Rx Only

cobas® SARS-CoV-2 & Influenza A/B

Nucleic acid test for use on the cobas® Liat® System

For in vitro diagnostic use

CLIA Complexity: WAIVED

cobas® SARS-CoV-2 & Influenza A/B	P/N: 09211101190
cobas® SARS-CoV-2 & Influenza A/B Quality Control Kit	P/N: 09211128190

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Intended use

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

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

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

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

Summary and explanation of the test

Background

Coronavirus disease 2019 (COVID-19) is a respiratory illness caused by a novel human coronavirus, named SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2) by the World Health Organization.[1-3] COVID-19 has been declared a public health emergency of international concern and is the first pandemic caused by coronavirus.[4,5] Amidst global concerns over COVID-19, influenza A and B viruses continue to circulate and also cause acute respiratory disease. COVID-19 and influenza are potentially fatal infections that result in significant worldwide morbidity and mortality.[6]

Rapid and accurate diagnosis and differentiation of SARS-CoV-2 and influenza infections is important in individuals suspected of a respiratory infection. The seasonality of COVID-19 and influenza overlap and the clinical manifestations of the two diseases can be similar, ranging from asymptomatic or mild "influenza-like" illness (such as fever, cough, shortness of breath, or myalgia) in a majority of individuals to more severe and life-threatening disease.[7-9] The current widespread implementation of rapid point of care (POC) testing for influenza underscores the importance of prompt and accurate detection.[10] Rapid and accurate detection of both SARS-CoV-2 and influenza can help to inform time-critical medical decision-making, facilitate infection control efforts, promote efficient resourcing, optimize use of targeted therapies and antimicrobials, and reduce ancillary testing or procedures.[11,12]

Explanation of the test

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

Principles of the procedure

The **cobas**® SARS-CoV-2 & Influenza A/B assay is performed on the **cobas**® Liat® Analyzer which automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence in biological samples using real-time RT-PCR assays. The assay targets both the ORF1 a/b non-structural region and nucleocapsid protein gene that are unique to SARS-CoV-2, a well-conserved region of the matrix gene of influenza A, and the non-structural protein gene of influenza B. An Internal Process Control (IPC) is also included. The IPC is present to control for adequate processing of the target virus through steps of sample purification, nucleic acid amplification, and to monitor the presence of inhibitors in the RT-PCR processes.

Reagents and materials

The materials provided for **cobas**® SARS-CoV-2 & Influenza A/B can be found in Table 1 and Table 2. Reagent handling and storage can be found in Table 3. Materials required, but not provided can be found in Table 4 and Table 5.

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

cobas® SARS-CoV-2 & Influenza A/B reagents and controls

All unopened assay tubes and controls shall be stored as recommended in Table 1 to Table 3.

Table 1 **cobas**® SARS-CoV-2 & Influenza A/B
Store at 2-8°C
20 tests (P/N 09211101190)
2 **cobas**® transfer pipette packs (12 pipettes/pack - P/N 9329676001)
1 Package Insert Barcode card

Reagents in cobas® SARS-CoV-2 & Influenza A/B assay tube	Reagent ingredients	Safety symbol and warning[a]
cobas® Liat® Internal Process Control	Tris buffer, tween-80, polyethylene glycol, EDTA, < 0.001% stock bacteriophage MS2 (inactivated), 0.002% carrier RNA, 0.01% ProClin® 300 preservative[b]	EUH210 Safety data sheet available on request. EUH208 Contains reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1). May produce an allergic reaction.
Proteinase K	100% Proteinase K	N/A
cobas® Liat® Magnetic Glass Particles	Magnetic Glass Particles	N/A

Reagents in cobas® SARS-CoV-2 & Influenza A/B assay tube	Reagent ingredients	Safety symbol and warning[a]
cobas® Liat® Lysis Buffer	Citric acid, sodium phosphate, 42.6% guanidinium isothiocyanate[b], 5% decaethylene glycol monododecyl ether[b], dithiothreitol	 **DANGER** H302 + H332 Harmful if swallowed or if inhaled. 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. P261 Avoid breathing dust/fume/gas/mist/ vapours/spray. P273 Avoid release to the environment. P280 Wear protective gloves/protective clothing/ eye protection/face 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. 593-84-0 Guanidinium thiocyanate 9002-92-0 Brij 35
cobas® Liat® Wash Buffer	Glycine, potassium fluoride, 0.01% ProClin® 300 preservative	N/A
cobas® Liat® Elution Buffer	Trehalose, tris buffer, magnesium sulfate, bovine serum albumin, 0.01% ProClin® 300 preservative[b]	EUH210 Safety data sheet available on request. EUH208 Contains reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1). May produce an allergic reaction.
cobas® Liat® SARS-CoV-2 & Influenza A/B Master Mix-1	Tween-80, tris buffer, trehalose, potassium chloride, bovine serum albumin, dATP, dCTP, dGTP, dUTP, 0.01% ProClin® 300 preservative[b], < 0.001% Downstream *SARS-CoV-2, influenza A, influenza B* and Internal Process Control primers	EUH210 Safety data sheet available on request. EUH208 Contains reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1). May produce an allergic reaction.
cobas® Liat® SARS-CoV-2 & Influenza A/B Master Mix-2	Tween-80, tween-20, tris buffer, glycerol, potassium chloride, EDTA, dithiothreitol, < 0.01% Z05 polymerase with aptamer, 0.23% MMLV Reverse Transcriptase	N/A

Reagents in cobas® SARS-CoV-2 & Influenza A/B assay tube	Reagent ingredients	Safety symbol and warning[a]
cobas® Liat® SARS-CoV-2 & Influenza A/B Master Mix-3	Tween-80, tris buffer, EDTA, trehalose, potassium chloride, bovine serum albumin, < 0.001% upstream *SARS-CoV-2, influenza A, influenza B* and Internal Control primers, < 0.01% fluorescent-labeled *SARS-CoV-2, influenza A, influenza B* and Internal Control probes, 0.004% Taq DSC 2.0 DNA polymerase, 0.01% ProClin® 300 preservative[b]	EUH210 Safety data sheet available on request. EUH208 Contains reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1). May produce an allergic reaction.

[a] Product safety labeling primarily follows EU GHS guidance

[b] Hazardous substance or mixture

Table 2 **cobas®** SARS-CoV-2 & Influenza A/B Quality Control Kit

Store at 2-8°C
(P/N 09211128190)
11 transfer pipettes
1 Control Kit Barcode card

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning[a]
cobas® SARS-CoV-2 & Influenza A/B Positive Control **SARS-CoV-2 (+) C** (P/N 09212078001)	Tris buffer, EDTA, < 0.003% Poly rA (synthetic), < 0.01% non-infectious plasmid DNA (microbial) containing SARS-CoV-2 sequence, < 0.05% sodium azide	3 X 0.25 mL	N/A
cobas® SARS-CoV-2 & Influenza A/B Positive Control **FLU A/B (+) C** (P/N 07758448001)	Magnesium chloride, polyethylene glycol, bovine serum albumin, phosphate buffer saline, < 0.01% Poly rA, (synthetic), 5% non-infectious influenza AH1 stock and 1% Non-infectious influenza B stock (micro-organism purified and chemically inactivated), < 0.01% ProClin® 300 preservative[b], Phenol red	3 X 10 µL	 EUH210 Safety data sheet available on request. EUH208 Contains reaction mass of: 5-chloro-2- methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3- one [EC no. 220-239-6] (3:1). May produce an allergic reaction.
cobas® Dilution UTM **Dilution UTM (-) C** (P/N 08053669001)	N/A	3 X 0.3 mL	N/A

[a] Product safety labeling primarily follows EU GHS guidance

[b] Hazardous substance or mixture

Reagent storage and handling

Reagents shall be stored and will be handled as specified in Table 3.

Do not freeze materials listed below. Do not open individual assay tube packaging until operator is ready to perform testing.

Table 3 Reagent storage and handling

Reagent	Storage Temperature	Storage Time
cobas® SARS-CoV-2 & Influenza A/B	2–8°C	Stable until the expiration date indicated
cobas® SARS-CoV-2 & Influenza A/B Quality Control Kit	2-8°C	Stable until the expiration date indicated

Additional materials required

Table 4 Materials required but not provided

Specimen Collection Kit	P/N
Nasopharyngeal Swab Collection Kits: Flexible minitip FLOQSwab™ with Universal Transport Media™ (UTM®) from Copan Diagnostics OR BD™ Universal Viral Transport (UVT) 3-mL collection kit with a flocked flexible minitip swab	 305C 220531
Nasal Swab Collection Kits: Regular FLOQSwab™ with Universal Transport Media™ (UTM®) from Copan Diagnostics OR BD™ Universal Viral Transport (UVT) 3-mL collection kit with a regular flocked swab Copan Universal Transport Medium (UTM-RT®), without beads	 306C 220528 3C047N
Thermo Fisher™ Scientific Remel™ M4RT Thermo Fisher™ Scientific Remel™ M4 Thermo Fisher™ Scientific Remel™ M5 Thermo Fisher™ Scientific Remel™ M6 Thermo Fisher™ Scientific Remel™ M4RT® tube, without beads	R12565, R12566, R12567 R12550 R12555 R12563, R12568, R12569 R12622, R12591
Pre-aliquotted 3 mL 0.9% Physiological saline Thomas Scientific MANTACC™ 0.9% Saline Solution, 3 mL in 10mL Tube, 50 Tubes per Pack	 20A00K984

Instrumentation and software required

The **cobas®** Liat® System Software is installed on the instrument(s).

Table 5 Equipment and software required but not provided

Equipment and Software
cobas® Liat® Analyzer (P/N 07341920190) Including **cobas®** Liat® System Software (Core) Version 3.3 or higher
cobas® SARS CoV-2 & Influenza A/B Assay Script v1.2 or higher

Note: For additional information regarding the **cobas®** Liat® Analyzer, please refer to the **cobas®** Liat® System User Guide.

Precautions and handling requirements

Warnings and precautions

- For in vitro diagnostic use.
- CLIA Complexity: WAIVED
  A Certificate of Waiver is required to perform this test in a CLIA Waived setting. To obtain CLIA waiver information and a Certificate of Waiver, please contact your state health department. Additional CLIA waiver information is available at the Centers for Medicare and Medicaid website at www.cms.hhs.gov/CLIA. Failure to follow the instructions or modification to the test system instructions will result in the test no longer meeting the requirements for waived classification.
- Before using the **cobas**® SARS-CoV-2 & Influenza A/B test, operator should carefully read Instructions For Use (IFU) and the **cobas**® Liat® System User Guide.
- Treat all biological specimens, including used **cobas**® SARS-CoV-2 & Influenza A/B assay tubes and transfer pipettes, as if capable of transmitting infectious agents. It is often impossible to know which specimens might be infectious; all biological specimens should be treated with universal precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention, Clinical and Laboratory Standards Institute and World Health Organization.[13-17]
- If infection with SARS-CoV-2 is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions. Refer to the CDC Interim Guidelines for Collecting and Handling of Clinical Specimens for COVID-19 Testing for more information. (https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html) Viral cultures should not be attempted in cases of positive results for SARS-CoV-2 and/or any similar microbial agents unless a facility with an appropriate level of laboratory biosafety (e.g., BSL 3 and BSL 3+, etc.) is available to receive and culture specimens.
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected using appropriate infection control precautions for novel virulent influenza viruses and sent to state health departments for testing. Virus culture should not be attempted in these cases unless a BSL-3 facility is available to receive and culture specimens.
- Positive results for SARS-CoV-2 or suspected novel influenza should be reported to state, local, or federal health departments according to local reporting requirements.
- Do not use a damaged **cobas**® SARS-CoV-2 & Influenza A/B assay tube.
- Do not use a **cobas**® SARS-CoV-2 & Influenza A/B assay tube that has been dropped after removal from its foil pouch.
- Do not open the cap of the **cobas**® SARS-CoV-2 & Influenza A/B assay tube during or after the run on the **cobas**® Liat Analyzer.
- Do not use Negative Control if the color has changed from light orange-red.
- For additional warnings, precautions and procedures to reduce the risk of contamination for the **cobas**® Liat® Analyzer, consult the **cobas**® Liat® System User Guide.
- Ensure any additional labels are only placed on the back of the tube sleeve, do not place labels over barcodes or over the top of the assay tube cap.
- Dispose of a used **cobas**® SARS-CoV-2 & Influenza A/B assay tube, pipette and specimen tube according to your institution's safety guidelines for hazardous material.

- On request Safety Data Sheets (SDS) are available from your local Roche representative.
- Due to the high sensitivity of the assays run on the **cobas**® Liat® Analyzer, contamination of the work area with previous positive samples may cause false positive results. Handle samples according to standard laboratory practices. Clean instruments and surrounding surfaces according to instructions provided in the cleaning section of the **cobas**® Liat® System User Guide. If spills occur on the **cobas**® Liat® Analyzer, follow the appropriate instructions in the **cobas**® Liat® System User Guide to clean.
- Specimen collection must be performed using the recommended swab types. Inadequate or inappropriate sample collection, storage, and transport may yield incorrect or invalid test results. DO NOT use cotton or calcium alginate swabs, or swabs with wood shafts.
- Ensure there is no sign of leakage from the collection tube prior to running the test.
- When using pre-aliquotted 3 mL of sterile 0.9% physiological saline solution, ensure that the swab height is appropriate for the collection and the score mark is not higher than the height of the collection tube.
- Use only the transfer pipettes provided in either **cobas**® Liat® Assay Tube Kits or **cobas**® Liat® Quality Control Kits to transfer controls and samples into the assay tube. Use of alternative transfer pipettes may lead to invalid results.
- Good laboratory practices and careful adherence to the procedures specified in this Instructions For Use document are necessary. Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed prior to taking transfer pipette out of the **cobas**® transfer pipette pack, between handling samples, **cobas**® SARS-CoV-2 & Influenza A/B assay tube, and **cobas**® SARS-CoV-2 Quality Control Kit to avoid contamination of reagents and pipettes.
- After handling samples and kit reagents, remove gloves and wash hands thoroughly.
- Performance characteristics have been determined with specimens from human patients with signs and symptoms of respiratory infection.

Sample collection, transport, and storage

Note: Handle all samples and controls as if they are capable of transmitting infectious agents. Do not use cotton or calcium alginate swab, or swab with wood shafts.

Sample collection

- Collect specimen using a sterile flocked swab with a synthetic tip according to applicable manufacturer instructions and/or standard collection technique using 3 mL of viral transport media or sterile 0.9% physiological saline.

Transport and storage

Transportation of collected specimens must comply with all applicable regulations for the transport of etiologic agents. Transport and test specimens as soon as possible after collection.

- If transportation is required, specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential SARS-CoV-2 or influenza virus specimens. Store specimens at 2-8°C and ship overnight on ice pack. If a specimen in transport media (UTM, VTM, M4, M4RT, M5, and M6) is frozen at ≤-70°C, ship overnight on dry ice.
- Specimen transferred into the **cobas**® SARS-CoV-2 & Influenza A/B assay tube should be run as soon as possible on the Analyzer. Once the sample has been added to the **cobas**® SARS-CoV-2 & Influenza A/B assay tube it may be stored at room temperature for up to 4 hours.
- Specimens collected in transport media (UTM or UVT, M4, M4RT, M5 and M6) may be stored up to 4 hours at room temperature or up to 72 hours at 2-8°C if immediate testing is not possible. Freezing at -70°C or colder (and transportation on dry ice) is required for specimen storage or transportation beyond 72 hours prior to the specimen being added to the assay tube for testing.
- Specimens collected in 0.9% physiological saline solution may be stored up to 4 hours at room temperature or up to 72 hours at 2-8°C if immediate testing is not possible.
- Nasal and nasopharyngeal swabs collected in Thomas Scientific MANTACC™ premeasured 3 mL 0.9% physiological saline solution are compatible for use with **cobas**® SARS-CoV-2 & Influenza A/B test. Performance of the **cobas**® SARS-CoV-2 & Influenza A/B test with specimens collected in 0.9% physiological saline has been established in analytical studies, however, clinical performance of the assay in this media types was not established.

Instructions for use

Procedural notes

- Do not use **cobas**® SARS-CoV-2 & Influenza A/B assay tube and **cobas**® SARS-CoV-2 & Influenza A/B Quality Control Kit after their expiry dates.
- Do not reuse assay tubes and transfer pipettes. They are for one-time use only.
- Refer to the **cobas**® Liat® System User Guide for detailed operation and routine cleaning of instruments.

Running cobas® SARS-CoV-2 & Influenza A/B

Use the transfer pipette to load approximately 0.2 mL of the specimen into the **cobas**® SARS-CoV-2 & Influenza A/B assay tube. The **cobas**® Liat® Analyzer will adjust the sample volume if more sample was loaded.

Always use caution when transferring specimens from a sample collection tube to the assay tube.

Use transfer pipettes from the cobas® transfer pipette pack included in the kit to handle specimens.

Ensure clean gloves are used when removing transfer pipettes from the cobas® transfer pipette pack.

Reseal the cobas® transfer pipette pack immediately after removing the necessary pipette(s).

The cobas® transfer pipette pack may be stored at room temperature following first removal from the kit.

Always use a new transfer pipette for each specimen.

The test procedure is described in detail in the **cobas**® Liat® System User Guide. Figure 1 below summarizes the procedure.

Test procedure

Figure 1 **cobas**® SARS-CoV-2 & Influenza A/B procedure

"Lot Validation" workflow

1	Start up the system and login
2	Obtain Controls and assay tubes
3	Under "Assay Menu", choose "New Lot"
4	Scan the Package Insert barcode from the Package Insert Barcode card
5	Scan and run Negative Control
6	Scan and run Positive Control

cobas® SARS CoV-2 & Influenza A/B workflow

1	Start up the system and login
2	Obtain samples and assay tubes
3	On the Main menu, choose "Run Assay"
4	Scan the **cobas**® SARS-CoV-2 & Influenza A/B assay tube barcode
5	Scan or enter the sample ID
6	Add specimen to the **cobas**® SARS-CoV-2 & Influenza A/B assay tube using the transfer pipette and re-cap the tube
7	Re-scan the **cobas**® SARS-CoV-2 & Influenza A/B assay tube barcode
8	Start run
9	Review results*
10	Unload and dispose of the used **cobas**® SARS-CoV-2 & Influenza A/B assay tube

* Refer to **cobas**® Liat® System User Guide for details of result uploading to LIS or DMS.

cobas® SARS-CoV-2 & Influenza A/B assay tube Lot Validation

Before using a new lot of **cobas**® SARS-CoV-2 & Influenza A/B assay tubes, a Lot Validation procedure must be performed on the **cobas**® Liat® Analyzer to validate the **cobas**® SARS-CoV-2 & Influenza A/B assay tube lot at your site. The procedure includes running a Negative Control sample and a Positive Control sample.

Note: Refer to the **cobas**® Liat® System User Guide for detailed operating instructions.

Materials needed for Lot Validation

The following materials are needed:

Materials needed to validate Negative Control:	**Materials needed to validate Positive Control:**
□ 1 Dilution UTM vial[2]	□ 1 **cobas**® SARS-CoV-2 Positive Control vial[2]
□ 1 **cobas**® SARS-CoV-2 & Influenza A/B assay tube from this lot[1]	□ 1 **cobas**® Influenza A/B Positive Control vial[2] (pellet comprising dried positive control material at bottom of vial)
□ 1 transfer pipette[1 or 2]	□ 1 **cobas**® SARS-CoV-2 & Influenza A/B assay tube from this lot[1]
□ Package Insert Barcode card[1]	□ 2 transfer pipettes[1 or 2]
□ Negative Control barcode on the Control Kit Barcode card[2]	□ Positive Control barcode on the Control Kit Barcode card[2]

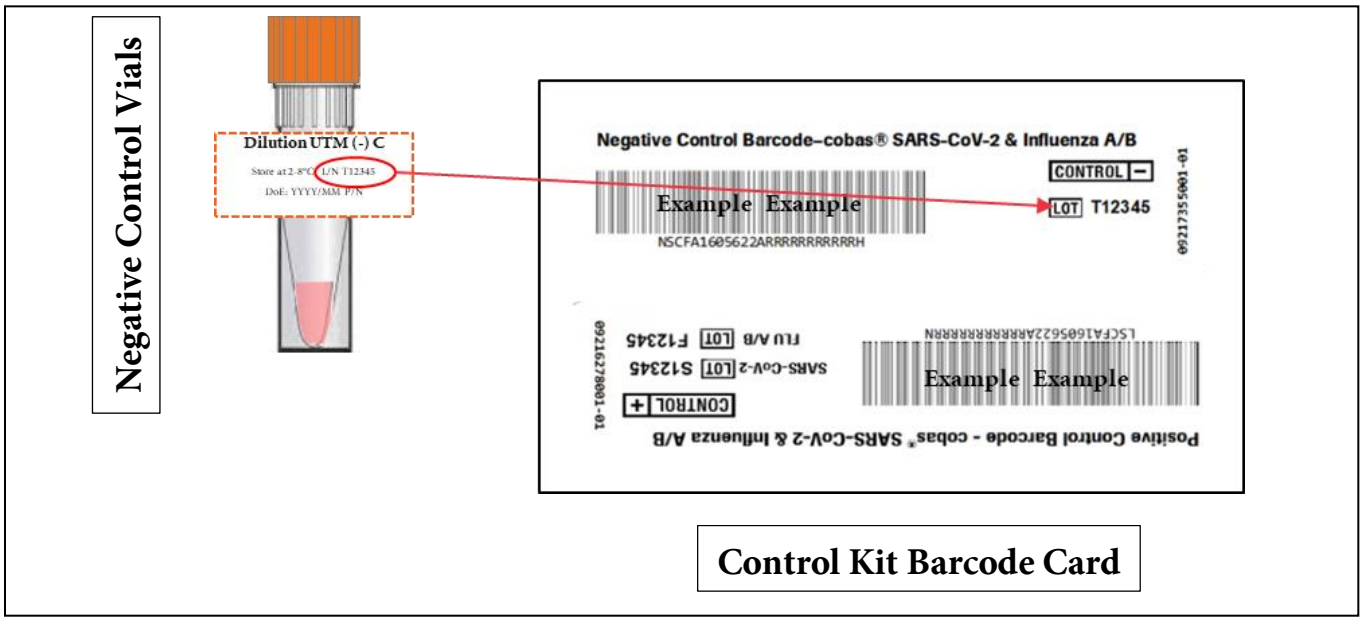
[1] Contained in **cobas**® SARS-CoV-2 & Influenza A/B assay tube Kit

Package Insert Barcode card: This barcode is lot-specific; match the lot number next to the barcode with the lot number on the **cobas**® SARS-CoV-2 assay tubes.

[2] Contained in **cobas**® SARS-CoV-2 & Influenza A/B Quality Control Kit

Note: *Following Figure 2, match the lot number (L/N) of the Dilution UTM vial label to the lot number (LOT) of the Negative Control barcode on the Control Kit Barcode card, and then use the Negative Control barcode (on the Control Kit Barcode card) as the sample ID when performing a negative control run.*

Figure 2 Schematic diagram for illustrating Negative Control vial and Control Kit Barcode Card



Assay tube Lot Validation workflow

1. Press the power on/off button to start the **cobas**® Liat® Analyzer.
2. Choose **"Login"** on the screen of the **cobas**® Liat® Analyzer.
3. Enter user name when prompted, choose "**OK**".
4. Enter user password when prompted, choose "**OK**".

   Note: You may be prompted to confirm you have read the User Manual, (i.e., cobas® Liat® System User Guide).
5. From the Main menu, choose "**Assay Menu**".
6. Choose "**New Lot**" at the bottom of the list.
7. When prompted to **Scan insert ID,** choose "**Scan**" and scan the **cobas**® SARS-CoV-2 & Influenza A/B Package Insert barcode from the Package Insert Barcode card. Ensure that the red scan light is over the entire barcode.

   Note: You may be prompted to confirm you have read Instructions For Use.
8. When prompted to **Scan negative control ID**, choose "**Scan**" and scan the Negative Control barcode from the Control Kit Barcode card included with the control kit. Ensure that the red scan light is over the entire barcode. Next, the **cobas**® Liat® Analyzer will prompt with the message "**Add negative control & scan tube ID**".
9. Hold a Dilution UTM vial upright and lightly tap on a flat surface to collect liquid at the bottom of the vial. Visually check that the Dilution UTM has pooled at the bottom of the vial.
10. Open up a **cobas**® SARS-CoV-2 & Influenza A/B assay tube foil pouch (from the lot to be added) and remove the contents.

11. Use a transfer pipette provided in the kit to add the Negative Control to the **cobas**® SARS-CoV-2 & Influenza A/B assay tube. Firmly squeeze the bulb of the pipette until the bulb is fully flat, then insert the tip of the pipette into the liquid and draw up the sample by slowly releasing the bulb.

    Note: Only use transfer pipettes provided in either cobas® Liat® Assay Tube Kits or cobas® Liat® Quality Control Kits to transfer controls and samples into the assay tube.

12. Carefully remove the cap of the **cobas**® SARS-CoV-2 & Influenza A/B assay tube and insert the pipette into the opening. Place the pipette tip near the bottom of the open segment.

13. Slowly squeeze the bulb to empty the contents of the pipette into the **cobas**® SARS-CoV-2 & Influenza A/B assay tube. Avoid creating bubbles in the sample. Do not release the pipette bulb while the pipette is still in the **cobas**® SARS-CoV-2 & Influenza A/B assay tube.

    Note: Do not puncture the cobas® SARS-CoV-2 & Influenza A/B assay tube or the seal at the bottom of the sample compartment. If either of these are damaged, discard both the cobas® SARS-CoV-2 & Influenza A/B assay tube and the transfer pipette, and restart the testing procedure with a new cobas® SARS-CoV-2 & Influenza A/B assay tube and pipette.

14. Screw the cap back onto the **cobas**® SARS-CoV-2 & Influenza A/B assay tube. Dispose of the transfer pipette as biohazardous material.

15. Choose "**Scan**" and place the **cobas**® SARS-CoV-2 & Influenza A/B assay tube horizontally on the table beneath the barcode reader so that the red scan light is over the entire barcode. The tube entry door on top of the **cobas**® Liat® Analyzer will open automatically once the barcode is read.

16. Remove the **cobas**® SARS-CoV-2 & Influenza A/B assay tube sleeve and immediately insert the **cobas**® SARS-CoV-2 & Influenza A/B assay tube into the **cobas**® Liat® Analyzer until the tube clicks into place.

    Note: The cobas® SARS-CoV-2 & Influenza A/B assay tube only fits in one way - the grooved side of the cobas® SARS-CoV-2 & Influenza A/B assay tube must be on the left while the cap is on top.

17. If the tube is not inserted by the time the door closes, re-scan the **cobas**® SARS-CoV-2 & Influenza A/B assay tube barcode and insert the **cobas**® SARS-CoV-2 & Influenza A/B assay tube again. Once the **cobas**® SARS-CoV-2 & Influenza A/B assay tube is properly inserted, the **cobas**® Liat® Analyzer will close the door automatically and begin the test.

18. During the test, the **cobas**® Liat® Analyzer displays the running status and estimated time remaining. Once the test is complete, the **cobas**® Liat® displays the message, "*Remove the assay tube slowly and carefully.*" and opens the tube entry door automatically. Slowly lift the **cobas**® SARS-CoV-2 & Influenza A/B assay tube out of the **cobas**® Liat® Analyzer. Dispose of the used **cobas**® SARS-CoV-2 & Influenza A/B assay tube as biohazardous material.

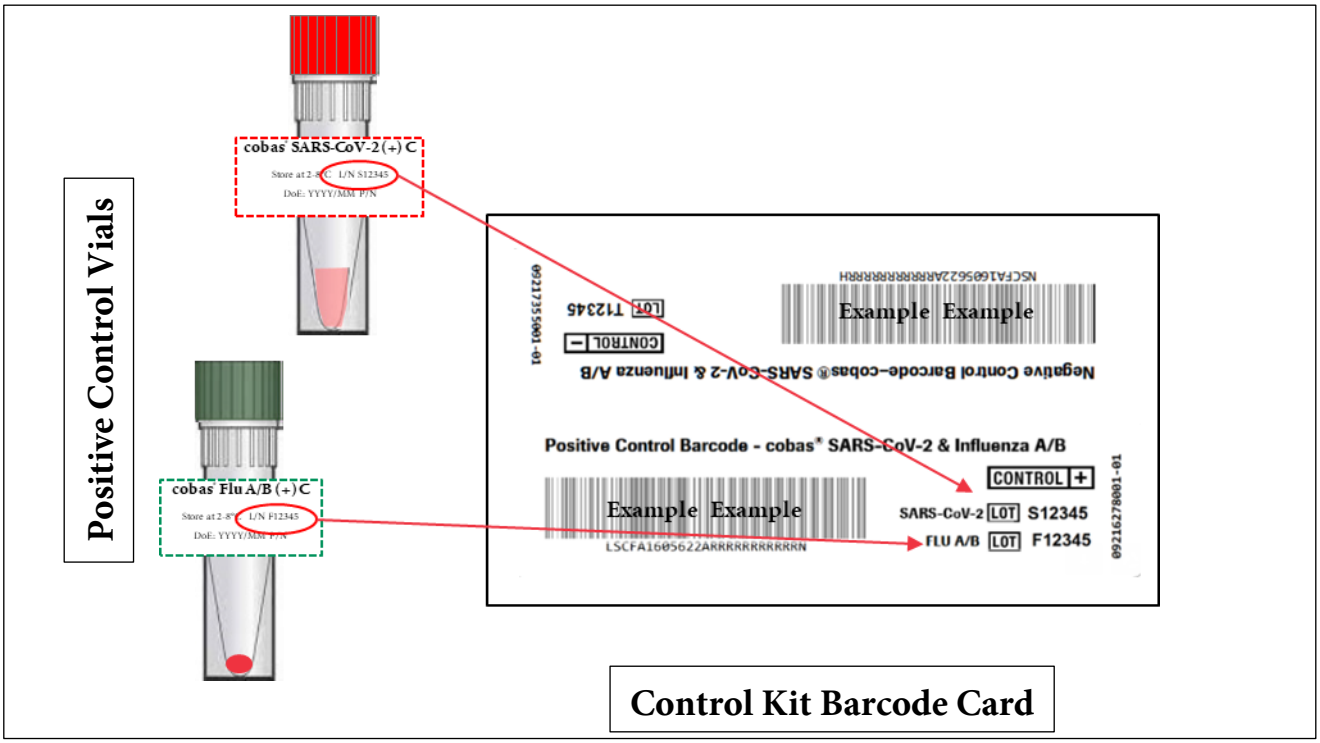
19. If **"Negative control result accepted."** is displayed at the end of the run, choose **"Confirm"**. If the result is rejected, repeat the negative control run (steps 8-19). If repeated control runs do not produce the expected results, contact your local Roche representative.

20. Choose **"Back"** to proceed with the **cobas**® SARS-CoV-2 & Influenza A/B Positive Control test on the same instrument.

21. Prepare positive control sample as follows.

Note: *Prior to resuspending the Positive Control, match the lot numbers (L/N) of the Positive Control vial label for* ***cobas***® *SARS-CoV-2 &* ***cobas***® *Influenza A/B to the lot number(LOT) of the Positive Control barcode on the Control Kit Barcode card as shown in Figure 3. Use the Positive Control barcode (on the Control Kit Barcode card) as the sample ID when performing a positive control run.*

Figure 3 Schematic diagram illustrating **cobas**® SARS-CoV-2 & **cobas**® Influenza A/B Positive Control vials and Control Kit Barcode Card



1. After opening **cobas**® Influenza A/B Positive Control pouch, discard desiccant packet.
2. After opening **cobas**® SARS-CoV-2 Positive Control pouch, hold the vial upright and lightly tap on a flat surface to collect liquid at the bottom of the vial. Visually check that the liquid has pooled at the bottom of the vial.
3. Use the provided transfer pipette to transfer approximately 0.2 mL of the liquid from the **cobas**® SARS-CoV-2 Positive Control vial to the **cobas**® Influenza A/B Positive Control vial.
    a) Check that the **cobas**® Influenza A/B Positive Control pellet is at the bottom of the vial prior to addition of the **cobas**® SARS-CoV-2 Positive Control. Do not use the **cobas**® Influenza A/B Positive Control if a pellet is not visible prior to rehydration.
    b) Squeeze the pipette bulb until the bulb is fully flat. While holding the bulb fully flat, insert the pipette tip into the liquid just below the liquid surface in the **cobas**® SARS-CoV-2 Positive Control vial.
    c) Slowly release the bulb completely while keeping the pipette tip below the liquid surface. You will see the liquid rising into the pipette. After releasing the bulb completely, withdraw the pipette from the **cobas**® SARS-CoV-2 Positive Control vial. A small volume of liquid may remain in the vial after the bulb is fully released.
    d) Insert pipette into the **cobas**® Influenza A/B Positive Control vial until the tip is at the bottom of the vial.

e) Slowly squeeze the bulb to empty the contents of pipette. Avoid creating bubbles in the sample. Do not release the pipette bulb.

f) While still squeezing the pipette bulb, withdraw the pipette from the vial. Dispose of the **cobas**® SARS-CoV-2 Positive Control vial and transfer pipette according to your institution's guidelines for safe disposal of hazardous material. Do not reuse transfer pipettes.

g) Cap the **cobas**® Influenza A/B Positive Control vial. Hold the **cobas**® Influenza A/B Positive Control vial by the cap and shake down the liquid in the vial using a quick, sharp, downward wrist motion.

4. Let the **cobas**® Influenza A/B Positive Control vial sit for 5 minutes to begin dissolving the dried material.
5. After the Positive Control vial has sat for 5 minutes, use another transfer pipette from the kit to slowly pipette the sample up and down 10 times to dissolve and mix the positive control sample. Avoid generating bubbles. Re-cap the **cobas**® Influenza A/B Positive Control vial and dispose of the transfer pipette as biohazardous material.
6. Similarly, follow **Lot Validation** workflow steps 8 to 18 with the resuspended **cobas**® SARS-CoV-2 & Influenza A/B Positive Control in place of the Negative Control.
7. If **"Positive control result accepted. Lot … added"** is displayed at the end of the run, choose "**Confirm"** and then choose **"Back"** to return to Main menu. If the result is rejected, repeat the **cobas**® SARS-CoV-2 & Influenza A/B Positive Control test. If repeated control runs do not produce the expected results, contact your local Roche representative.
8. Choose **"Assay Menu"** to verify that the new lot has been added.

Transferring assay tube lot information

After Lot Validation workflow is completed on one analyzer, use the Advanced Tools to transfer the lot information to the other analyzers at your site. This allows the other analyzers to use this **cobas**® SARS-CoV-2 & Influenza A/B assay tube lot without performing Lot Validation on each analyzer. Consult the **cobas**® Liat® System User Guide for details of operation.

cobas® SARS-CoV-2 & Influenza A/B on clinical specimens testing

Material needed for running cobas® SARS-CoV-2 & Influenza A/B

- **cobas**® SARS-CoV-2 & Influenza A/B assay foil pouch which includes the **cobas**® SARS-CoV-2 & Influenza A/B assay tube
- 1 transfer pipette
- One specimen in collection media

Procedure

1. Ensure that the **cobas**® Liat® Analyzer is powered on.
2. Choose **"Login"** on the screen of the **cobas**® Liat® Analyzer.
3. Enter user name when prompted, choose **"OK"**.
4. Enter user password when prompted, choose **"OK"**.

   Note: You may be prompted to confirm you have read the User Manual (i.e., cobas® Liat® System User Guide).
5. From the Main Menu, choose "**Run Assay**".
6. Open up a **cobas**® SARS-CoV-2 & Influenza A/B assay tube pouch and take out the assay tube. When prompted to **Scan tube ID,** choose **"Scan"** and place the SARS-CoV-2 & Influenza A/B assay tube horizontally on the table beneath the barcode reader so that the red scan light is over the entire barcode.
7. When prompted to **Scan sample ID**, choose "**Scan**" to scan the sample barcode. In the case that the sample cannot be scanned, choose "**Enter**" to manually enter the sample ID.
    a. **Note:** If patient verification is activated, the Analyzer will display the status of verification.
        i. If patient verification is successful, the Analyzer may prompt confirmation of entered information before proceeding with running the assay.
        ii. If patient verification fails, the Analyzer may display a notification that verification failed:
            1. And may require acknowledgement before proceeding with running the assay or
            2. If unable to proceed with running the assay contact your lab administrator.
8. Carefully remove one transfer pipette from the **cobas**® transfer pipette pack and avoid touching other pipettes in the pack. Re-seal the pack.
9. When prompted to add the sample**,** use the transfer pipette provided in the assay kit to transfer specimen. Firmly squeeze the bulb of the pipette until the bulb is fully flat, then insert the tip of the pipette into the liquid and draw up the sample by slowly releasing the bulb.
10. Carefully remove the cap of the **cobas**® SARS-CoV-2 & Influenza A/B assay tube and insert the pipette into the opening. Place the pipette tip near the bottom of the open segment.
11. Slowly squeeze the bulb to empty the contents of the pipette into the **cobas**® SARS-CoV-2 & Influenza A/B assay tube. Do not release the pipette bulb while the pipette is still in the **cobas**® SARS-CoV-2 & Influenza A/B assay tube.

    Note: Do not puncture the cobas® SARS-CoV-2 & Influenza A/B assay tube or the seal at the bottom of the sample compartment. If either of these are damaged, discard both the cobas® SARS-CoV-2 & Influenza A/B assay tube and

the transfer pipette, and restart the testing procedure with a new cobas® SARS-CoV-2 & Influenza A/B assay tube and pipette.

12. Re-cap the **cobas**® SARS-CoV-2 & Influenza A/B assay tube and dispose of the transfer pipette as biohazardous material.

Note: Avoid contaminating gloves, equipment and work surfaces with the residual contents of the pipette.

13. Choose "**Scan"** and rescan the same **cobas**® SARS-CoV-2 & Influenza A/B assay tube barcode. The tube entry door on top of the **cobas**® Liat® Analyzer will open automatically.
14. Remove the **cobas**® SARS-CoV-2 & Influenza A/B assay tube sleeve and immediately insert the **cobas**® SARS-CoV-2 & Influenza A/B assay tube into the **cobas**® Liat® Analyzer until the tube clicks into place.

Note: The SARS-CoV-2 & Influenza A/B assay tube only fits in one way - the grooved side of the cobas® SARS-CoV-2 & Influenza A/B assay tube must be on the left while the cap is on top.

15. If the assay tube is not inserted by the time the door closes, re-scan the **cobas**® SARS-CoV-2 & Influenza A/B assay tube barcode and insert the **cobas**® SARS-CoV-2 & Influenza A/B assay tube again. Once the **cobas**® SARS-CoV-2 & Influenza A/B assay tube is properly inserted, the **cobas**® Liat® Analyzer will close the door automatically and begin the test.
16. During the test, the **cobas**® Liat® Analyzer displays the running status and estimated time remaining. Once the test is complete, the **cobas**® Liat® Analyzer displays the message, "*Remove the assay tube slowly and carefully."* and opens the tube entry door automatically. Slowly lift the **cobas**® SARS-CoV-2 & Influenza A/B assay tube out of the **cobas**® Liat® Analyzer. Dispose of the used **cobas**® SARS-CoV-2 & Influenza A/B assay tube as biohazardous material.
17. Choose "**Report"** to see the Result Report. If applicable, choose "**Print"** to print the report.
18. Choose "**Back"**, and then "**Main"** to return to the Main menu to perform the next test.

Performing additional control runs

In accordance with local, state, federal and/or accrediting organization requirements, additional control runs may be performed with a lot of **cobas**® SARS-CoV-2 & Influenza A/B assay tubes that has already been added through the "Lot Validation" workflow. Use the **cobas**® SARS-CoV-2 & Influenza A/B Quality Control Kit for use on the **cobas**® Liat® System to conduct these runs.

Materials needed for additional control runs

- **cobas**® SARS-CoV-2 & Influenza A/B assay tubes
- Transfer pipette(s)
- **cobas**® Liat® SARS-CoV-2 & Influenza A/B Positive Controls and/or Negative Control
- Corresponding barcodes for the **cobas**® SARS-CoV-2 & Influenza A/B Positive Controls and/or the Negative Control

Procedure

Use the procedure outlined under the section "**cobas**® SARS-CoV-2 & Influenza A/B on clinical specimens testing" to perform additional control runs. In step **7**, be sure to use the provided control barcodes included in **cobas**® SARS-CoV-2 & Influenza A/B Control Kit to scan as sample ID barcode. Interpretation of results for **cobas**® SARS-CoV-2 & Influenza A/B when running additional **cobas**® SARS-CoV-2 & Influenza A/B Positive Controls or Negative Controls are shown in the "Interpretation of results" section (Table 6 through Table 8). Using barcodes other than the control barcodes provided may lead to incorrect control results.

Results

Quality control and interpretation of results

Table 6 Interpretation of results of **cobas®** SARS-CoV-2 & Influenza A/B when running "Lot Validation" procedure

cobas® Liat® Analyzer Display	**Interpretation**
Negative Control Valid	**Negative Control Valid** Control is negative for the presence of SARS-CoV-2, Influenza type A virus and Influenza type B virus RNA.
Negative Control Invalid. Repeat Run	**Negative Control Invalid** Result is Invalid. The Negative Control should be re-tested to obtain valid result. Repeat Run.
Positive Control Valid	**Positive Control Valid** Control is positive for the presence of SARS-CoV-2, Influenza type A virus and Influenza type B virus RNA.
Positive Control Invalid. Repeat Run	**Positive Control Invalid** Result is Invalid. The Positive Control should be re-tested to obtain valid result. Repeat Run.

Note: If the repeated run is still invalid, contact your local Roche representative.

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Table 7 Interpretation of results of **cobas®** SARS-CoV-2 & Influenza A/B when running a sample

Result Report	Result Report	Interpretation
SARS-CoV-2	SARS-CoV-2 Not Detected	Negative test for SARS-CoV-2 (no SARS-CoV-2 RNA detected)
SARS-CoV-2	SARS-CoV-2 Detected	Positive test for SARS-CoV-2 (SARS-CoV-2 RNA present)
SARS-CoV-2	SARS-CoV-2 Invalid	Presence or absence of SARS-CoV-2 cannot be determined. If clinically indicated, repeat assay with same sample or, if possible, collect new sample for testing.
Influenza A	Influenza A Not Detected	Negative test for Influenza A (no Influenza A RNA detected)
Influenza A	Influenza A Detected	Positive test for Influenza A (Influenza A RNA present)
Influenza A	Influenza A Invalid	Presence or absence of Influenza A cannot be determined. If clinically indicated, repeat assay with same sample or, if possible, collect new sample for testing.
Influenza B	Influenza B Not Detected	Negative test for Influenza B (no Influenza B RNA detected)
Influenza B	Influenza B Detected	Positive test for Influenza B (Influenza B RNA present)
Influenza B	Influenza B Invalid	Presence or absence of Influenza B cannot be determined. If clinically indicated, repeat assay with same sample or, if possible, collect new sample for testing.
Assay Invalid	Assay Invalid	Presence or absence of SARS-CoV-2, Influenza A, and Influenza B cannot be determined. Repeat assay with same sample or, if possible, collect new sample for testing.
Assay Aborted by System	Assay Aborted by System	Run failed or aborted by system. Repeat assay with same sample or, if possible, collect new sample for testing.
Assay aborted by script: Script aborted	Assay aborted by script: Script aborted	Run failed or aborted by script. Repeat assay with same sample or, if possible, collect new sample for testing.
Assay Aborted by User	Assay Aborted by User	Run aborted by user.

Influenza A and influenza B negative results should be considered presumptive in samples that have a positive SARS-CoV-2 result.

Competitive inhibition studies showed that SARS-CoV-2 virus, when present at concentrations above 3.6E+04 copies/mL, can inhibit the detection and amplification of influenza A and influenza B virus RNA if present at or below 1.8E+02 copies/mL or 4.9E+02 copies/mL, respectively, and may lead to false negative influenza virus results. If co-infection with influenza A or influenza B virus is suspected in samples with a positive SARS-CoV-2 result, the sample should be re-tested with another FDA cleared, approved, or authorized influenza test, if influenza virus detection would change clinical management.

Table 8 Interpretation of results when running additional controls after following "Lot Validation" procedure

Positive control

cobas® Liat® Analyzer Display	Interpretation
Positive Control Valid	**Positive Control Valid** Control is positive for the presence of SARS-CoV-2 Influenza type A and Influenza type B RNA.
Positive Control Invalid	**Positive Control Invalid** Result is Invalid. The Positive Control should be re-tested to obtain valid result. Repeat Run.

Note: If the repeated run is still invalid, contact your local Roche representative.

Negative control

cobas® Liat® Analyzer Display	Interpretation
Negative Control Valid	**Negative Control Valid** Control is negative for the presence of SARS-CoV-2, Influenza type A and Influenza type B RNA.
Negative Control Invalid	**Negative Control Invalid** Result is Invalid. The Negative Control should be re-tested to obtain valid result. Repeat Run.

Note: If the repeated run is still invalid, contact your local Roche representative.

Procedural limitations

- **cobas**® SARS-CoV-2 & Influenza A/B test has been evaluated only for use in combination with the **cobas**® SARS-CoV-2 & Influenza A/B Quality Control Kit and this Instructions For Use document. Modifications to these procedures may alter the performance of the test.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. One hundred percent agreement between the results should not be expected due to aforementioned differences between technologies. Users should follow their own specific policies/procedures.
- This test is intended to be used for the detection of SARS-CoV-2, Influenza A and Influenza B RNA in nasal and nasopharyngeal swab samples collected in a Copan UTM System (UTM) or BD™ Universal Viral Transport System (UVT) or Thermo Fisher™ Scientific Remel™ media. Testing of other sample or media types may lead to inaccurate results.
- **cobas**® SARS-CoV-2 & Influenza A/B test has not been validated for the testing of pooled specimens or the screening of specimens from asymptomatic individuals that do not have signs and symptoms of respiratory infection.
- As with other tests, negative results do not preclude SARS-CoV-2, influenza A or influenza B, infection and should not be used as the sole basis for treatment or other patient management decisions. Test results should be interpreted in conjunction with other clinical and laboratory data available to the clinician.
- Positive and negative predictive values are highly dependent on prevalence. The likelihood of a negative result being false is higher during peak activity when prevalence of disease is high. The likelihood of a positive result being false is higher during periods when prevalence is moderate to low.
- False negative results may occur if a specimen is improperly collected, transported or handled, if there is insufficient RNA to be detected, or if one or more target viruses inhibits amplification of other targets.
- Invalid results may be obtained if there is insufficient sample volume or if the specimen contains inhibitory substances that prevent nucleic acid target extraction and/or amplification and detection.
- Mutations within the target regions of **cobas**® SARS-CoV-2, influenza A, and influenza B could affect primer and/or probe binding that results 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**® SARS-CoV-2 & Influenza A/B to help identify the specimens containing substances that may interfere with nucleic acid isolation and PCR amplification.
- This test does not differentiate influenza A subtypes (i.e., H1N1, H3N2); additional testing is required to differentiate any specific influenza A subtypes or strains, in consultation with local public health departments.
- Recent administration of nasal vaccines (e.g., FluMist®) within 6 weeks prior to collection were not evaluated; **cobas**® SARS-CoV-2 & Influenza A/B test may detect the agents in those vaccines but may not represent infection by those viruses.
- Performance characteristics for influenza A were established when influenza A/H1 and A/H3 were predominant. When other influenza A viruses are emerging, performance characteristics may differ.
- Due to the small number or absence of positive results during the prospective clinical study, performance characteristics for influenza B were established primarily with retrospective clinical specimens.
- The clinical performance has not been established for all circulating variants of SARS-CoV-2 but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

Non-clinical performance – SARS-CoV-2

Analytical sensitivity

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

WHO International Standard

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

WHO Standard IS was serially diluted in pooled negative nasopharyngeal swabs matrix. Five concentration levels were tested with 24 replicates at each level across three lots of assay tubes (8 replicates per lot). Three independent dilution series were used in the study with an approximately equal numbers of replicates per dilution series. The LoD was determined by 95% hit rate to be 62.5 IU/mL.

The results of the hit rate and LoD are shown in Table 9 below.

Table 9 Hit rate and mean Ct results of SARS-CoV-2 LoD determination

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

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

*Calculations only include positive results.

SARS-CoV-2 viral culture

To determine the LoD for SARS-CoV-2, a heat inactivated cultured virus of an isolate from a US patient (USA-WA1/2020, lot number 324047, 3.16E+06 $TCID_{50}$/mL, ZeptoMetrix, NY, USA) was serially diluted in pooled negative nasopharyngeal swab matrix. Five concentration levels were tested with 20 replicates except for the highest concentration level, which was tested with 10 replicates. Three lots of assay tubes (approximately equal numbers of replicates per lot), and two independent dilution series (equal numbers of replicates per dilution series) were used in the study.

As shown in Table 10, the concentration level with observed hit rates greater than or equal to 95% was 0.012 $TCID_{50}$/mL (12 copies/mL) for SARS-CoV-2.

Table 10 LoD determination Using USA-WA1/2020 strain

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

Concentration [$TCID_{50}$/mL]	Concentration [copies/mL]	Total valid results	Hit rate [%]	Mean Ct*
0.048	49	10	100	32.6
0.024	24	20	100	33.5
0.012	12	20	100	35.2
0.006	6	20	70	35.7
0.003	3	20	25	36.7

* Calculations only include positive results.

Reactivity/inclusivity

The inclusivity study evaluates the ability of the assay to detect SARS-CoV-2 isolates/variants. The reactivity/inclusivity was evaluated with 16 SARS-CoV-2 isolates/variants. The isolates/variants were tested as inactivated viruses diluted into pooled clinical negative nasopharyngeal swab matrix. The isolates/variants tested in the study and the concentrations that they can be detected are listed in Table 11. *In silico* analysis of additional SARS-CoV-2 sequences indicates that >99.9% of sequences for SARS-CoV-2 have no changes in primer/probe binding sites at both target regions simultaneously. All known sequences are predicted to be detected by at least one of the two target regions.

Table 11 Results of Testing SARS-CoV-2 Isolate/Variant

Isolate/Variant Name	Pango Lineage	WHO Label	Test Concentration (copies/mL)	SARS-CoV-2	Influenza A	Influenza B
SARS-CoV-2 Italy-INMI1	not listed	N/A	2.0E+01	+	-	-
SARS-CoV-2 Hong Kong/VM20001061/2020	A	N/A	2.0E+01	+	-	-
SARS-CoV-2 England/204820464/2020	B.1.1.7	Alpha	5.0E+00	+	-	-
SARS-CoV-2 South Africa/KRISP-K005325/2020	B.1.351	Beta	2.0E+01	+	-	-
USA/COR-22-063113/2022	BA5.5	Omicron	6.00E+00	+	-	-
USA/GA-EHC-2811C/2021	BA.1	Omicron	1.50E+00	+	-	-
hCoV-19/USA/MD-HP40900/2022	B.1.1.529, XBB.1.5	Omicron	6.00E+00	+	-	-
hCoV-19/USA/MD-HP38861/2022	B.1.1.529, BQ.1.1	Omicron	1.20E+01	+	-	-
hCoV-19/USA/MD-HP38288/2022	B.1.1.529, BF.7	Omicron	1.20E+01	+	-	-
hCoV-19/USA/MD-HP30386/2022	B.1.1.529, BA.4	Omicron	6.00E+00	+	-	-
USA/MD-HP24556/2022	BA.2.3	Omicron	1.20E+01	+	-	-
USA/MD-HP20874/2021	B.1.1.529	Omicron	6.00E+00	+	-	-
hCoV-19/USA/CA-Stanford-15_S02/2021	B.1.617.1	Kappa	1.20E+01	+	-	-

Isolate/Variant Name	Pango Lineage	WHO Label	Test Concentration (copies/mL)	SARS-CoV-2	Influenza A	Influenza B
USA/NY-Wadsworth-21025952/2021	B.1.526	Iota	3.60E+01	+	-	-
hCoV-19/USA/PHC658/2021	B.1.617.2	Delta	1.20E+01	+	-	-
hCoV-19/Japan/TY7-503/2021	P.1	Gamma	1.20E+01	+	-	-

Cross reactivity

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

None of the organisms tested interfered with **cobas**® SARS-CoV-2 performance.

Table 12 Cross-reactivity

Microorganisms	Testing conc.*	SARS-CoV-2 result	Influenza A result	Influenza B result
Adenovirus	1.00E+05	Not Detected	Not Detected	Not Detected
Cytomegalovirus	1.00E+05	Not Detected	Not Detected	Not Detected
Epstein-Barr virus	1.00E+05	Not Detected	Not Detected	Not Detected
Human Enterovirus D	1.00E+05	Not Detected	Not Detected	Not Detected
Human Coronavirus 229E	1.00E+05	Not Detected	Not Detected	Not Detected
Human Coronavirus HKU1	1.00E+05	Not Detected	Not Detected	Not Detected
Human Coronavirus NL63	1.00E+05	Not Detected	Not Detected	Not Detected
Human Coronavirus OC43	1.00E+05	Not Detected	Not Detected	Not Detected
MERS-Coronavirus	1.00E+05	Not Detected	Not Detected	Not Detected
SARS Coronavirus	1.00E+05	Not Detected	Not Detected	Not Detected
Human Rhinovirus B	1.00E+05	Not Detected	Not Detected	Not Detected
Human Metapneumovirus 27	1.00E+05	Not Detected	Not Detected	Not Detected
Measles	1.00E+05	Not Detected	Not Detected	Not Detected
Mumps	1.00E+05	Not Detected	Not Detected	Not Detected
Parainfluenzavirus Type 1	1.00E+05	Not Detected	Not Detected	Not Detected
Parainfluenzavirus Type 2	1.00E+05	Not Detected	Not Detected	Not Detected
Parainfluenzavirus Type 3	1.00E+05	Not Detected	Not Detected	Not Detected
Parainfluenzavirus Type 4A	1.00E+05	Not Detected	Not Detected	Not Detected
Respiratory Syncytial Virus A2	1.00E+05	Not Detected	Not Detected	Not Detected
Aspergillus Flavus var. flavus	1.00E+06	Not Detected	Not Detected	Not Detected
Bordetella pertussis	1.00E+06	Not Detected	Not Detected	Not Detected
Bordetella parapertussis	1.00E+06	Not Detected	Not Detected	Not Detected
Candida albicans	1.00E+06	Not Detected	Not Detected	Not Detected

Microorganisms	Testing conc.*	SARS-CoV-2 result	Influenza A result	Influenza B result
Chlamydia pneumoniae	1.00E+06	Not Detected	Not Detected	Not Detected
Corynebacterium flavescens	1.00E+06	Not Detected	Not Detected	Not Detected
Escherichia coli	1.00E+06	Not Detected	Not Detected	Not Detected
Fusobacterium necrophorum subsp. Necrophorum	1.00E+06	Not Detected	Not Detected	Not Detected
Haemophilus influenzae	1.00E+06	Not Detected	Not Detected	Not Detected
Lactobacillus crispatus	1.00E+06	Not Detected	Not Detected	Not Detected
Legionella pneumophila	1.00E+06	Not Detected	Not Detected	Not Detected
Moraxella catarrhalis	1.00E+06	Not Detected	Not Detected	Not Detected
Mycoplasma genitalium	1.00E+06	Not Detected	Not Detected	Not Detected
Mycoplasma pneumoniae	1.00E+06	Not Detected	Not Detected	Not Detected
Mycobacterium tuberculosis	1.00E+06	Not Detected	Not Detected	Not Detected
Neisseria flava	1.00E+06	Not Detected	Not Detected	Not Detected
Neisseria meningitidis	1.00E+06	Not Detected	Not Detected	Not Detected
Pneumocystis jirovecii	5.00E+03	Not Detected	Not Detected	Not Detected
Pneumocystis jirovecii clinical Sample	1:10 diluted	Not Detected	Not Detected	Not Detected
Pseudomonas aeruginosa	1.00E+06	Not Detected	Not Detected	Not Detected
Staphylococcus epidermis	1.00E+06	Not Detected	Not Detected	Not Detected
Staphylococcus aureus	1.00E+06	Not Detected	Not Detected	Not Detected
Streptococcus pneumoniae	1.00E+06	Not Detected	Not Detected	Not Detected
Streptococcus pyogenes	1.00E+06	Not Detected	Not Detected	Not Detected
Streptococcus salivarius	1.00E+06	Not Detected	Not Detected	Not Detected
Nasal wash	1:10 diluted	Not Detected	Not Detected	Not Detected

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

Microbial interference

Microbial Interference of **cobas**® SARS-CoV-2 & Influenza A/B was evaluated by testing a panel of multiple unique sub-species of microorganisms (Table 13) in the presence of 3x LoD concentrations of SARS-CoV-2, influenza A and influenza B viruses. High titer stocks of the potentially interfering microorganisms were spiked into pooled negative nasopharyngeal swab clinical matrix with spiked 3x LoD concentrations of SARS-CoV-2, influenza A and influenza B viruses.

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

Table 13 Microbial interference

Microorganisms	Testing conc.*	SARS-CoV-2 result	Influenza A result	Influenza B result
Adenovirus	1.00E+05	Detected	Detected	Detected
Cytomegalovirus	1.00E+05	Detected	Detected	Detected
Epstein-Barr virus	1.00E+05	Detected	Detected	Detected
Human Enterovirus D	1.00E+05	Detected	Detected	Detected
Human Coronavirus 229E	1.00E+05	Detected	Detected	Detected
Human Coronavirus HKU1	1.00E+05	Detected	Detected	Detected
Human Coronavirus NL63	1.00E+05	Detected	Detected	Detected
Human Coronavirus OC43	1.00E+05	Detected	Detected	Detected
MERS-Coronavirus	1.00E+05	Detected	Detected	Detected
SARS Coronavirus	1.00E+05	Not Detected	Detected	Detected
	1.00E+04	Detected	Detected	Detected
Human Rhinovirus B	1.00E+05	Detected	Detected	Detected
Human Metapneumovirus 27	1.00E+05	Detected	Detected	Detected
Measles	1.00E+05	Detected	Detected	Detected
Mumps	1.00E+05	Detected	Detected	Detected
Parainfluenzavirus Type 1	1.00E+05	Detected	Detected	Detected
Parainfluenzavirus Type 2	1.00E+05	Detected	Detected	Detected
Parainfluenzavirus Type 3	1.00E+05	Detected	Detected	Detected
Parainfluenzavirus Type 4A	1.00E+05	Detected	Detected	Detected
Respiratory Syncytial Virus A2	1.00E+05	Detected	Detected	Detected
Aspergillus Flavus var. flavus	1.00E+06	Detected	Detected	Detected
Bordetella pertussis	1.00E+06	Detected	Detected	Detected
Bordetella parapertussis	1.00E+06	Detected	Detected	Detected
Candida albicans	1.00E+06	Detected	Detected	Detected
Chlamydia pneumoniae	1.00E+06	Detected	Detected	Detected
Corynebacterium flavescens	1.00E+06	Detected	Detected	Detected
Escherichia coli	1.00E+06	Detected	Detected	Detected
Fusobacterium necrophorum subsp. Necrophorum	1.00E+06	Detected	Detected	Detected
Haemophilus influenzae	1.00E+06	Detected	Detected	Detected
Lactobacillus crispatus	1.00E+06	Detected	Detected	Detected
Legionella pneumophila	1.00E+06	Detected	Detected	Detected
Moraxella catarrhalis	1.00E+06	Detected	Detected	Detected
Mycoplasma genitalium	1.00E+06	Detected	Detected	Detected
Mycoplasma pneumoniae	1.00E+06	Detected	Detected	Detected
Mycobacterium tuberculosis	1.00E+06	Detected	Detected	Detected
Neisseria flava	1.00E+06	Detected	Detected	Detected
Neisseria meningitidis	1.00E+06	Detected	Detected	Detected
Pneumocystis jirovecii	5.00E+03	Detected	Detected	Detected
Pneumocystis jirovecii clinical Sample	1:10 diluted	Detected	Detected	Detected
Pseudomonas aeruginosa	1.00E+06	Detected	Detected	Detected
Staphylococcus epidermis	1.00E+06	Detected	Detected	Detected
Staphylococcus aureus	1.00E+06	Detected	Detected	Detected

Microorganisms	Testing conc.*	SARS-CoV-2 result	Influenza A result	Influenza B result
Streptococcus pneumoniae	1.00E+06	Detected	Detected	Detected
Streptococcus pyogenes	1.00E+06	Detected	Detected	Detected
Streptococcus salivarius	1.00E+06	Detected	Detected	Detected
Nasal wash	1:10 diluted	Detected	Detected	Detected

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

Endogenous and exogenous interference

Potentially interfering substances that may be commonly encountered in respiratory specimens were evaluated. Medically and/or physiologically relevant concentrations of potential interferents were tested with 1 influenza A strain, 1 influenza B strain and 1 SARS-CoV-2 strain at ~3x LoD. Each substance was tested, by introducing interferents into pooled negative nasopharyngeal swab specimens (NNPS) in UTM and tested with and without target strains. As shown in Table 14, substances at the concentrations tested did not interfere in the detection of SARS-CoV-2, influenza A and influenza B.

Table 14 Interference testing results

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

Co-infection (competitive inhibition)

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

Inactivated SARS-CoV-2 (USA-WA1/2020), cultured influenza A (Brisbane/59/07) virus, and cultured influenza B (Florida/04/06 and Colorado/06/2017) were prepared in pooled negative nasopharyngeal swabs eluted in UTM sample matrix. Three replicates were tested per condition. The concentrations tested in the dilution experiments are presented in both ID_{50}/mL and copies/mL.

The concentration of each viral stock in copies/mL was quantified using the RT-ddPCR (Reverse transcriptase droplet digital PCR) in a single target, single-plex assay with target specific PCR primers and probe sets designed to independently amplify influenza A, influenza B, or SARS-CoV-2 using the One-Step RT-ddPCR Advanced Kit for Probes (Bio Rad, cat # 1864021).

Summary of testing results are shown in the table below (Table 15). Influenza A high target sample exhibited an average Ct of 12, while the influenza B and SARS-CoV-2 target samples yielded an average Ct between 20-24. The low target concentrations (Target 2 and 3) were ~ 3x LoD.

Table 15 Competitive inhibition – Simulated co-infection study of influenza A, influenza B and SARS-CoV-2 targets

Co-Infection Condition	Target 1 (High)				Target 2			Target 3			Hit Rate		
	Description	Concentration ID_{50}/mL	Concentration Copies/mL	~ Ct	Description	Concentration ID_{50}/mL	Concentration Copies/mL	Description	Concentration ID_{50}/mL	Concentration Copies/mL	Influenza A	Influenza B	SARS-CoV-2
1	Influenza A (A/Brisbane/59/07)	1.40E+04	8.30E+08	12	Influenza B (B/Florida/04/06)	1.20E-02	4.85E+02	SARS-CoV-2 (USA-WA1/2020)	3.60E-02	3.60E+01	3/3	3/3	3/3
2	Influenza B (B/Florida/04/06)	3.20E+02	1.30E+07	17	Influenza A (A/Brisbane/59/07)	3.00E-03	1.79E+02	SARS-CoV-2 (USA-WA1/2020)	3.60E-02	3.60E+01	2/3	3/3	0/3
3	Influenza B (B/Florida/04/06)	1.60E+02	6.50E+06	18	Influenza A (A/Brisbane/59/07)	3.00E-03	1.79E+02	SARS-CoV-2 (USA-WA1/2020)	3.60E-02	3.60E+01	3/3	3/3	0/3
4	Influenza B (B/Florida/04/06)	4.00E+01	1.60E+06	20	Influenza A (A/Brisbane/59/07)	3.00E-03	1.79E+02	SARS-CoV-2 (USA-WA1/2020)	3.60E-02	3.60E+01	3/3	3/3	2/3
5	Influenza B (B/Florida/04/06)	2.00E+01	8.10E+05	21	Influenza A (A/Brisbane/59/07)	3.00E-03	1.79E+02	SARS-CoV-2 (USA-WA1/2020)	3.60E-02	3.60E+01	3/3	3/3	3/3
6	Influenza B (B/Colorado/06/2017)	1.40E+04	1.70E+06	19	Influenza A (A/Brisbane/59/07)	NT	NT	SARS-CoV-2 (USA-WA1/2020)	3.60E-02	3.60E+01	NT	3/3	1/3
7	Influenza B (B/Colorado/06/2017)	7.00E+03	8.50E+05	20	Influenza A (A/Brisbane/59/07)	NT	NT	SARS-CoV-2 (USA-WA1/2020)	3.60E-02	3.60E+01	NT	3/3	3/3
8	SARS-CoV-2 (USA-WA1/2020)	4.80E+01	4.90E+04	23	Influenza A (A/Brisbane/59/07)	3.00E-03	1.79E+02	Influenza B (B/Florida/04/06)	1.20E-02	4.85E+02	2/3	2/3	3/3
9	SARS-CoV-2 (USA-WA1/2020)	3.60E+01	3.60E+04	24	Influenza A (A/Brisbane/59/07)	3.00E-03	1.79E+02	Influenza B (B/Florida/04/06)	1.20E-02	4.85E+02	3/3	3/3	3/3

NT = not tested

Results of the study showed that influenza B at concentrations above 8.10E+05 copies/mL caused inhibition of SARS-CoV-2 detection, and SARS-CoV-2 concentrations above 3.60E+04 copies/mL caused inhibition of both influenza A and influenza B detection, when present at low concentrations (~3x LoD) in a sample.

Additional competitive inhibition testing was executed with higher target concentrations of influenza B (3.9E+07 and 4.04E+07 copies/mL) and SARS-CoV-2 (2.9E+06 and 5.0E+06 copies/mL) RNA (Ct 15-16). In the presence of these high target concentrations of influenza B, the detection of SARS-CoV-2 virus was achieved at 4.6E+02 copies/mL (Table 16); the impact on influenza A virus detection was not evaluated. In the presence of high target concentrations of SARS-CoV-2, the detection of influenza A and influenza B viruses was achieved at 4.8E+04 copies/mL and between 1.2-1.3E+05 copies/mL, respectively.

Table 16 Competitive inhibition with high target concentrations – Simulated co-infection study of influenza A, influenza B and SARS-CoV-2 targets

Co-Infection Condition	Target 1 (High)				Target 2			Target 3			Hit Rate		
	Description	Concentration ID_{50}/mL	Concentration Copies/mL	~ Ct	Description	Concentration ID_{50}/mL	Concentration Copies/mL	Description	Concentration ID_{50}/mL	Concentration Copies/mL	Influenza A	Influenza B	SARS-CoV-2
1	Influenza B (B/Florida/04/06)	1.00E+03	4.04E+07	15	Influenza A (A/Brisbane/59/07)	NT	NT	SARS-CoV-2 (USA-WA1/2020)	4.50E-01	4.56E+02	NT	3/3	3/3
2	Influenza B (B/Colorado/06/2017)	3.20E+05	3.90E+07	15	Influenza A (A/Brisbane/59/07)			SARS-CoV-2 (USA-WA1/2020)	4.50E-01	4.56E+02	NT	3/3	3/3
3	SARS-CoV-2 (USA-WA1/2020)	8.50E+03	2.91E+06	16	Influenza A (A/Brisbane/59/07)			Influenza B (B/Florida/04/06)	3.00E+00	1.21E+05	NT	3/3	3/3
4	SARS-CoV-2 (USA-WA1/2020)	5.00E+03	5.07E+06	16	Influenza A (A/Brisbane/59/07)			Influenza B (B/Florida/04/06)	3.20E+00	1.29E+05	NT	3/3	3/3
5	SARS-CoV-2 (USA-WA1/2020)	8.50E+03	2.91E+06	16	Influenza A (A/Brisbane/59/07)	8.00E-01	4.77E+04	Influenza B (B/Florida/04/06)	NT	NT	3/3	NT	3/3
6	SARS-CoV-2 (USA-WA1/2020)	5.00E+03	5.07E+06	16	Influenza A (A/Brisbane/59/07)	8.00E-01	4.77E+04	Influenza B (B/Florida/04/06)	NT	NT	3/3	NT	3/3

NT = not tested

Levels tested below the listed concentration for Targets 2 and 3 resulted in less than 3/3 replicates detected for these targets, indicating competitive inhibition had occurred.

Matrix equivalency – UTM, Remel Media and Saline

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

For each matrix, 10 replicates of negative samples, 30 replicates of positive samples at 2x LoD and 10 replicates of positive samples at 5x LoD were tested. The expected positive hit rate was 0% for negative samples, ≥95% for positive samples at 2x LoD and 100% for positive samples at 5x LoD. The results showed that the assay was able to correctly detect the presence of the viral targets suspended in all matrices (Table 17) demonstrating that UTM, M4RT, and Saline media are acceptable collection and transport media for use with the **cobas**® SARS-CoV-2 & Influenza A/B for use on the **cobas**® Liat® System.

Table 17 Summary of matrix equivalency study results

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

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

FDA SARS-CoV-2 reference panel testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The extraction method and instrument used were the **cobas**® SARS-CoV-2 & Influenza A/B on the **cobas**® Liat® System. The results are summarized in Table 18.

Table 18 Summary of LoD confirmation result using the FDA SARS-CoV-2 reference panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal Clinical Sample	5.4 x10^3 NDU/mL	N/A
MERS-CoV	Nasopharyngeal Clinical Sample	N/A	ND

NDU/mL = RNA NAAT detectable units/mL

N/A: Not applicable

ND: Not detected

Non-clinical performance – Influenza A/B

Analytical sensitivity

The Limit of Detection (LoD) was evaluated using 3 strains of influenza A and 2 strains of influenza B. The LoD was determined by limiting dilution studies using these titered viruses. The viruses were spiked into negative nasopharyngeal swab (NPS) in UTM sample matrix. The LoD was determined to be 2×10^{-3} - 2×10^{-2} $TCID_{50}$/mL for influenza A strains, and 2×10^{-3} - 4×10^{-3} $TCID_{50}$/mL for influenza B strains (Table 19).

Table 19 LoD determination for influenza A and influenza B strains

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

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

Reactivity/inclusivity

The reactivity study evaluates the ability to detect influenza strains representing temporal and geographical diversity. The reactivity/inclusivity was evaluated with 28 influenza A and 15 influenza B strains. Influenza A strains included 14 influenza A/H1 strains (including 3 H1N1 pdm09 strains), 12 influenza A/H3 strains (including 1 H3N2v strain), 1 influenza A/H7N9 strain, and 1 influenza A/H5N1 reassortant strain. Influenza B strains included that from both the Victoria lineage and Yamagata lineage. All strains were detected at the concentrations tested (Table 20). *In silico* analysis of influenza A and influenza B sequences predicted that the **cobas**® SARS-CoV-2 & Influenza A/B Test detects all the recorded circulating strains as of January 2023.

Table 20 Results of testing influenza A and influenza B strains

Virus Strain	Type / Subtype	Test Concentration	Inf A Result	Inf B Result
A/Aichi/2/68	Influenza A/H3N2	1.0×10^{2} $CEID_{50}$/mL	+	–
A/Alice	Influenza A/H3N2	5.0×10^{1} $CEID_{50}$/mL	+	–
A/Anhui/1/2013	Influenza A/H7N9 (Eurasian lineage)	1.0×10^{3} $TCID_{50}$/mL	+	–
A/Brisbane/10/07	Influenza A/H3N2	2.0×10^{-2} $TCID_{50}$/mL	+	–
A/Brisbane/59/07	Influenza A/H1N1	2.0×10^{-3} $TCID_{50}$/mL	+	–
A/Cambodia/X0810301/2013(H5N1)-PR8-IDCDC-RG34B	Influenza A/H5N1 reassortant	2.5×10^{1} $CEID_{50}$/mL	+	–
A/Denver/1/57	Influenza A/H1N1	1.0×10^{2} $CEID_{50}$/mL	+	–
A/FM/1/47	Influenza A/H1N1	1.0×10^{2} $CEID_{50}$/mL	+	–
A/H3/Perth/16/09	Influenza A/H3N2	2.5×10^{-1} $TCID_{50}$/mL	+	–

Virus Strain	Type / Subtype	Test Concentration	Inf A Result	Inf B Result
A/Hong Kong/8/68	Influenza A/H3N2	1.0×10^{2} $TCID_{50}$/mL	+	–
A/Indiana/8/2011	Influenza A/H3N2v	5.0×10^{-1} $TCID_{50}$/mL	+	–
A/Mal/302/54	Influenza A/H1N1	4.0×10^{2} $CEID_{50}$/mL	+	–
A/MRC2	Influenza A/H3	1.0×10^{2} $CEID_{50}$/mL	+	–
A/New Caledonia/20/99	Influenza A/H1N1	1.0×10^{2} $TCID_{50}$/mL	+	–
A/New Jersey/8/76	Influenza A/H1N1	1.0×10^{1} $CEID_{50}$/mL	+	–
A/NY/01/2009	Influenza A/H1N1 pdm09	2.0×10^{-2} $TCID_{50}$/mL	+	–
A/NY/02/2009	Influenza A/H1N1 pdm09	2.5×10^{-2} $TCID_{50}$/mL	+	–
A/NY/03/2009	Influenza A/H1N1 pdm09	2.0×10^{-1} $TCID_{50}$/mL	+	–
A/Port Chalmers/1/73	Influenza A/H3N2	1.0×10^{2} $CEID_{50}$/mL	+	–
A/PR/8/34	Influenza A/H1N1	5.0×10^{0} $TCID_{50}$/mL	+	–
A/Solomon Island/3/2006	Influenza A/H1N1	5.0×10^{-2} $TCID_{50}$/mL	+	–
A/Swine/1976/31	Influenza A/H1N1	1.0×10^{1} $CEID_{50}$/mL	+	–
A/Swine/Iowa/15/30	Influenza A/H1N1	1.0×10^{2} $CEID_{50}$/mL	+	–
A/Texas/50/2012	Influenza A/H3N2	1.0×10^{-1} $TCID_{50}$/mL	+	–
A/Victoria/3/75	Influenza A/H3N2	1.0×10^{2} $CEID_{50}$/mL	+	–
A/Victoria/361/2011	Influenza A/H3N2	2.0×10^{-2} $TCID_{50}$/mL	+	–
A/Weiss/43	Influenza A/H1N1	1.0×10^{3} $TCID_{50}$/mL	+	–
A/Wisconsin/67/05	Influenza A/H3N2	5.0×10^{-1} $TCID_{50}$/mL	+	–
B/Allen/45	Influenza B	5.0×10^{-1} $TCID_{50}$/mL	–	+
B/Brisbane/60/2008	Influenza B (Victoria lineage)	1.0×10^{-2} $TCID_{50}$/mL	–	+
B/Florida/04/06	Influenza B (Yamagata lineage)	2.0×10^{-3} $TCID_{50}$/mL	–	+
B/Florida/07/04	Influenza B (Yamagata lineage)	5.0×10^{-2} $TCID_{50}$/mL	–	+
B/GL/1739/54	Influenza B	2.0×10^{0} $TCID_{50}$/mL	–	+
B/HongKong/5/72	Influenza B	2.5×10^{-1} $TCID_{50}$/mL	–	+
B/Lee/40	Influenza B	2.5×10^{-1} $TCID_{50}$/mL	–	+
B/Malaysia/2506/04	Influenza B (Victoria lineage)	4.0×10^{-3} $TCID_{50}$/mL	–	+
B/Maryland/1/59	Influenza B	2.0×10^{-2} $TCID_{50}$/mL	–	+
B/Mass/3/66	Influenza B	1.0×10^{1} $TCID_{50}$/mL	–	+
B/Massachusetts/2/2012	Influenza B (Yamagata lineage)	5.0×10^{-3} $TCID_{50}$/mL	–	+
B/Nevada/03/2011	Influenza B (Victoria lineage)	2.5×10^{-1} $CEID_{50}$/mL	–	+
B/Taiwan/2/62	Influenza B	2.0×10^{-1} $TCID_{50}$/mL	–	+
B/Texas/6/2011	Influenza B (Yamagata lineage)	1.0×10^{-1} $TCID_{50}$/mL	–	+
B/Wisconsin/1/2010	Influenza B (Yamagata lineage)	5.0×10^{-1} $TCID_{50}$/mL	–	+

Cross reactivity

Cross-reactivity study evaluates potential cross reactivity with non-influenza microorganisms that may be present in nasopharyngeal swab samples. The cross reactivity was evaluated against a panel comprising human genomic DNA and 35 microorganisms. Bacteria and *Candida albicans* were tested at ≥ 10^6 CFU/mL. Viruses were tested at ≥ 10^5 $TCID_{50}$/mL, or the highest available concentration. No cross reactivity was observed for the human genomic DNA or the microorganisms at the concentrations tested (Table 21).

Table 21 Influenza A/B cross-reactivity testing results

Microorganism	Test Concentration	Inf A Result	Inf B Result
Adenovirus Type 1	9.0×10^5 $TCID_{50}$/mL	–	–
Adenovirus Type 7	1.4×10^5 $TCID_{50}$/mL	–	–
Cytomegalovirus	4.5×10^4 $TCID_{50}$/mL	–	–
Epstein Barr Virus	2.5×10^5 $TCID_{50}$/mL	–	–
Herpes Simplex Virus	1.4×10^5 $TCID_{50}$/mL	–	–
Human Coronavirus 229E	8.0×10^3 $TCID_{50}$/mL	–	–
Human Coronavirus OC43	8.0×10^4 $TCID_{50}$/mL	–	–
Human Enterovirus 68	1.0×10^5 $TCID_{50}$/mL	–	–
Human Metapneumovirus	7.0×10^3 $TCID_{50}$/mL	–	–
Human Parainfluenza Type 1	3.7×10^5 $TCID_{50}$/mL	–	–
Human Parainfluenza Type 2	7.5×10^5 $TCID_{50}$/mL	–	–
Human Parainfluenza Type 3	4.5×10^5 $TCID_{50}$/mL	–	–
Human Rhinovirus Type 1A	8.0×10^5 $TCID_{50}$/mL	–	–
Measles	8.0×10^4 $TCID_{50}$/mL	–	–
Mumps Virus	8.0×10^4 $TCID_{50}$/mL	–	–
Varicella-Zoster Virus	4.4×10^3 $TCID_{50}$/mL	–	–
Bordetella pertussis	2.2×10^6 CFU/mL	–	–
Candida albicans	4.2×10^6 CFU/mL	–	–
Chlamydia pneumoniae	8.0×10^4 $TCID_{50}$/mL	–	–
Corynebacterium sp	3.6×10^6 CFU/mL	–	–
Escherichia coli	1.9×10^6 CFU/mL	–	–
Haemophilus influenzae	2.3×10^6 CFU/mL	–	–
Lactobacillus sp	1.9×10^6 CFU/mL	–	–
Legionella pneumophila	6.7×10^6 CFU/mL	–	–
Moraxella catarrhalis	2.5×10^6 CFU/mL	–	–
Mycobacterium tuberculosis	2.8×10^6 copies/mL[†]	–	–
Mycoplasma pneumoniae	2.9×10^6 copies/mL[†]	–	–
Neisseria elongate	2.0×10^6 CFU/mL	–	–
Neisseria meningitidis	2.2×10^6 CFU/mL	–	–
Pseudomonas aeruginosa	2.3×10^6 CFU/mL	–	–
Staphylococcus aureus	2.4×10^6 CFU/mL	–	–
Staphylococcus epidermidis	1.9×10^6 CFU/mL	–	–
Streptococcus pneumoniae	1.8×10^6 CFU/mL	–	–
Streptococcus pyogenes	2.5×10^6 CFU/mL	–	–
Streptococcus salivarius	4.3×10^6 CFU/mL	–	–
Human genomic DNA	1.0×10^4 copies/mL	–	–

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

Interfering microorganisms

Interfering microorganism study evaluates whether non-influenza microorganisms that may be present in nasopharyngeal swab samples can interfere in the detection of influenza A or influenza B. The panel comprising human genomic DNA and 35 microorganisms tested in the cross-reactivity study was tested for potential interference. Bacteria and *Candida albicans* were tested at ≥ 10^6 CFU/mL and viruses were tested at ≥ 10^5 $TCID_{50}$/mL or the highest available concentration, in the presence of 1 influenza A strain and 1 influenza B strain at ~3x LoD concentration in negative NPS in UTM matrix. Results show that the presence of human genomic DNA or the microorganisms at the concentrations tested did not interfere with the detection of influenza A or influenza B (Table 22).

Table 22 Influenza A/B interfering microorganisms study results

Microorganism	Test Concentration	1 Influenza A & 1 Influenza B strain at ~3x LoD	
		Inf A Result	Inf B Result
Adenovirus Type 1	9.0×10^5 $TCID_{50}$/mL	+	+
Adenovirus Type 7	1.4×10^5 $TCID_{50}$/mL	+	+
Cytomegalovirus	4.5×10^4 $TCID_{50}$/mL	+	+
Epstein Barr Virus	2.5×10^5 $TCID_{50}$/mL	+	+
Herpes Simplex Virus	1.4×10^5 $TCID_{50}$/mL	+	+
Human Coronavirus 229E	8.0×10^3 $TCID_{50}$/mL	+	+
Human Coronavirus OC43	8.0×10^4 $TCID_{50}$/mL	+	+
Human Enterovirus 68	1.0×10^5 $TCID_{50}$/mL	+	+
Human Metapneumovirus	7.0×10^3 $TCID_{50}$/mL	+	+
Human Parainfluenza Type 1	3.7×10^5 $TCID_{50}$/mL	+	+
Human Parainfluenza Type 2	7.5×10^5 $TCID_{50}$/mL	+	+
Human Parainfluenza Type 3	4.5×10^5 $TCID_{50}$/mL	+	+
Human Rhinovirus Type 1A	8.0×10^5 $TCID_{50}$/mL	+	+
Measles	8.0×10^4 $TCID_{50}$/mL	+	+
Mumps Virus	8.0×10^4 $TCID_{50}$/mL	+	+
Varicella-Zoster Virus	4.4×10^3 $TCID_{50}$/mL	+	+
Bordetella pertussis	2.2×10^6 CFU/mL	+	+
Candida albicans	4.2×10^6 CFU/mL	+	+
Chlamydia pneumoniae	8.0×10^4 $TCID_{50}$/mL	+	+
Corynebacterium sp	3.6×10^6 CFU/mL	+	+
Escherichia coli	1.9×10^6 CFU/mL	+	+
Haemophilus influenzae	2.3×10^6 CFU/mL	+	+
Lactobacillus sp	1.9×10^6 CFU/mL	+	+
Legionella pneumophila	6.7×10^6 CFU/mL	+	+
Moraxella catarrhalis	2.5×10^6 CFU/mL	+	+
Mycobacterium tuberculosis	2.8×10^6 copies/mL[†]	+	+
Mycoplasma pneumoniae	2.9×10^6 copies/mL[†]	+	+
Neisseria elongata	2.0×10^6 CFU/mL	+	+
Neisseria meningitidis	2.2×10^6 CFU/mL	+	+
Pseudomonas aeruginosa	2.3×10^6 CFU/mL	+	+
Staphylococcus aureus	2.4×10^6 CFU/mL	+	+
Staphylococcus epidermidis	1.9×10^6 CFU/mL	+	+
Streptococcus pneumoniae	1.8×10^6 CFU/mL	+	+
Streptococcus pyogenes	2.5×10^6 CFU/mL	+	+
Streptococcus salivarius	4.3×10^6 CFU/mL	+	+
Human Genomic DNA	1.0×10^4 copies/mL	+	+

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

Clinical performance evaluation

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

Prospective clinical specimens were collected and tested February–June 2022. In total, prospectively collected specimens from 640 evaluable symptomatic individuals were included in the analysis population for the evaluation of **cobas**® SARS-CoV-2 & Influenza A/B. No coinfections with SARS-CoV-2 and influenza A/B were detected by the comparator method. No prospective fresh specimens tested in this performance evaluation were influenza B positive by the comparator method. Additionally, to supplement the prospective data for influenza A and influenza B, retrospective frozen positive and negative NPS (n=178) and NS (n=190) specimens prospectively obtained during the 2013-2014, 2014-2015, and 2019-2020 flu seasons and during the COVID-19 pandemic (March–June 2021) were distributed to 4 of the 10 sites and worked into the daily workflow of sites for testing.

Clinical performance evaluation using nasopharyngeal swab specimens

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

In total, 828 test results from NPS samples were obtained with **cobas**® SARS-CoV-2 & Influenza A/B during the clinical evaluation, including samples that required repeat testing in accordance with this IFU. Of these, a total of 6 (0.7%) failed tests and 6 (0.7%) invalid results were obtained. An additional 10 tests experienced protocol deviations, leaving a total of 806 (97.3%) valid NPS results obtained with **cobas**® SARS-CoV-2 & Influenza A/B during the clinical evaluation.

As shown in Table 23 for prospective symptomatic subjects, 101 NPS specimens tested positive for SARS-CoV-2 with both the **cobas**® SARS-CoV-2 & Influenza A/B test on **cobas**® Liat System and the composite comparator; five SARS-CoV-2-positive specimens tested negative for SARS-CoV-2 with the **cobas**® SARS-CoV-2 & Influenza A/B test. A total of 507 NPS specimens tested negative for SARS-CoV-2 with both the **cobas**® SARS-CoV-2 & Influenza A/B test and the composite comparator; three SARS-CoV-2-negative specimens tested positive for SARS-CoV-2 with the **cobas**® SARS-CoV-2 & Influenza A/B test. All discordant SARS-CoV-2 results showed late Ct values, which are indicative of NPS specimens from individuals with viral loads near or below the limit of detection of both **cobas**® SARS-CoV-2 & Influenza A/B and the

composite comparator methods.

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

Table 23 Clinical performance comparison – SARS-CoV-2 for prospective NPS specimens

		Composite Comparator Method SARS-CoV-2 Result	
		Positive	Negative
cobas® SARS-CoV-2 & Influenza A/B on **cobas**® Liat® System **Nasopharyngeal Swab (NPS)**	Positive	101	3
	Negative	5	507

PPA	95.3% (95% CI: 89.4% - 98.0%)
NPA	99.4% (95% CI: 98.3% - 99.8%)

As shown in Table 24 for prospective symptomatic subjects, 18 NPS specimens tested positive for influenza A with both the **cobas**® SARS-CoV-2 & Influenza A/B test on **cobas**® Liat System and the comparator assay; one influenza A-positive specimen tested negative for influenza A with the **cobas**® SARS-CoV-2 & Influenza A/B test. A total of 595 NPS specimens tested negative for influenza A with both the **cobas**® SARS-CoV-2 & Influenza A/B test and the comparator assay; two influenza A-negative specimens tested positive for influenza A with the **cobas**® SARS-CoV-2 & Influenza A/B test.

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

Table 24 Clinical performance comparison – Influenza A for prospective NPS specimens

		Comparator Method Influenza A Result	
		Positive	Negative
cobas® SARS-CoV-2 & Influenza A/B on **cobas**® Liat® System **Nasopharyngeal Swab (NPS)**	Positive	18	2
	Negative	1	595

PPA	94.7% (95% CI: 75.4% - 99.1%)
NPA	99.7% (95% CI: 98.8% - 99.9%)

As shown in Table 25 for retrospective NPS specimens, the results of the clinical performance evaluation for influenza A demonstrated 97.7% PPA (43/44; 95% score CI: 88.2% - 99.6%) and 99.2% NPA (131/132; 95% score CI: 95.8% – 99.9%) as compared to the comparator method.

Table 25: Clinical performance comparison – Influenza A for retrospective NPS specimens

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

PPA 97.7% (95% CI: 88.2% - 99.6%)

NPA 99.2% (95% CI: 95.8% - 99.9%)

As shown in Table 26 for retrospective NPS specimens, the results of the clinical performance evaluation for influenza B demonstrated 100.0% PPA (22/22; 95% score CI: 85.1% - 100.0%) and 100.0% NPA (151/151; 95% score CI: 97.5% - 100.0%) as compared to the comparator method.

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

Table 26 Clinical performance comparison – Influenza B for retrospective NPS specimens

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

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

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

Clinical performance evaluation using nasal swab specimens

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

In total, 834 test results from NS samples were obtained with **cobas**® SARS-CoV-2 & Influenza A/B during the clinical evaluation, including samples that required repeat testing in accordance with this IFU. Of these, a total of 1 (0.1%) failed test and 7 (0.8%) invalid results were obtained. An additional 11 tests experienced protocol deviations, leaving a total of 815 (97.7%) valid NS results obtained with **cobas**® SARS-CoV-2 & Influenza A/B during the clinical evaluation.

As shown in Table 27 for prospective symptomatic subjects, 105 NS specimens tested positive for SARS-CoV-2 with both the **cobas**® SARS-CoV-2 & Influenza A/B test on **cobas**® Liat System and the composite comparator; four SARS-CoV-2-positive specimens tested negative for SARS-CoV-2 with the **cobas**® SARS-CoV-2 & Influenza A/B test. A total of 503 NS specimens tested negative for SARS-CoV-2 with both the **cobas**® SARS-CoV-2 & Influenza A/B test and the composite comparator; four SARS-CoV-2-negative specimens tested positive for SARS-CoV-2 with the **cobas**® SARS-CoV-2 & Influenza A/B test. All eight of the discordant SARS-CoV-2 results showed late Ct values, which are indicative of NS specimens from individuals with viral loads near or below the limit of detection of both **cobas**® SARS-CoV-2 & Influenza A/B and the composite comparator methods.

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

Table 27 Clinical performance comparison – SARS-CoV-2 for prospective NS specimens

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

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

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

As shown in Table 28 for prospective symptomatic subjects, all 20 NS specimens tested positive for influenza A with both the **cobas**® SARS-CoV-2 & Influenza A/B test on **cobas**® Liat System and the comparator assay. A total of 595 NS specimens tested negative for influenza A with both the **cobas**® SARS-CoV-2 & Influenza A/B test and the comparator assay; one influenza A-negative specimens tested positive for influenza A with the **cobas**® SARS-CoV-2 & Influenza A/B test.

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

Table 28 Clinical performance comparison – Influenza A for prospective NS specimens

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

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

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

As shown in Table 29 for retrospective NS specimens, the results of the clinical performance evaluation for influenza A demonstrated 97.2% PPA (35/36; 95% score CI: 85.8% - 99.5%) and 100.0% NPA (150/150; 95% score CI: 97.5% - 100.0%) as compared to the comparator method.

Table 29 Clinical performance comparison – Influenza A for retrospective NS specimens

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

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

As shown in Table 30 for retrospective NS specimens, the results of the clinical performance evaluation for influenza B demonstrated 100.0% PPA (32/32; 95% score CI: 89.3% - 100.0%) and 100.0% NPA (154/154; 95% score CI: 97.6% - 100.0%) as compared to the comparator method.

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

Table 30 Clinical performance comparison – Influenza B for retrospective NS specimens

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

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

Expected Values

For the prospective clinical performance evaluation of **cobas**® SARS-CoV-2 & Influenza A/B, paired NPS and NS specimens from 640 evaluable subjects, including 616 evaluable results, were freshly collected and tested at 10 point-of-care clinical sites in the United States during February–June 2022. Expected value (as determined by **cobas**® SARS-CoV-2 & Influenza A/B) summaries for prospective specimens, stratified by specimen collection/testing site are presented for SARS-CoV-2 and influenza A targets in Table 31 and Table 32, respectively. No prospective fresh specimens tested in this performance evaluation were influenza B positive by either **cobas**® SARS-CoV-2 & Influenza A/B or comparator test methods.

Table 31 Expected value summary by clinical site for prospective clinical evaluation for SARS-CoV-2 (as determined by **cobas**® SARS-CoV-2 & Influenza A/B)

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

Table 32 Expected value summary by clinical site for prospective clinical evaluation for influenza A (as determined by **cobas®** SARS-CoV-2 & Influenza A/B)

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

Reproducibility

Reproducibility study assesses the total variability of the assay in detecting SARS-CoV-2, influenza A, and influenza B across operators, study sites, testing days, Analyzers, and assay tube lots. The reproducibility was evaluated at 3 study sites. Two operators at each of the 3 sites tested a 3-member reproducibility panel in triplicate on 5 different days, for a total of ~270 runs (3 panel members x 3 replicates x 2 operators x 5 days x 3 sites). Nine Analyzers and 3 assay tube lots were used. The reproducibility panel comprises a low positive and a moderate positive for each of SARS-CoV-2, influenza A, and influenza B, in addition to a negative sample. The expected result for the true negative panel member is "Not Detected," while the expected result for the low positive and moderate positive panel member is "Detected." Percent agreement with expected result, mean Ct, Ct SD, and Ct %CV are shown in Table 33–Table 35.

Table 33 SARS-CoV-2 reproducibility

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

Table 34 Influenza A reproducibility

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

Table 35 Influenza B reproducibility

Number of Valid Test Runs	Number of Valid Test Runs	Negative	Influenza B Low Positive	Influenza B Moderate Positive
		266	263	268
Ct	**Mean**	-	30.2	29.3
Ct	**SD**	-	0.92	1.05
Ct	**CV (%)**	-	3.1	3.6
Site	**1**	100.0% (89/89)	100.0% (90/90)	98.9% (88/89)
Site	**2**	100.0% (90/90)	100.0% (90/90)	100.0% (89/89)
Site	**3**	100.0% (87/87)	100.0% (83/83)	100.0% (90/90)
Overall Hit Rate	**Agreement (n/N)**	100.0% (266/266)	100.0% (263/263)	99.6% (267/268)
Overall Hit Rate	**95% CI**	98.6% - 100.0%	98.6% - 100.0%	97.9% - 99.9%

Failure codes

The result report may contain failure codes as described in Table 36, depending on potential run failures. For any questions, please contact your Roche Service representative.

Table 36 Failure codes and definitions

Failure Code Summary Failure Codes	Failure Code Summary Sample	Failure Code Summary Negative Control	Failure Code Summary Positive Control
g0*	IPC out of range. Repeat run.	IPC out of range. Repeat run.	IPC out of range. Repeat run.
g1	IPC out of range. Repeat run.	IPC out of range. Repeat run.	IPC out of range. Repeat run.
g2	IPC out of range. Repeat run.	IPC out of range. Repeat run.	IPC out of range. Repeat run.
g3	IPC out of range. Repeat run.	IPC out of range. Repeat run.	IPC out of range. Repeat run.
g4	IPC out of range. Repeat run.	IPC out of range. Repeat run.	IPC out of range. Repeat run.
x4	One or more targets out of range. Repeat run.	N/A	N/A
FP	N/A	One or more targets out of range. Repeat run.	N/A
b1	N/A	N/A	Target out of range. Repeat run.
b2	N/A	N/A	Target out of range. Repeat run.
b4	N/A	N/A	Target out of range. Repeat run.
a1	N/A	N/A	Target out of range. Repeat run.
a2	N/A	N/A	Target out of range. Repeat run.
a4	N/A	N/A	Target out of range. Repeat run.
r1	N/A	N/A	Target out of range. Repeat run.
r2	N/A	N/A	Target out of range. Repeat run.
r3	N/A	N/A	Target out of range. Repeat run.
r4	N/A	N/A	Target out of range. Repeat run.

Note: * Failure code g0 does not appear for Positive Control

Additional information

Key test features

Sample type	Nasopharyngeal and Nasal swab samples collected with the Copan UTM System or the BD™ UVT System or Thermo Fisher™ Remel (M4®, M4RT®, M5®, M6®) MicroTest Tubes, or in premeasured 3 mL 0.9% physiological saline, such as Thomas Scientific MANTACC™
Minimum amount of sample required	Approximately 0.2 mL
Test duration	Results are available within approximately 20 minutes after loading the sample on the instrument.

Symbols

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

Table 37 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 distributor

Table 38 Manufacturer and distributor

Roche Molecular Systems, Inc.
1080 US Highway 202 South
Branchburg, NJ 08876 USA
www.roche.com

Made in USA

Distributed by

Roche Diagnostics
9115 Hague Road
Indianapolis, IN 46250-0457 USA
(For Technical Assistance call the
Roche Response Center
toll-free: 1-800-800-5973)

Trademarks and patents

See https://diagnostics.roche.com/us/en/about-us/patents

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Document revision

Document Revision Information	
Doc Rev. 5.0 01/2024	Added Non-clinical and Clinical Performance study data for regulatory submission. Change PC tubes to PC vials. Removed references to emergency use authorization and indicated the 'CLIA- waived' status. Updated **Warnings and precautions** section to add additional precautions. Updated for clearance of 510k and CLIAW application. Please contact your local Roche Representative if you have any questions.