerberding JL. Actual causes of death in the United States, 2000. *JAMA*. 2004;291:1238-45.
6. Passioti M, Maggina P, Megremis S, Papadopoulos NG. The common cold: Potential for future prevention or cure. *Curr Allergy Asthma Rep*. 2014;14:413.
7. Heikkinen T, Jarvinen A. The common cold. *Lancet*. 2003;361:51-9.
8. Florin TA, Plint AC, Zorc JJ. Viral bronchiolitis. *Lancet*. 2017;389:211-24.
9. Prina E, Ranzani OT, Torres A. Community-acquired pneumonia. *Lancet*. 2015;386:1097-108.
10. Dasaraju PV, Liu C. Infections of the respiratory system. In: Baron S, editor. *Medical Microbiology*. 4th Edition. Galveston, TX (USA): University of Texas Medical Branch at Galveston; 1996.
11. Dinnes J, Deeks JJ, Berhane S, et al. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. *Cochrane Database Syst Rev*. 2021;3:CD013705.
12. Azar MM, Landry ML. Detection of influenza A and B viruses and respiratory syncytial virus by use of Clinical Laboratory Improvement Amendments of 1988 (CLIA)-waived point-of-care assays: A paradigm shift to molecular tests. *J Clin Microbiol*. 2018;56.
13. Gonzales R, Bartlett JG, Besser RE, et al. Principles of appropriate antibiotic use for treatment of nonspecific upper respiratory tract infections in adults: Background. *Ann Intern Med*. 2001;134:490-4.
14. Thompson WW, Shay DK, Weintraub E, et al. Mortality associated with influenza and respiratory syncytial virus in the United States. *JAMA*. 2003;289:179-86.
15. Bloom-Feshbach K, Alonso WJ, Charu V, et al. Latitudinal variations in seasonal activity of influenza and respiratory syncytial virus (RSV): A global comparative review. *PLoS One*. 2013;8:e54445.
16. Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. *Lancet*. 2020;395:470-3.
17. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA*. 2020;323:1061-9.
18. World Health Organization. WHO COVID-19 dashboard. Accessed: 29 Jan 2024. https://data.who.int/dashboards/covid19/cases?n=c.

19. Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet*. 2020;395:565-74.

20. American Academy of Pediatrics. Coronaviruses, including SARS and MERS. In: Kimberlin DW, Brady MT, Jackson MA, Long SS, editors. Red Book: 2018. Report of the Committee on Infectious Diseases. 31st Edition. American Academy of Pediatrics; 2018:297-301.

21. World Health Organization. Influenza (seasonal) [Fact sheet]. Published: 3 Oct 2023; Accessed: 29 Jan 2024. https://www.who.int/en/news-room/fact-sheets/detail/influenza-(seasonal).

22. Uyeki TM. Influenza. *Ann Intern Med*. 2017;167:ITC33-ITC48.

23. Heikkinen T, Ojala E, Waris M. Clinical and socioeconomic burden of respiratory syncytial virus infection in children. *J Infect Dis*. 2017;215:17-23.

24. Chosewood LC, Wilson DE, editors. *Biosafety in Microbiological and Biomedical Laboratories.* 5th Edition. HHS Publication No. (CDC) 21-1112. Atlanta, GA (USA): Centers for Disease Control and Prevention; 2009.

25. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections*. 4th Edition. M29-A4. Wayne, PA (USA): Clinical and Laboratory Standards Institute; 2014.

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The summary of safety and performance report can be found using the following link: https://ec.europa.eu/tools/eudamed