Rx Only

cobas® Influenza A/B & RSV UC

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

For in vitro diagnostic use

cobas® Influenza A/B & RSV UC	P/N: 09233962190
cobas® Influenza A/B & RSV UC Control Kit	P/N: 09356525190
cobas omni Utility Channel Reagent Kit	P/N: 09052011190
cobas® Buffer Negative Control Kit	P/N: 07002238190

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Intended use

The **cobas®** Influenza A/B & RSV UC Qualitative Nucleic Acid test for use with the **cobas omni** Utility Channel on the **cobas®** 6800/8800 Systems is an automated, multiplex, real-time reverse transcription polymerase chain reaction (RT-PCR) assay for the timely in vitro qualitative detection and discrimination of influenza A virus, influenza B virus, and respiratory syncytial virus (RSV) RNA in nasopharyngeal swab specimens from patients with signs and symptoms of respiratory infection in conjunction with clinical and epidemiological risk factors. The test is intended for use as an aid in the diagnosis and differentiation of influenza A, influenza B, and RSV in humans and is not intended to detect influenza C virus.

Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions. Conversely, positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

The **cobas®** Influenza A/B & RSV UC Qualitative Nucleic Acid test for use with the **cobas omni** Utility Channel on the **cobas®** 6800/8800 Systems is intended for professional use in a clinical laboratory setting.

Summary and explanation of the test

Background

Influenza and lower respiratory tract infections are significant causes of worldwide morbidity and mortality.[1-4] Influenza virus is globally estimated to cause over one billion infections and 500,000 deaths each year, with the highest burdens in infants and young children, the elderly, and those with underlying medical conditions such as chronic lung disease.[3,4] 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.[3,4]

Respiratory syncytial virus is a leading cause of lower respiratory tract infections and hospitalizations in infants and children, with most children having had an RSV infection by two years of age.[5-7] In children five years of age or younger, there are over 3 million hospitalizations and over 100,000 globally estimated deaths from lower respiratory RSV infections.[5] More recently, due in part to diagnostic improvements, RSV has also been associated with a substantial disease and health economic burden in older adults.[8]

Effective diagnosis and differentiation of influenza and RSV infection from other respiratory pathogens in vulnerable patients is needed to address this substantial burden.[9] The global seasonality of influenza and RSV epidemics overlap, with peaks of infectious activity occurring in the respective winter months for temperate climates in the Northern and Southern hemispheres.[10] Moreover, the infectious signs and symptoms of influenza and RSV can often be insufficient to definitively diagnose or clinically distinguish between “influenza like” and “common cold” symptoms such as fever, cough, congestion, or tiredness that can be present in patients infected with either influenza virus or RSV, along with many other viral and bacterial respiratory pathogens.[9] Prompt 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.[9]

Rationale for PCR testing

Traditional diagnostics like rapid antigen detection tests for influenza virus and RSV have lower sensitivity than newer rapid molecular methods.[11,12] To allow for rapid medical management and effective infection control, a fast, accurate, high

throughput, and user friendly diagnostic solution is needed to detect influenza virus and RSV in at-risk patients of all ages with acute respiratory symptoms.[13] Based on their improved clinical performance, molecular nucleic acid amplification test (NAAT) methods such as real-time reverse transcriptase polymerase chain reaction (RT PCR) have become the preferred laboratory method for detecting influenza and RSV over time intensive, culture based methods.[14,15]

Explanation of the test

cobas® Influenza A/B & RSV UC Qualitative Nucleic Acid test for use with the **cobas omni** Utility Channel on the **cobas**® 6800/8800 Systems is an automated, multiplex, real-time reverse transcription polymerase chain reaction (RT-PCR) assay for the rapid in vitro qualitative detection and discrimination of influenza A virus, influenza B virus, and respiratory syncytial virus (RSV) RNA in nasopharyngeal swab specimens collected in Copan Universal Transport Medium System (UTM-RT), BD™ Universal Viral Transport System (UVT), or equivalent. The RNA Internal Control, 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 (low titer positive control and a negative control).

Principles of the procedure

cobas® Influenza A/B & RSV UC for use with the **cobas omni** Utility Channel, is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. 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**® 6800/8800 Systems software, which assigns test 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 with each **cobas**® Influenza A/B & RSV UC run.

cobas® Influenza A/B & RSV UC contains the influenza A, influenza B, and RSV primers and probes which are used in combination with the **cobas omni** Utility Channel Master Mix Reagent 2 (UC MMX-R2) and the 192-test cassette included in the **cobas omni** Utility Channel Reagent Kit. The 192-test cassette contains an internal control recognized by specific primers and probes included in the **cobas omni** Utility Channel Master Mix Reagent 2 (UC MMX-R2).

Selective amplification of Influenza A and Influenza B target nucleic acid from the sample is achieved by the use of target-specific forward and reverse primers for the matrix proteins 1 and 2 (M1/M2) for influenza A and the nuclear export protein (NEP) / nonstructural protein 1(NS1) genes for influenza B, respectively. For RSV, selective amplification of target nucleic acid from the sample is achieved by the use of target-specific forward and reverse primers for the RSV matrix protein sequences. Selective amplification of RNA Internal Control is achieved by the use of non-competitive sequence specific forward and reverse primers which have no homology with the RSV or influenza genomes. Amplified target is detected by cleavage of fluorescently labeled oligonucleotide probe. A thermostable DNA polymerase enzyme is used for amplification.

The prepared **cobas**® Influenza A/B & RSV UC master mix contains detection probes which are specific for influenza A virus, influenza B virus, RSV and the RNA Internal Control nucleic acid. Influenza A, influenza B, RSV and RNA Internal Control detection probes are each labeled with unique fluorescent dyes that act as a reporter. Each probe

also has a second dye which acts as a quencher. When not bound to the target sequence, the fluorescent signals of the intact probes are suppressed by the quencher dye. 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. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly. Each reporter dye is measured at defined wavelengths, which enables simultaneous detection and discrimination of the amplified target and the RNA Internal Control. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine 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 **cobas**® Influenza A/B & RSV can be found in Table 1. Materials required, but not provided can be found in Table 2, Table 3, Table 4, Table 5, and Table 9.

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

cobas® Influenza A/B & RSV UC reagents and controls

All unopened reagents and controls shall be stored as recommended in Table 1 to Table 5.

Table 1 cobas® Influenza A/B & RSV UC (primers and probes)

Store at 2-8°C
Primers and Probes (P/N 09233962190)

Kit components	Reagent ingredients	Quantity per kit 192 tests
INFLUENZA A/B & RSV UC PP (FluA/B-RSV)	TE buffer, < 0.02% upstream and downstream influenza A primers, < 0.02% upstream and downstream influenza B primers, < 0.02% upstream and downstream RSV primers,< 0.01% fluorescent-labeled oligonucleotide probes specific for influenza A, influenza B, RSV and the RNA Internal Control, < 0.1% sodium azide	1 x 0.65 mL

Table 2 cobas® Influenza A/B & RSV UC Control Kit

Store at 2–8°C
(P/N 09356525190)

Kit components	Reagent ingredients	Quantity per kit
INFLUENZA A/B & RSV UC (+) C (FluA/B-RSV (+) C)	< 0.001% synthetic (plasmid) influenza A DNA, influenza B DNA and RSV DNA in Tris buffer, < 0.1% sodium azide, EDTA, < 0.002% Poly rA RNA (synthetic)	16 mL (10 × 1.6 mL)

Table 3 cobas omni Utility Channel Reagent Kit (UC)

Store at 2–8°C
(P/N 09052011190)

Reagents	Reagent ingredients	Quantity per kit 192 tests
192 test cassette		
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase EUH210: Safety data sheet available on request. EUH208: Contains Subtilisin. May produce an allergic reaction.	22.3 mL
RNA Internal Control (RNA-QS)	Tris buffer, <0.05% EDTA, <0.001% 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
R2 Empty Vessel (R2 EV)	N/A	1
Master Mix Reagent 2 bottle		
cobas omni Utility Channel Master Mix Reagent 2 (UC MMX-R2)	Tricine buffer, potassium acetate, < 18% dimethyl sulfoxide, glycerol, < 0.1% Tween 20, EDTA, < 0.12% dATP, dCTP, dGTP, dUTPs, < 0.01% internal control forward and reverse primers, < 0.01% fluorescent-labeled oligonucleotide probes specific for RNA-IC, < 0.01% oligonucleotide aptamer, < 0.01% Z05D DNA polymerase, < 0.1% AmpErase (uracil-N-glycosylase) enzyme (microbial), < 0.1% sodium azide	19.6 mL (2 x 9.8 mL)

Table 4 cobas® Buffer Negative Control Kit

(BUF (-) C)

Store at 2-8°C

(P/N 07002238190)

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 1mL)

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 × 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 × 875 mL	 **DANGER** H302 + H332: Harmful if swallowed or if inhaled. H314: Causes severe skin burns and eye damage. H411: Toxic to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P273: Avoid release to the environment. P280: Wear protective gloves/protective clothing/eye protection/face protection/hearing protection. 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. P391: Collect spillage. 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**® Influenza A/B & RSV UC kit. See listing of additional materials required (Table 8).

** Product safety labeling primarily follows EU GHS guidance

***Hazardous substance

Reagent storage and handling requirements

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

When reagents are not loaded on the **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® Influenza A/B & RSV UC[a]	2–8°C
cobas® Influenza A/B & RSV UC Control Kit	2–8°C
cobas omni Utility Channel Reagent 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

[a] The prepared reagent cassette can be stored for up to 30 days at 2-8 °C before first usage. After first usage, please refer to expiry conditions of the **cobas omni** Utility Channel Reagent Kit in Table 7.

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 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®** 6800/8800 Systems.

Table 7 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 omni Utility Channel Reagent Kit	Date not passed[a]	90 days from first usage	Max 40 runs	Max 40 hours
cobas® Buffer Negative Control Kit	Date not passed[a]	Not applicable[b]	Not applicable	Max 10 hours
cobas omni Lysis Reagent	Date not passed[a]	30 days from loading[c]	Not applicable	Not applicable
cobas omni MGP Reagent	Date not passed[a]	30 days from loading[c]	Not applicable	Not applicable
cobas omni Specimen Diluent	Date not passed[a]	30 days from loading[c]	Not applicable	Not applicable
cobas omni Wash Reagent	Date not passed[a]	30 days from loading[c]	Not applicable	Not applicable

[a] Reagents are not expired

[b] Single use reagents

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

Additional materials required

Table 8 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 Solid Waste Update	07435967001 and 07094361001 or 08030073001 and 08387281001
cobas omni Secondary Tubes 13x75 (optional)	06438776001

Instrumentation and software required

The **cobas**® 6800/8800 software and **cobas**® Influenza A/B & RSV UC analysis package shall be installed on the instrument(s). The Instrument Gateway (IG) server will be provided with the system.

Table 9 Instrumentation

Equipment	P/N
cobas® 6800 System (Option Moveable)	05524245001 and 06379672001
cobas® 6800 System (Fix)	05524245001 and 06379664001
cobas® 8800 System	05412722001
Sample Supply Module	06301037001
Instrument Gateway	06349595001
TWN3 Legic NFC USB (RFID Reader/Writer)	07450460001
External PC with remote connection provided by the customer	N/A
Barcode Printer	N/A

For additional information, please refer to the **cobas**® 6800/8800 Systems – User Assistance and/or User Guide.

Note: Contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack trays accepted on the instruments.

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 only.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.[16,17] Only personnel proficient in handling infectious materials and the use of **cobas**® Influenza A/B & RSV UC and **cobas**® 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 with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures.
- 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 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 reagent cassette and vials, 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 contains 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**® Influenza A/B & RSV UC test kit, **cobas**® Influenza A/B & RSV UC Control kit, **cobas omni** Utility Channel 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, which contains 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**® Influenza A/B & RSV UC kits**, cobas**® Influenza A/B & RSV UC Control kits, **cobas omni** Utility Channel Reagent kits, **cobas**® Buffer Negative Control kits 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 (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas**® 6800/8800 instrument, follow the instructions in the **cobas**® 6800/8800 Systems – User Assistance and/or User Guide 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.
Ensure samples are equilibrated to room temperature prior to transfer into a **cobas omni** Secondary Tube.

Samples collection

- Nasopharyngeal specimens should be collected according to standard collection technique using flocked swabs and immediately place in 3 mL of Copan Universal Transport Medium (UTM-RT), BD™ Universal Viral Transport (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.
- After collection, specimens can be stored in primary tubes for up to 48 hours at 2-25°C followed by up to 3 days at 2-8°C and at ≤ -15°C for up to 30 days.
- Specimens are stable for up to three freeze/thaw cycles when frozen in primary tubes at ≤ -15°C.

Instructions for use

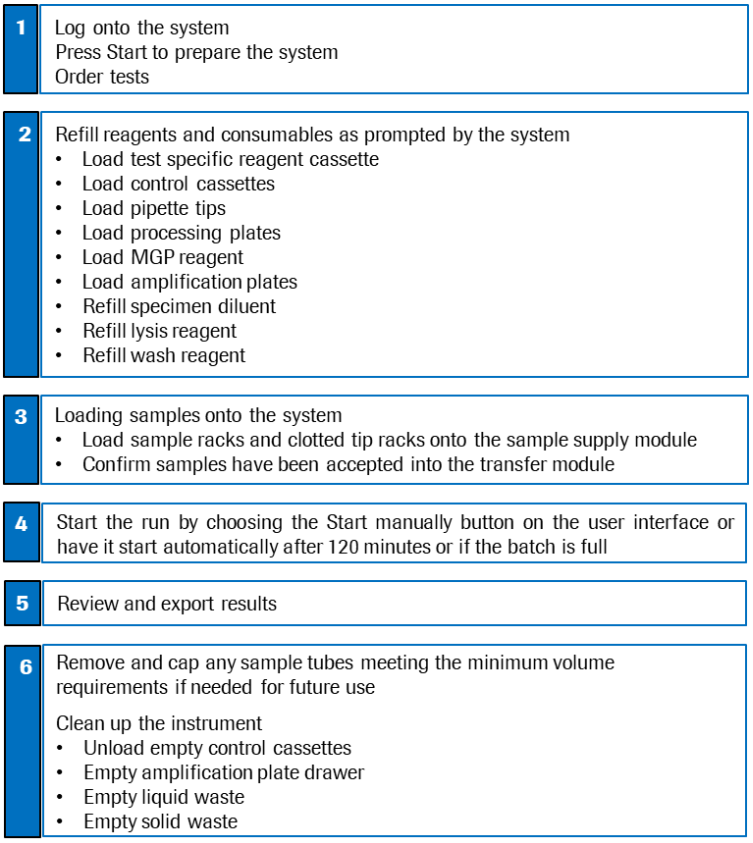
Procedural notes

- The assay is only intended for use with **cobas**® UC_FluAB_RSV USAP or **cobas**® RSV USAP from Roche.
- Do not use **cobas**® Influenza A/B & RSV UC reagents, **cobas**® Influenza A/B & RSV UC Control Kit, **cobas omni** Utility Channel Reagent kit, **cobas**® Buffer Negative Control Kit, or **cobas omni** reagents after their expiry dates.
- Only use the UC MMx-R2 bottles provided with the reagent cassette.
- 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**® 6800/8800 Systems User Guide for proper barcode specifications and additional information on loading sample tubes.
- Refer to the **cobas**® 6800/8800 Systems – User Assistance and/or User Guide for proper maintenance of instruments.

Running cobas® Influenza A/B & RSV UC

cobas® Influenza A/B & RSV UC can be run with a minimum required sample volume of 0.6 mL in the **cobas omni** secondary tube for specimens collected in Copan Universal Transport Medium (UTM-RT), BD™ Universal Viral Transport (UVT) or equivalent.

Figure 1 **cobas**® Influenza A/B & RSV UC test procedure

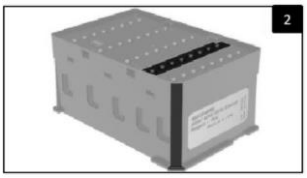


Preparing the reagent cassette

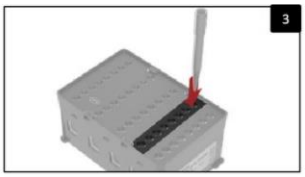
The PCR MMX R2 is prepared from the mix of Master Mix Reagent 2 (UC-MMX-R2) and **cobas**® Influenza A/B & RSV UC primers and probes loaded in the **cobas omni** Utility Channel Reagent kit 192-test cassette.

- Remove the Master Mix Reagent 2 (UC-MMX-R2, see picture 1) from **cobas omni** Utility Channel Reagent Kit and **cobas**® Influenza A/B & RSV UC primers and probes from their 2-8°C storage location.
- Mix UC-MMx-R2 on the roller mixer for 5 minutes at room temperature.
  Note: If no roller mixer is available, invert the bottle 20 times.
- Transfer 10 mL of UC MMX-R2 reagent to a light-protected polypropylene tube.
  Note: Refer to the **cobas omni** Utility Channel User Assistance for details on transfer option steps.
- Mix the **cobas**® Influenza A/B & RSV UC primers and probes by inverting 20 times.
- Add 0.600 mL of the **cobas**® Influenza A/B & RSV UC primers and probes (refer Table 1) to the light-protected polypropylene tube.
- Mix the polypropylene tube for 5 minutes on the roller mixer.
  Note: If no roller mixer is available, invert the bottle 20 times.

The reagent cassette is prepared by loading the PCR Mix into the reagent cassette from the **cobas omni** Utility Channel Reagent Kit.

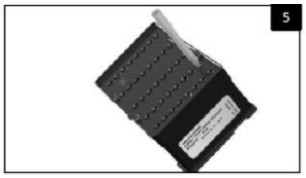


- Position the cassette by placing the slanted edge to the lower right corner (see picture 2).
  Note: The second row from the right side contains the empty MMX container.



- Place a 1 mL pipette tip into the top septum hole row 2 (see picture 3).
  Note: The pipette tip allows air pressure in the vessel to adjust while the prepared PCR Mix is added.
- Take a repeater pipette with a 10 mL pipette tip. Load the pipette tip with 9.7 mL of the prepared PCR Mix.

- Insert the loaded pipette into the bottom septum hole of the reagent cassette. Puncture the septum deeply enough to avoid spillage in the row 2 (see picture 4).
- Tilt the reagent cassette to a 45° angle lengthwise from the bottom. Make sure the cassette is tipped along the edge where the pipette with the 10 mL tip is inserted (see picture 5).



- Slowly and carefully pipette 9.7 mL of the prepared PCR Mix through the bottom septum into the empty container in row 2 (see picture 5). If possible, dispense the prepared PCR Mix in a single movement. Ensure that the correct volume of prepared PCR Mix is pipetted.
- Ensure that there is no fluid in the 1 mL pipette tip and the remove it from the septum.
  Note: if there is fluid in the tip, carefully rotate the tip to release the fluid from the tip back into the cassette. If fluid still remains in the 1 mL tip, perform the following: Using the repeater pipette with a 10 mL tip, remove some of the pipetted PCR Mix from the cassette vessel until no fluid remains in the 1 mL tip. Slowly and carefully pipette any fluid in the 10 mL pipette tip back into the vessel. Once both tips are empty, the tips can be removed from the cassette.

- Slowly tilt the reagent cassette 20 times to remove any air bubbles from the newly filled container (see picture 6).
- On the label of the 192-test **cobas omni** Utility Channel Reagent Kit document the assay name (Influenza A/B & RSV UC), the date the cassette was prepared, the lot number of the assay kits primers and probes used

(P&P Mix Lot) and check the box 'P&P Added' to confirm that primers and probes mix has been added.

The RFID label of the prepared **cobas omni** Utility Channel Reagent Kit reagent cassette is labelled as follows:

- Open the **cobas omni** Utility Channel Tool using the Roche Utility Channel Tool start icon on the desktop.
- Click the "Open UC analysis package" button and select the USAP.zip file from the Recently used UC specific analysis packages section or load the **cobas**® Flu AB_RSV USAP or the **cobas**® RSV USAP via "Open published UC analysis package to write on reagent cassette RFID tag." The UC analysis package screen in UCAP tab opens up.
- On the UC analysis package panel, click the "Reagent cassette" button.
- Enter the **cobas omni** Utility Channel Reagent Kit lot number in the field corresponding to Reagent cassette lot ID.
- Place the RFID reader/writer next to the RFID tag of the Utility Channel reagent cassette to be written on.
- Click the "Write data on the RFID tag" button to write the RFID label.
- Load the prepared reagent cassette onto the **cobas**® 6800/8800 Systems.
- The prepared reagent cassette can be stored for up to 30 days at 2-8 °C before first usage. After first usage, please refer to expiry conditions of the **cobas omni** Utility Channel Reagent Kit in Table 7.

Prepare samples and controls

One positive control vial contains sufficient volume to perform two runs. The control has to be loaded as sample in each run and for each new reagent cassette. To guarantee that each control batch contains a positive control, it is recommended to use the entire **cobas omni** Utility Channel reagent cassette before loading a new **cobas omni** Utility Channel reagent cassette.

Follow the steps below to transfer the positive control into a **cobas omni** Secondary Tube:

- Vortex the control vial for 3-5 seconds.
- Transfer 0.6 mL into the prepared barcoded secondary tube.
- Transfer secondary tube to a rack. Close the control vial tube cap.

The opened control vial can be stored for up to 30 days at 2-8 °C after first usage

Specimens collected in Copan Universal Transport Medium (UTM-RT), BD™ Universal Viral Transport (UVT) or equivalent must be transferred into a **cobas omni** Secondary tube prior to processing on the **cobas**® 6800/8800 Systems. Samples transferred to **cobas omni** Secondary tubes should be processed using the 'VTM' sample type selection.

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 patient sample from a primary collection tube into a **cobas omni** Secondary Tube:

- Unscrew the primary sample tube cap.
- Transfer 0.6 mL into the prepared barcoded secondary tube.
- Transfer secondary tube to a rack. Close the primary sample tube cap.

Define test ordering

Create a test order as described in **cobas**® 6800/8800 Systems User Guide.

- In the Sample type field, select **"VTM"** from the drop down menu.
- In the Test region, select "**UC_FluAB_RSV**" test or "RSV" from the drop down menu.
- In the Volume region, ensure that the volume equals "**400 µL**".
- Save and perform the test as described in the **cobas**® 6800/8800 Systems User Guide.

Refer to the **cobas**® 6800/8800 Systems User Guide for more details.

Results

The **cobas**® 6800/8800 Systems automatically detect the influenza A, influenza B and RSV, for each individually processed sample and control, displaying individual target results for samples and the positive control, as well as test validity and overall results for the negative control.

Quality control and validity of results

- One **cobas**® Buffer Negative Control [BUF (-) C] and one **cobas**® Influenza A/B & RSV UC Control [FluA/B-RSV (+) C] need to be processed with each batch.
- In the **cobas**® 6800/8800 software and/or report, check for flags and their associated results to ensure the batch validity.
- All flags are described in the **cobas**® 6800/8800 Systems User Guide.
- The batch is valid if no flags appear for the negative control and if the positive control is positive for all targets. If the batch is invalid, repeat testing of the entire batch.

Validation of results is performed automatically by the **cobas**® 6800/8800 software based on negative control performance. The positive control validation has to be performed by the operator based on the positive control performance.

To determine this validity, interpret the results from the controls and IC as described in Table 10 below.

Table 10 Run and result validity interpretation

Validity	Control	Valid	Invalid	Validation
Run	BUF (-) C	Indicated as "Valid" in Test Result column	Indicated as "Invalid" in Test Result column (All samples of the run must be retested)	**cobas**® 6800/8800 Systems
Run	FluA/B-RSV (+) C	Ct value indicated in each Target column	Indicated as "Invalid" or "Negative" in one of the Target columns (2, 3 OR 4) (All samples of the run must be retested)	Operator
Sample Result	IC	Indicated as "Yes" in Valid column	Indicated as "No" in Valid column AND Target 2, 3 AND 4: Invalid (Invalidated sample must be retested)	**cobas**® 6800/8800 Systems

Interpretation of results

If the run and sample are valid, the result interpretation for each target is based on the results provided by the **cobas**® 6800/8800 Systems and described in Table 11. Invalid results for one or more target combinations are possible and are reported out specifically for each channel on the **cobas**® 6800/8800 Systems. In these cases, original sample should be re-tested to obtain a valid target result. If the target result is still invalid, a new sample should be obtained.

Results and their corresponding interpretation for detecting influenza A/B & RSV are shown below (Table 11).

Table 11 cobas® Influenza A/B & RSV UC results interpretation (Flu = influenza)

Target 2 (RSV)	Target 3 (Flu A)	Target 4 (Flu B)	Interpretation
RSV Negative	Any	Any	Target Result for RSV is valid. Result for RSV RNA is Not Detected.
RSV Ct value	Any	Any	Target Result for RSV is valid. Result for RNA A RNA is Detected.
Invalid	Any	Any	Target Result for RSV is invalid. Sample should be retested. If the result is still invalid, a new specimen should be obtained.
Any	**Flu A Negative**	Any	Target Result for Flu A is valid. Result for Flu A RNA is Not Detected.
Any	**Flu A Ct value**	Any	Target Results for Flu A is valid. Result for Flu A RNA is Detected.
Any	**Invalid**	Any	Target Result for Flu A is invalid. Sample should be retested. If the result is still invalid, a new specimen should be obtained.
Any	Any	**Flu B Negative**	Target Result for Flu B is valid. Result for Flu B RNA is Not Detected.
Any	Any	**Flu B Ct value**	Target Results for Flu B is valid. Result for Flu B RNA is Detected.
Any	Any	**Invalid**	Target Result for Flu B is invalid. Sample should be retested. If the result is still invalid, a new specimen should be obtained.
Invalid	**Invalid**	**Invalid**	None of the Targets Results are valid. Sample should be retested. If the result is still invalid, a new specimen should be obtained.

Procedural limitations

- **cobas**® Influenza A/B & RSV UC has been evaluated only for use in combination with the **cobas**® Influenza A/B & RSV UC Control Kit, **cobas omni** Utility Channel Reagent 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**® 6800/8800 Systems.
- The assay is only intended for use with **cobas**® UC_FluAB_RSV USAP or **cobas**® RSV USAP from Roche.
- Reliable results depend on proper sample collection, storage and handling procedures.
- This test is to be used for the detection of influenza A, influenza B, and RSV RNA in nasopharyngeal swab samples collected in a Copan UTM-RT System (UTM-RT), BD™ Universal Viral Transport System (UVT) or equivalent. Testing of other sample types with **cobas**® Influenza A/B & RSV UC may result in inaccurate results.
- Detection of influenza A, influenza B, and RSV RNA 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**® Influenza A/B & RSV UC 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**® Influenza A/B & RSV UC (in the **cobas omni** Utility Channel Reagent kit) 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 omni** Utility Channel Master Mix reagent enables selective amplification of target RNA; 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

Limit of Detection (LoD)

The LoD study determines the lowest detectable concentration of influenza A, influenza B and RSV at which greater or equal to 95% of all (true positive) replicates test positive.

To determine the LoD, six cultured viruses – two each of influenza A, influenza B and RSV – were serially diluted in simulated clinical matrix to build two co-formulated target panels with one strain per virus. Six concentration levels, with two-fold serial dilutions between the levels, were prepared on three days and tested with a total of 63 replicates per concentration across three reagent lots. Table 12 to Table 14 summarize the established LoD values.

Table 12 Summary of LoD for influenza A

Viral Strain	Kit Lot	95% Probit [$TCID_{50}$/mL]	95% CI of Probit [$TCID_{50}$/mL]	Hit Rate ≥ 95% [$TCID_{50}$/mL]	Mean CT at ≥ 95% Hit Rate
A/Brisbane/02/2018 (H1N1) Cat No 0810585CF Lot 323771	Lot 1	0.029	0.018 – 0.076	0.024	37.2
	Lot 2	0.017	0.011 – 0.38	0.024	37.1
	Lot 3	0.016	0.011 – 0.034	0.024	36.9
	Lot 1-3	**0.020**	0.015 – 0.031	0.024	37.1
A/Kansas/14/2017 (H3N2) Cat No 0810586CF Lot 324412	Lot 1	0.56	0.39 – 1.05	0.50	37.3
	Lot 2	0.78	0.49 – 1.80	1.00	35.8
	Lot 3	0.52	0.37 – 0.98	0.50	37.5
	Lot 1-3	**0.61**	0.48 – 0.86	1.00	36.0

Table 13 Summary of LoD for influenza B

Viral Strain	Kit Lot	95% Probit [$TCID_{50}$/mL]	95% CI of Probit [$TCID_{50}$/mL]	Hit Rate ≥ 95% [$TCID_{50}$/mL]	Mean CT at ≥ 95% Hit Rate
B/Colorado/06/17 (Victoria lineage) Cat No 0810573CF Lot 323459	Lot 1	0.022	0.015 – 0.043	0.026	36.0
	Lot 2	0.020	0.013 – 0.045	0.026	35.6
	Lot 3	0.017	0.012 – 0.034	0.013	37.0
	Lot 1-3	**0.020**	0.015 – 0.028	0.026	35.9
B/Phuket/3073/13 (Yamagata lineage) Cat No 0810515CF Lot 324397	Lot 1	0.010	0.0062 – 0.022	0.010	36.1
	Lot 2	0.0054	0.0036 – 0.011	0.010	36.7
	Lot 3	0.0079	0.0052 – 0.017	0.010	36.5
	Lot 1-3	**0.0077**	0.0059 – 0.011	0.010	36.4

Table 14 Summary of LoD for RSV

Viral Strain	Kit Lot	95% Probit [$TCID_{50}$/mL]	95% CI of Probit [$TCID_{50}$/mL]	Hit Rate ≥ 95% [$TCID_{50}$/mL]	Mean CT at ≥ 95% Hit Rate
2/2015 Isolate #2 (Type A) Cat No 0810474CF Lot 317572 (sublot: 526637)	Lot 1	0.074	0.046 – 0.18	0.080	36.2
	Lot 2	0.11	0.067 – 0.28	0.080	36.5
	Lot 3	0.073	0.048 – 0.15	0.080	36.0
	Lot 1-3	**0.085**	0.063 – 0.13	0.080	36.2
3/2015 Isolate #2 (Type B) Cat No 0810480CF Lot 318797 (sublot: 531071)	Lot 1	0.015	0.0098 – 0.033	0.020	36.8
	Lot 2	0.019	0.012 – 0.046	0.020	36.3
	Lot 3	0.014	0.0088 – 0.034	0.010	37.6
	Lot 1-3	**0.016**	0.012 – 0.024	0.020	36.5

Precision – within laboratory

Within-laboratory precision was examined using six cultured viruses – two each of influenza A, influenza B and RSV – which were serially diluted in simulated clinical matrix to build two co-formulated target panels. Sources of variability were examined with two panels consisting of three concentration levels of approximately 0.3 x, 1 x and 3 x of the LoD of **cobas**® Influenza A/B & RSV UC. All negative panel members were tested negative throughout the study.

Testing was performed for the following variability components:

- day-to-day variability over 12 days
- run-to-run variability
- lot-to-lot variability using 3 different reagent lots of **cobas**® Influenza A/B & RSV UC
- instrument-to-instrument variability using 3 different **cobas**® 6800/8800 Systems by 3 operators

Twenty-four replicates were tested with each of the 3 panel members for each reagent lot for a total of 72 replicates over all reagent lots per target. Precision results were evaluated by calculating the percentage of reactive test results at each concentration level for each of the variability components analyzed.

The limits of two-sided 95% confidence intervals for each reactive rate were calculated for each of the three levels of the different influenza A, influenza B and RSV strains tested across 12 days, 3 reagent lots and 3 **cobas**® 6800/8800 Systems / operators. **cobas**® Influenza A/B & RSV UC is reproducible over multiple days, reagent lots and instruments / operators. The results from reagent lot-to-lot variability are summarized in Table 15 to Table 17.

Table 15 Reagent lot-to-lot precision summary for influenza A

Analyte	Concentration	Reagent lot	% Reactive (reactive/valid replicates)	Lower limit of 95% confidence interval	Upper limit of 95% confidence interval
A/Brisbane/02/2018 (H1N1)	~0.3 × LoD	1	83.3% (20/24)	62.6%	95.3%
A/Brisbane/02/2018 (H1N1)	~0.3 × LoD	2	75.0% (18/24)	53.3%	90.2%
A/Brisbane/02/2018 (H1N1)	~0.3 × LoD	3	70.8% (17/24)	48.9%	87.4%
A/Brisbane/02/2018 (H1N1)	~1 × LoD	1	95.8% (23/24)	78.9%	99.9%
A/Brisbane/02/2018 (H1N1)	~1 × LoD	2	100% (24/24)	85.8%	100%
A/Brisbane/02/2018 (H1N1)	~1 × LoD	3	100% (24/24)	85.8%	100%
A/Brisbane/02/2018 (H1N1)	~3 × LoD	1	100% (24/24)	85.8%	100%
A/Brisbane/02/2018 (H1N1)	~3 × LoD	2	100% (24/24)	85.8%	100%
A/Brisbane/02/2018 (H1N1)	~3 × LoD	3	100% (24/24)	85.8%	100%
A/Kansas/14/2017 (H3N2)	~0.3 × LoD	1	66.7% (16/24)	44.7%	84.4%
A/Kansas/14/2017 (H3N2)	~0.3 × LoD	2	75.0% (18/24)	53.3%	90.2%
A/Kansas/14/2017 (H3N2)	~0.3 × LoD	3	62.5% (15/24)	40.6%	81.2%
A/Kansas/14/2017 (H3N2)	~1 × LoD	1	95.8% (23/24)	78.9%	99.9%
A/Kansas/14/2017 (H3N2)	~1 × LoD	2	95.8% (23/24)	78.9%	99.9%
A/Kansas/14/2017 (H3N2)	~1 × LoD	3	100% (24/24)	85.8%	100%
A/Kansas/14/2017 (H3N2)	~3 × LoD	1	100% (24/24)	85.8%	100%
A/Kansas/14/2017 (H3N2)	~3 × LoD	2	100% (24/24)	85.8%	100%
A/Kansas/14/2017 (H3N2)	~3 × LoD	3	100% (24/24)	85.8%	100%

Table 16 Reagent lot-to-lot precision summary for influenza B

Analyte	Concentration	Reagent lot	% Reactive (reactive/valid replicates)	Lower limit of 95% confidence interval	Upper limit of 95% confidence interval
B/Colorado/06/2017 (Victoria lineage)	~0.3 × LoD	1	79.2% (19/24)	57.8%	92.9%
B/Colorado/06/2017 (Victoria lineage)	~0.3 × LoD	2	83.3% (20/24)	62.6%	95.3%
B/Colorado/06/2017 (Victoria lineage)	~0.3 × LoD	3	95.8% (23/24)	78.9%	99.9%
B/Colorado/06/2017 (Victoria lineage)	~1 × LoD	1	100% (24/24)	85.8%	100%
B/Colorado/06/2017 (Victoria lineage)	~1 × LoD	2	100% (24/24)	85.8%	100%
B/Colorado/06/2017 (Victoria lineage)	~1 × LoD	3	100% (24/24)	85.8%	100%
B/Colorado/06/2017 (Victoria lineage)	~3 × LoD	1	100% (24/24)	85.8%	100%
B/Colorado/06/2017 (Victoria lineage)	~3 × LoD	2	100% (24/24)	85.8%	100%
B/Colorado/06/2017 (Victoria lineage)	~3 × LoD	3	100% (24/24)	85.8%	100%
B/Phuket/3073/2013 (Yamagata lineage)	~0.3 × LoD	1	75.0% (18/24)	53.3%	90.2%
B/Phuket/3073/2013 (Yamagata lineage)	~0.3 × LoD	2	83.3% (20/24)	62.6%	95.3%
B/Phuket/3073/2013 (Yamagata lineage)	~0.3 × LoD	3	79.2% (19/24)	57.8%	92.9%
B/Phuket/3073/2013 (Yamagata lineage)	~1 × LoD	1	100% (24/24)	85.8%	100%
B/Phuket/3073/2013 (Yamagata lineage)	~1 × LoD	2	95.8% (23/24)	78.9%	99.9%
B/Phuket/3073/2013 (Yamagata lineage)	~1 × LoD	3	100% (24/24)	85.8%	100%
B/Phuket/3073/2013 (Yamagata lineage)	~3 × LoD	1	100% (24/24)	85.8%	100%
B/Phuket/3073/2013 (Yamagata lineage)	~3 × LoD	2	100% (24/24)	85.8%	100%
B/Phuket/3073/2013 (Yamagata lineage)	~3 × LoD	3	100% (24/24)	85.8%	100%

Table 17 Reagent lot-to-lot precision summary for RSV

Analyte	Concentration	Reagent lot	% Reactive (reactive/valid replicates)	Lower limit of 95% confidence interval	Upper limit of 95% confidence interval
2/2015 Isolate #2 (Type A)	~0.3 × LoD	1	75.0% (18/24)	53.3%	90.2%
2/2015 Isolate #2 (Type A)	~0.3 × LoD	2	75.0% (18/24)	53.3%	90.2%
2/2015 Isolate #2 (Type A)	~0.3 × LoD	3	83.3% (20/24)	62.6%	95.3%
2/2015 Isolate #2 (Type A)	~1 × LoD	1	100% (24/24)	85.8%	100%
2/2015 Isolate #2 (Type A)	~1 × LoD	2	100% (24/24)	85.8%	100%
2/2015 Isolate #2 (Type A)	~1 × LoD	3	100% (24/24)	85.8%	100%
2/2015 Isolate #2 (Type A)	~3 × LoD	1	100% (24/24)	85.8%	100%
2/2015 Isolate #2 (Type A)	~3 × LoD	2	100% (24/24)	85.8%	100%
2/2015 Isolate #2 (Type A)	~3 × LoD	3	100% (24/24)	85.8%	100%
3/2015 Isolate #2 (Type B)	~0.3 × LoD	1	79.2% (19/24)	57.8%	92.9%
3/2015 Isolate #2 (Type B)	~0.3 × LoD	2	66.7% (16/24)	44.7%	84.4%
3/2015 Isolate #2 (Type B)	~0.3 × LoD	3	95.8% (23/24)	78.9%	99.9%
3/2015 Isolate #2 (Type B)	~1 × LoD	1	91.7% (22/24)	73.0%	99.0%
3/2015 Isolate #2 (Type B)	~1 × LoD	2	95.8% (23/24)	78.9%	99.9%
3/2015 Isolate #2 (Type B)	~1 × LoD	3	100% (24/24)	85.8%	100%
3/2015 Isolate #2 (Type B)	~3 × LoD	1	100% (24/24)	85.8%	100%
3/2015 Isolate #2 (Type B)	~3 × LoD	2	100% (24/24)	85.8%	100%
3/2015 Isolate #2 (Type B)	~3 × LoD	3	100% (24/24)	85.8%	100%

Inclusivity

The inclusivity of **cobas**® Influenza A/B & RSV UC for the detection of influenza A, influenza B and RSV was confirmed by testing ten influenza A, five influenza B and nine RSV strains. The lowest concentration of target analyte at which all four tested replicates were positive are reported in Table 18.

Table 18 Summary of inclusivity

Viral Target	Strain	Catalog Number	Lot Number	Lowest Concentration detected
Influenza A	A/Canada/6294/09 (H1N1)	0810109CFJ	306161 (sublot: 511440)	0.010 $TCID_{50}$/mL
	A/California/07/09 (H1N1)	0810165CF	325194	0.055 $TCID_{50}$/mL
	A/Mexico/4108/09 (H1N1)	0810166CF	313217 (sublot: 514161)	0.0079 $TCID_{50}$/mL
	A/Singapore/63/04 (H1N1)	0810246CF	313221 (sublot: 514205)	2.72 $TCID_{50}$/mL
	A/Michigan/45/15 (H1N1)	0810538CF	321053 (sublot: 533618)	0.056 $TCID_{50}$/mL
	A/Texas/50/12 (H3N2)	0810238CF	325079	0.20 $TCID_{50}$/mL
	A/Perth/16/09 (H3N2)	0810251CF	325143	0.0072 $TCID_{50}$/mL
	A/Wisconsin/67/05 (H3N2)	0810252CF	325407	0.098 $TCID_{50}$/mL
	A/Switzerland/9715293/13 (H3N2)	0810511CF	325276	0.018 $TCID_{50}$/mL
	A/Hong Kong/4801/14 (H3N2)	0810526CF	325191	0.095 $TCID_{50}$/mL
Influenza B	B/Florida/78/2015 (Victoria lineage)	VR-1931	70020870	0.23 $TCID_{50}$/mL
	B/Brisbane/60/08 (Victoria lineage)	0810254CF	313257 (sublot: 513438)	0.0029 $TCID_{50}$/mL
	B/Alabama/2/17 (Victoria lineage)	0810572CF	322548	0.022 $TCID_{50}$/mL
	B/Wisconsin/1/2010 (Yamagata lineage)	VR-1883	70012127	0.20 $CEID_{50}$/mL
	B/Utah/9/14 (Yamagata lineage)	0810516CF	323752	0.0039 $TCID_{50}$/mL
RSV	Long (Type A)	VR-26PQ	70024412	0.79 $TCID_{50}$/mL
	2/2015 Isolate #3 (Type A)	0810475CF	317870 (529910)	0.26 $TCID_{50}$/mL
	4/2015 Isolate #1 (Type A)	0810481CF	322739 (534595)	0.058$TCID_{50}$/mL
	ATCC-2012-10 (Type B)	VR-1794	61635142	0.045 $TCID_{50}$/mL
	18537 (Type B)	VR-1580PQ	70025292	2.49 $TCID_{50}$/mL
	9320 (Type B)	VR-955	70030486	3.25 $TCID_{50}$/mL
	12/2014 Isolate #1(Type B)	0810450CF	318798 (531073)	0.0054 $TCID_{50}$/mL
	3/2015 Isolate #1 (Type B)	0810479CF	325279	0.049 $TCID_{50}$/mL
	11/2014 Isolate #2 (Type B)	0810451CF	318796 (531072)	0.036 $TCID_{50}$/mL

Analytical specificity (cross-reactivity and microbial interference)

A panel of 44 viruses, bacteria, and fungi (including those commonly found in respiratory tract) were tested with **cobas**® Influenza A/B & RSV UC to assess analytical specificity. The organisms listed in Table 19 were spiked at concentrations of 1 x 10^5 units/mL for viruses and 1 x 10^6 units/mL for other organisms, unless otherwise noted. Testing was performed with each potential interfering organism in the absence and presence of influenza A, influenza B, and RSV target (spiked co-formulated at ~3 x LoD – 0.060, 0.057 and 0.23 $TCID_{50}$/mL, respectively). None of the organisms interfered with the test performance by generating false positive results. Detection of influenza A, influenza B, and RSV targets was not affected in the presence of the organisms tested. Potential cross-reactivity of influenza C, *Leptospira interrogans*, *Chlamydia psittaci*, *Bacillus anthracis* and *Coxiella burnetii* was evaluated in silico. Based on the in silico analyses, selected organisms are highly unlikely to interfere with the performance of **cobas**® Influenza A/B & RSV UC.

Table 19 Microorganisms tested for analytical specificity/cross reactivity

Microorganism	Concentration
Adenovirus	1.0E+05 $TCID_{50}$/mL
Bordetella pertussis	1.0E+06 CFU/mL
Candida albicans	1.0E+06 CFU/mL
Chlamydia pneumoniae	7.9E+04 $TCID_{50}$/mL
Corynebacterium diphteriae	1.0E+06 CFU/mL
Cytomegalovirus	1.0E+05 IU/mL
Epstein Barr Virus	1.0E+05 cp/mL
Escherichia coli	1.0E+06 CFU/mL
Haemophilus influenzae	1.0E+06 CFU/mL
Herpes Simplex Virus Type I	1.0E+05 cp/mL
Herpes Simplex Virus Type II	1.0E+05 cp/mL
Human coronavirus 229E	1.0E+05 $TCID_{50}$/mL
Human coronavirus HKU1	1.0E+05 genome cp/mL
Human coronavirus NL63	2.5E+04 $TCID_{50}$/mL
Human coronavirus OC43	1.0E+05 $TCID_{50}$/mL
Human Enterovirus	1.0E+05 $TCID_{50}$/mL
Human Metapneumovirus	1.0E+05 $TCID_{50}$/mL
Human Rhinovirus	1.0E+05 PFU/mL
Lactobacillus acidophilus	1.0E+06 CFU/mL
Legionella longbeachae	1.0E+06 CFU/mL
Legionella pneumophila	1.0E+06 CFU/mL
Measles virus	1.0E+05 $TCID_{50}$/mL
MERS-coronavirus	1.0E+05 cp/mL
Moraxella catarrhalis	1.0E+06 CFU/mL
Mumps Virus	1.0E+05 $TCID_{50}$/mL

Microorganism	**Concentration**
Mycobacterium tuberculosis	1.0E+06 CFU/mL
Mycoplasma pneumonia	1.0E+06 CCU/mL
Neisseria elongate	1.0E+06 CFU/mL
Neisseria meningitidis	1.0E+06 CFU/mL
Parainfluenza virus 1	1.0E+05 $TCID_{50}$/mL
Parainfluenza virus 2	1.0E+05 $TCID_{50}$/mL
Parainfluenza virus 3	1.0E+05 $TCID_{50}$/mL
Parainfluenza virus 4	1.0E+05 $TCID_{50}$/mL
Parechovirus	1.0E+05 U/mL
Pneumocystis jirovecii	5.0E+03 organisms/mL
Pseudomonas aeruginosa	1.0E+06 CFU/mL
SARS-coronavirus	1.0E+05 PFU/mL
SARS-CoV-2 (heat-inactivated)	1.0E+05 $TCID_{50}$/mL
Staphylococcus aureus	1.0E+06 CFU/mL
Staphylococcus epidermis	1.0E+06 CFU/mL
Streptococcus pneumonie	1.0E+06 CFU/mL
Streptococcus pyogenes	1.0E+06 CFU/mL
Streptococcus salivarius	1.0E+06 CFU/mL
Varicella Zoster Virus	1.0E+05 cp/mL

Interfering substances

The effect of exogenous substances potentially secreted into respiratory specimens was evaluated (Table 20). Each potentially interfering substance was tested at or above clinically relevant levels in negative simulated clinical matrix stabilized in UTM™ in absence and presence of influenza A, influenza B, and RSV target (spiked co-formulated at ~3 x LoD – 0.060, 0.057 and 0.23 $TCID_{50}$/mL, respectively).

None of the substances interfered with the test performance by generating false-negative or false-positive results. None of the substances interfered with the test performance by generating invalid results.

Table 20 List of exogenous substances tested for interference

Substance	Concentration
Oxymetazoline	0.011 mg/mL
Budesonide	0.039 mg/mL
Fluticasone propionate	0.167 mg/mL
Luffa operculata, Thryallis glauca	2.14 mg/mL
Histaminum, Sulfur	1.072 mg/mL
Benzocaine	5.0 mg/mL
Menthol	1.2 mg/mL
Glycerin	10.31 mg/mL
Phenol	0.47 mg/mL
Lidocaine	2.68 mg/mL
Mupirocin	0.2 mg/mL
Zanamivir	0.0015 mg/mL
Oseltamivir	0.0073 mg/mL
Tobramycin	0.018 mg/mL

Additionally, FluMist® Quadrivalent, a live quadrivalent vaccine for administration by intranasal spray, and containing two Influenza A and two Influenza B vaccine virus strains, was tested (Table 21) in negative simulated clinical matrix stabilized in UTM™ in absence and presence of influenza A, influenza B, and RSV target (spiked co-formulated at ~3 x LoD – 0.060, 0.057 and 0.23 $TCID_{50}$/mL, respectively). As expected, **cobas**® Influenza A/B & RSV UC generated positive results for the Influenza A and Influenza B targets and negative results for the RSV targets when solely testing FluMist® Quadrivalent and all positive results for influenza A, influenza B and RSV targets when additionally spiking with low levels of co-formulated influenza A, influenza B and RSV.

Table 21 FluMist® Quadrivalent tested for interference

Product	Substance	Concentration
FluMist® Quadrivalent (Influenza Vaccine Live, Intranasal)	influenza A virus A/Hawaii/6 6/20 19 (H1N1) live (attentuated) antigen influenza A virus A/Hong Kong/26 71/20 19 (H3N2) live (attentuated) antigen influenza B virus B/Phuket/30 73/20 13 live (attentuated) antigen influenza B virus B/Washington/0 2/20 19 live (attentuated) antigen	1336620.81 FFU/mL

Endogenous substances that may be present in respiratory specimens were tested for interference (Table 22). Each potentially interfering substance was tested at or above clinically relevant levels in negative simulated clinical matrix stabilized in UTM™ in absence and presence of influenza A, influenza B, and RSV target (spiked co-formulated at ~3 x LoD – 0.060, 0.057 and 0.23 $TCID_{50}$/mL, respectively).

The tested endogenous substances, have not shown interference with the test performance at or above the clinically relevant levels with the exception of mucin, which showed interference above the medically relevant level (0.4%).[18] However, interference was not observed below the medically relevant mucin concentrations (Table 22).

Table 22 List of endogenous substances tested for interference

Substance	Concentration with no interference
Mucin	0.3% (w/v)
Human Whole Blood	3.0% (v/v)

Co-infection (competitive interference)

To assess potential competitive interference between influenza A, influenza B, and RSV, samples were tested in replicates of 5 where low (approximately 3 x LoD) concentrations of any two targets were mixed with very high (1.0E+05 $TCID_{50}$/mL) concentrations of the third target. None of the targets present at very high concentration interfered with the detection of low levels of the other two targets.

Whole system failure

The whole system failure rate was assessed by testing 100 specimens of simulated clinical matrix co-spiked with one strain each for influenza A (A/Brisbane/02/2018 (H1N1)), influenza B (B/Colorado/06/2017 (Victoria lineage)) and RSV (2/2015 Isolate #2 (Type A)) to a concentration of approximately 3 x LoD of the respective target. The results of this study determined that all replicates were valid and positive for influenza A, influenza B and RSV, resulting in a whole system failure rate of 0% with an upper one-sided 95% confidence interval of 3.0%.

Clinical performance evaluation

The performance of **cobas**® Influenza A/B & RSV UC was evaluated in comparison with the **cobas**® Influenza A/B & RSV at one external site using archived nasopharyngeal swab (NPS) samples from patients with signs and symptoms of a respiratory infection, collected in UTM® or UVT. Clinical samples were collected by qualified personnel according to the package insert of the collection device.

The clinical evaluation study included a total of 377 NPS samples with valid results.

As shown in Table 23, the **cobas**® Influenza A/B & RSV UC demonstrated high percent agreement with the comparator test for the detection of influenza A, influenza B and RSV.

Table 23 Comparison of **cobas**® Influenza A/B & RSV UC with **cobas**® Influenza A/B & RSV for use on the **cobas**® Liat® System

Virus	Number of Samples	Test Results				Agreement Statistics		
		Concordant Positive (N)	Discordant Positive (N)	Concordant Negative (N)	Discordant Negative (N)	Agreement Parameter	Percent Agreement (%)	95% CI (LCL, UCL)*
Influenza A	377	91	6	280	0	PPA	100.0%	(95.9%, 100.0%)
						NPA	97.9%	(95.5%, 99.0%)
Influenza B	377	85	4	287	1	PPA	98.8%	(93.7%, 99.8%)
						NPA	98.6%	(96.5%, 99.5%)
RSV	377	98	2	277	0	PPA	100.0%	(96.2%, 100.0%)
						NPA	99.3%	(97.4%, 99.8%)

PPA = Positive Percent Agreement

NPA = Negative Percent Agreement

CI = confidence interval; LCL = Lower confidence Limit; UCL = Upper confidence Limit

*Confidence interval is calculated using Wilson's Score method

Discordant results between the **cobas**® Influenza A/B & RSV UC assay and the comparator method were observed for 13 samples. In 12 of these samples **cobas**® Influenza A/B & RSV UC detected six additional influenza A virus, four additional influenza B virus and two additional RSV positive samples compared to **cobas**® Influenza A/B & RSV for use on the **cobas**® Liat® System. With the exception of one sample all of these specimens had Ct values close to or below the LoD of the respective pathogen. Post-PCR analysis of the amplicons of these discordant positive samples confirmed the presence of influenza A, influenza B and RSV, respectively. One of the 13 samples was positive by the comparator alone. Additional testing showed positivity for influenza B by both the **cobas**® Influenza A/B & RSV UC assay and the comparator with Ct values close to the LoD of both tests.

Additional information

Key test features

Sample type	Nasopharyngeal swab samples collected in the Copan UTM-RT® System or the BD™ UVT System, or equivalent
Minimum amount of sample required	0.6 mL*
Sample process volume	0.4 mL
Test duration	Results are available within less than 3.5 hours after loading the sample on the system.

*Dead volume of 0.2 mL is identified for the **cobas omni** Secondary tubes. Other tubes compatible with **cobas®** 6800/8800 Systems (consult User Assistance Guide) may have different dead volume and require more or less minimum volume.

Symbols

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

Table 24 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 US Only: 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 25 Manufacturer and importer

Roche Molecular Systems, Inc.
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Made in USA

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

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IVD

EC REP Roche Diagnostics GmbH
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Document revision

Document Revision Information	
Doc Rev. 1.0 06/2021	First Publishing.
Doc Rev. 2.0 11/2021	Changed the name of FluAB_RSV USAP to UC_FluAB_RSV. Updated the harmonized symbol page. Updated to current Economic Operators. Please contact your local Roche Representative if you have any questions.
Doc Rev. 3.0 04/2022	Updated the prepare samples and controls section. Please contact your local Roche Representative if you have any questions.