Rx Only

cobas® SARS-CoV-2 & Influenza A/B

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

For use under the Emergency Use Authorization (EUA) only

For in vitro diagnostic use

Rx only

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 multiplex real-time RT-PCR assay intended for the simultaneous rapid in vitro qualitative detection and differentiation of SARS-CoV-2, influenza A, and influenza B virus RNA in healthcare provider-collected nasopharyngeal and nasal swabs, and self-collected nasal swabs (collected in a healthcare setting with instruction by a healthcare provider) from individuals suspected of respiratory viral infection consistent with COVID-19 by their healthcare provider. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2 and influenza can be similar.

cobas® SARS-CoV-2 & Influenza A/B is intended for use in the simultaneous rapid in vitro detection and differentiation of SARS-CoV-2, influenza A virus, and influenza B virus nucleic acids in clinical specimens and is not intended to detect influenza C virus. SARS-CoV-2, influenza A and influenza B viral RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of active infection but do not rule out bacterial infection or co-infection with other pathogens not detected by the test. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. The agent detected may not be the definite cause of disease.

Negative results do not preclude SARS-CoV-2, influenza A, and/or influenza B infection and should not be used as the sole basis for diagnosis, treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and/or epidemiological information.

cobas® SARS-CoV-2 & Influenza A/B is intended for use by health professionals or trained operators who are proficient in using the **cobas**® Liat System.

In the United States (US), testing with **cobas**® SARS-CoV-2 & Influenza A/B is authorized for laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform moderate or high complexity tests. cobas® SARS-CoV-2 & Influenza A/B is also authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation. Testing facilities within the U.S. and its territories are required to report all SARS-CoV-2 results to the appropriate public health authorities. In the U.S., **cobas**® SARS-CoV-2 & Influenza A/B is only for use under the Food and Drug Administration's Emergency Use Authorization.

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

cobas® SARS-CoV-2 & Influenza A/B Store at 2-8°C 20 tests (P/N 09211101190) 20 transfer pipettes 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

cobas® SARS-CoV-2 & Influenza A/B

Store at 2-8°C
20 tests (P/N 09211101190)
20 transfer pipettes
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® 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® SARS-CoV-2 & Influenza A/B Store at 2-8°C 20 tests (P/N 09211101190) 20 transfer pipettes 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® 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
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

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, or equivalent	 20A00K984

Note: If the viral transport media and saline listed in Table 4 are not available, CLIA certified moderate and high complexity laboratories only may prepare and package equivalent 3 mL of 0.9% physiological saline for use with **cobas**® SARS-CoV-2 & Influenza A/B test.

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 v1.0 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.
- This test has not been FDA cleared or approved in the United States; this test has been authorized by FDA under an EUA for use by CLIA Certified Moderate and High-Complexity laboratories and Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.
- This test has been authorized only for the simultaneous qualitative detection and differentiation of nucleic acid from SARS-CoV-2, influenza A virus and influenza B virus and not for any other viruses or pathogens.
- This test is only authorized in the United States for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- 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]
- 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.
- 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.
- For additional warnings, precautions and procedures to reduce the risk of contamination for the **cobas**® Liat® Analyzer, consult the **cobas**® Liat® System User Guide.
- 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.

- Use only the transfer pipettes contained in the **cobas**® SARS-CoV-2 & Influenza A/B assay pack and **cobas**® SARS-CoV-2 & Influenza A/B Quality Control Kit. 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 between handling samples and **cobas**® SARS-CoV-2 & Influenza A/B assay tube, **cobas**® SARS-CoV-2 Quality Control Kit to avoid contamination of reagents.
- 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 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.

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. **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 included in the kit to handle specimens.

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 barcode on the Package Insert ID 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 **cobas®** SARS-CoV-2 & Influenza A/B assay tube barcode
5	Scan or enter sample ID
6	Add specimen to **cobas®** SARS-CoV-2 & Influenza A/B assay tube using transfer pipette and re-cap the tube
7	Re-scan **cobas®** SARS-CoV-2 & Influenza A/B assay tube barcode
8	Start run
9	Review results*
10	Unload and dispose used **cobas®** SARS-CoV-2 & Influenza A/B assay tube

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

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

From **cobas**® SARS-CoV-2 & Influenza A/B assay tube Kit:

- Package Insert ID Barcode Card: contained in the **cobas**® SARS-CoV-2 & Influenza A/B assay tube Kit. This barcode is lot-specific; match the lot number next to the barcode with the lot number on the **cobas**® SARS-CoV-2 & Influenza A/B assay tubes.
- 2 **cobas**® SARS-CoV-2 & Influenza A/B assay tubes
- 2 transfer pipettes

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

- Negative Control: Negative Control Barcode (see Control Kit Barcode Card), 1 Dilution UTM tube (used as the negative control sample)
- Positive Control: Positive Control Barcode (see Control Kit Barcode Card), 1 **cobas**® SARS-CoV-2 Positive Control tube, 1 **cobas**® Influenza A/B Positive Control tube
- 1 transfer pipette

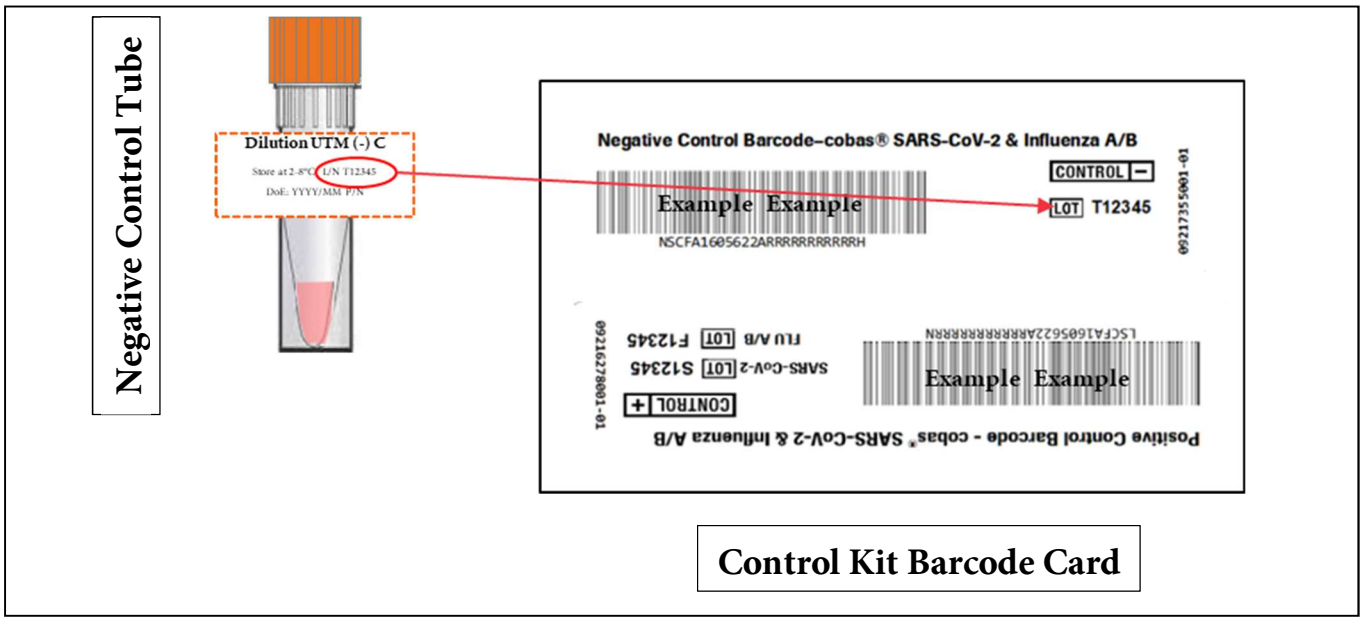
Prepare and test Negative Control sample

Materials needed:

- Package Insert Barcode on the Package Insert Barcode Card contained in the **cobas**® SARS-CoV-2 & Influenza A/B assay tube Kit
- Negative Control Barcode on the Control Kit Barcode Card
- 1 Dilution UTM tube
- 1 **cobas**® SARS-CoV-2 & Influenza A/B assay tube from this lot
- 1 transfer pipette

Note: Following Figure 2, match the lot number (L/N) of the Dilution UTM tube label to the lot number (LOT) of the Negative Control Barcode Label 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 negative control run.

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



Assay tube Lot Validation workflow

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

   Note: You may be prompted to confirm you have read the User Manual, *(i.e.,* ***cobas***® *Liat*® *System User Guide)****.***
5. Select "**Assay Menu**" on the main menu of a **cobas**® Liat® Analyzer.
6. Select "**New Lot**" at the bottom of the list.
7. When prompted to **Scan the Insert ID,** select "**Scan**" and scan the **cobas**® SARS-CoV-2 & Influenza A/B Package Insert ID 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 the Negative Control ID**, select "**Scan**" and scan the Negative Control 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 tube of Negative Control upright and lightly tap on a flat surface to collect liquid at the bottom of the tube. Visually check that the Dilution UTM has pooled at the bottom of the tube.
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 the transfer pipette provided in the pouch 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 the transfer pipette provided in the cobas® SARS-CoV-2 & Influenza A/B assay tube pouch to transfer controls and samples into the cobas® SARS-CoV-2 & Influenza A/B 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. Select "**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 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, select **"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. Select **"Confirm"** 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.

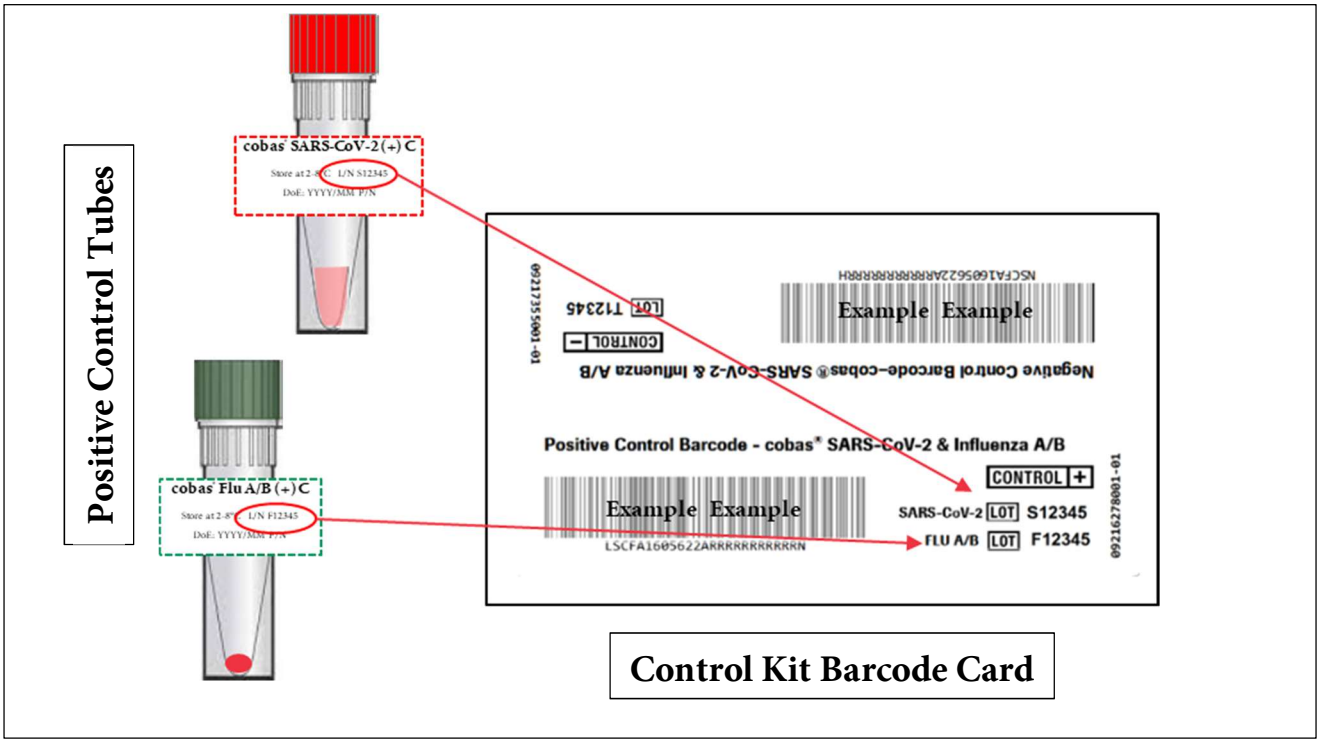
Prepare cobas® SARS-CoV-2 & Influenza A/B Positive Control sample and continue with Lot Validation

Materials needed:

- 1 transfer pipette (Use only transfer pipettes contained in the **cobas**® SARS-CoV-2 & Influenza A/B Quality Control Kit)
- 1 **cobas**® SARS-CoV-2 Positive Control
- 1 **cobas**® Influenza A/B Positive Control (pellet comprising dried positive control material at bottom of tube)

Note: Prior to resuspending the Positive control, match the lot numbers (L/N) of the Positive Control tube label for **cobas**® SARS-CoV-2 & **cobas**® Influenza A/B to the lot number(LOT) of the Positive Control Barcode Label 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 positive control run.

Figure 3: Schematic diagram illustrating cobas® SARS-CoV-2 & cobas® Influenza A/B Positive Control tubes 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 tube 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 tube.
3. Use the provided transfer pipette to transfer approximately 0.2 mL of the liquid from the **cobas**® SARS-CoV-2 Positive Control to the **cobas**® Influenza A/B Positive Control tube.
    a) Check that the **cobas**® Influenza A/B Positive Control pellet is at the bottom of the tube 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 tube.

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 tube after the bulb is fully released.

d) Insert pipette into the **cobas®** Influenza A/B Positive Control tube until the tip is at the bottom of the tube.

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 tube. Dispose of the **cobas®** SARS-CoV-2 Positive Control tube 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 tube. Hold the **cobas®** Influenza A/B Positive Control tube by the cap and shake down the liquid in the tube using a quick, sharp, downward wrist motion.

4. Let the **cobas®** Influenza A/B Positive Control tube sit for 5 minutes to begin dissolving the dried material.
5. After the Positive Control tube has sat for 5 minutes, use another transfer pipette from the **cobas®** SARS-CoV-2 & Influenza A/B Quality Control 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 tube and dispose of the transfer pipette as biohazardous material.
6. Similarly, follow **Lot Validation** workflow steps **8** to **19** with the resuspended **cobas®** SARS-CoV-2 & Influenza A/B Positive Control in place of the Negative Control.
7. If **"Positive control result accepted."** is displayed at the end of the run, select "**Confirm"** and then select **"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. Select "**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 software specific Advanced Tools 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. Select **"Login"** on the screen of the **cobas**® Liat® Analyzer.
3. Enter user name when prompted, select **"OK"**.
4. Enter user password when prompted, select **"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, select "**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 Liat Tube ID,** select **"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 the sample ID,** select "**Scan**" to scan the sample barcode. In the case that the sample cannot be scanned, select "**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. When prompted to add the sample**,** use the transfer pipette provided in the assay tube pouch 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.
9. 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.
10. 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.
11. 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.
12. Select "**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.

13. 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.

14. 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.

15. 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 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.

16. Select "**Report"** to see the Result Report. If applicable, select "**Print"** to print the report.

17. Select "**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
- 1 Transfer pipette
- **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.

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

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 Detected	Positive test for SARS-CoV-2 (SARS-CoV-2 RNA present)
	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 Detected	Positive test for Influenza A (Influenza A RNA present)
	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 Detected	Positive test for Influenza B (Influenza B RNA present)
	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		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.
[Error]. Assay Aborted		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.

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 virus, Influenza type A virus, and Influenza type B virus 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 virus, Influenza type A virus and Influenza type B virus 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, or Thomas Scientific MANTACC™ premeasured 3 mL 0.9% physiological saline solution. Testing of other sample or media types may lead to inaccurate results.
- Users in a point of care environment should not prepare (formulate, measure, aliquot) 0.9% physiological saline. CLIA certified moderate and high complexity laboratories may prepare and package equivalent 3 mL of 0.9% physiological saline for use with **cobas**® SARS-CoV-2 & Influenza A/B test, but performance with these alternative solutions has not been established. When using 0.9% physiological saline solution, ensure that the collection tube is an appropriate height for the swab such that the score mark on the swab is not higher than the height of the tube.
- 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.
- 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.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants 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.

Conditions of Authorization for the Laboratory (U.S. only)

The **cobas**® SARS-CoV-2 & Influenza A/B nucleic acid test for use on **cobas**® Liat System Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas.

However, to assist clinical laboratories using the **cobas**® SARS-CoV-2 & Influenza A/B nucleic acid test for use on **cobas**® Liat System (" **cobas**® SARS-CoV-2 & Influenza A/B" in the conditions below), the relevant Conditions of Authorization are listed below :

- Authorized laboratories[1] using **cobas**® SARS-CoV-2 & Influenza A/B will include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using **cobas**® SARS-CoV-2 & Influenza A/B will use **cobas**® SARS-CoV-2 & Influenza A/B as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use **cobas**® SARS-CoV-2 & Influenza A/B are not permitted.
- Authorized laboratories that receive **cobas**® SARS-CoV-2 & Influenza A/B will notify the relevant public health authorities of their intent to run **cobas**® SARS-CoV-2 & Influenza A/B prior to initiating testing.
- Authorized laboratories using **cobas**® SARS-CoV-2 & Influenza A/B will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories will collect information on the performance of **cobas**® SARS-CoV-2 & Influenza A/B and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Roche (https://www.roche.com/about/business/roche_worldwide.htm) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of **cobas**® SARS-CoV-2 & Influenza A/B of which they become aware.
- All laboratory personnel using **cobas**® SARS-CoV-2 & Influenza A/B must be appropriately trained in PCR techniques, the specific processes and instruments used in the **cobas**® SARS-CoV-2 & Influenza A/B and use appropriate laboratory and personal protective equipment when handling this kit, and use **cobas**® SARS-CoV-2 & Influenza A/B in accordance with the authorized labeling.
- Roche , authorized distributors, and authorized laboratories using **cobas**® SARS-CoV-2 & Influenza A/B will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1] The letter of authorization refers to, "laboratories certified under CLIA, to perform moderate or high complexity tests and use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation" as "authorized laboratories."

Non-clinical performance – SARS-CoV-2

Key performance characteristics

The **cobas**® SARS-CoV-2 & Influenza A/B assay was developed by mainly replacing the RSV primers and probes with those required to detect the SARS-CoV-2 targets in the existing **cobas**® Influenza A/B & RSV assay. The original studies of the **cobas**® Influenza A/B & RSV assay remain relevant for the performance of Influenza A/B targets in the **cobas**® SARS-CoV-2 & Influenza A/B assay.

Analytical sensitivity

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

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 9, 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 9: LoD determination Using USA-WA1/2020 strain

Strain	Concentration [$TCID_{50}$/mL]	Concentration [copies/mL]	Total valid results	Hit rate [%]	Mean Ct*
USA-WA1/2020 (stock concentration 3.16E+06 $TCID_{50}$/mL)	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	35.9
	0.003	3	20	25	36.7

* Calculations only include positive results.

Reactivity/inclusivity

In silico analysis concluded that **cobas**® SARS-CoV-2 & Influenza A/B will detect all analyzed SARS-CoV-2 sequences in NCBI and GISAID databases by using a dual target design (Table 10). Less than 1.44% of sequences analyzed had non-significant mismatches in the RdRp gene, of which all had 100% perfect match in the N gene. Conversely, less than 0.69% of sequences analyzed had non-significant mismatches in the N gene, of which all had 100% perfect match in the RdRp gene. One sequence was identified that had three mismatches close to the 5'-end of the probe binding region of N gene detection set. This sequence had 100% perfect match to the RdRp detection set, therefore no impact on assay performance is expected.

Table 10: In silico inclusivity analysis of SARS-CoV-2

Target	RdRp gene (ORF1ab)				N gene			
Database	**NCBI**		**GISAID**		**NCBI**		**GISAID**	
Number of Sequences	3552	100%	27350	100%	3342	100%	27175	100%
Sequences with mutation	51	1.44%	119	0.44%	23	0.69%	142	0.52%
Predicted no detection	0	0.00%	0	0.00%	0	0.00%	1	0.004%

Cross reactivity

The in silico analysis for possible cross reactions with all the organisms listed in Table 11 was conducted by mapping the primers and probes in **cobas**® SARS-CoV-2 & Influenza A/B to the sequences available from NCBI databases. The percent homology of sequences that partially aligned with the SARS-CoV-2 N and RdRp target primers and probes are shown in the table below. If any two of the primers were mapped to a sequence on opposite strands with short distance apart, potential amplifications were flagged. No potential unintended cross reactivity is expected based on this in silico analysis except for SARS-CoV-1 which has been additionally tested as shown in Table 11.

Table 11: Organisms with homology to SARS-CoV-2 N and RdRp primers and probes

Strain	Percent homology to N			Percent Homology to RdRp		
	Forward primer	**Probe**	**Reverse primer**	**Forward primer**	**Probe**	**Reverse primer**
Human coronavirus HKU1						81.50%
SARS-coronavirus (SARS-CoV-1)	100.00%	81.48%	94.74%	95.80%	87.50%	96.30%
MERS-coronavirus	80.00%					
Haemophilus influenzae	95.00%					
Legionella pneumophila	80.00%					
Streptococcus pyogenes	80.00%					
Mycoplasma pneumoniae				83.30%		
Candida albicans	90.00%			83.30%		
Staphylococcus epidermidis	85.00%					
Staphylococcus salivarius			89.47%			
Human coronavirus 229E/OC43/NL63	No alignment found*			No alignment found*		
Adenovirus (e.g., C1 Ad. 71)	No alignment found*			No alignment found*		
Human metapneumovirus (hMPV)	No alignment found*			No alignment found*		
Influenza A (all available sequences)	No alignment found*			No alignment found*		
Influenza B (all available sequences)	No alignment found*			No alignment found*		
Enterovirus (e.g. EV68)	No alignment found*			No alignment found*		
Respiratory syncytial virus (RSV)	No alignment found*			No alignment found*		
Rhinovirus	No alignment found*			No alignment found*		
Chlamydia pneumoniae	No alignment found*			No alignment found*		

Strain	Percent homology to N			Percent Homology to RdRp		
	Forward primer	Probe	Reverse primer	Forward primer	Probe	Reverse primer
Mycobacterium tuberculosis	No alignment found*			No alignment found*		
Streptococcus pneumoniae	No alignment found*			No alignment found*		
Bordetella pertussis	No alignment found*			No alignment found*		
Pneumocystis jirovecii (PJP)	No alignment found*			No alignment found*		
Pseudomonas aeruginosa	No alignment found*			No alignment found*		

* The primers and probes of SARS-CoV-2 N and RdRp targets were blasted against the exclusive sequences with cutoff of identity ≥ 80%. Identities ≥ 80% are shown in the table.

Cross reactivity with SARS-CoV-1

Cross reactivity with SARS-CoV-1 was evaluated by testing inactivated SARS-CoV-1 whole virus. Gamma irradiated cultured SARS-CoV-1 (Urbani strain, lot number 58542036, BEI Resources, VA, USA) was diluted into pooled negative nasopharyngeal swabs in UTM at 1.0E+05 PFU/mL. As shown in Table 12, SARS-CoV-1 did not interfere with the **cobas**® SARS-CoV-2 & Influenza A/B assay performance.

Table 12: SARS-CoV-2 cross reactivity with SARS-CoV-1

SARS-CoV-1 Concentration Tested	cobas® SARS-CoV-2 & Influenza A/B			
	SARS-CoV-2	Influenza A	Influenza B	IPC
	Result	Result	Result	Ct
1.00E+05 PFU/mL	Not Detected	Not Detected	Not Detected	31.6

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 13). 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 13: 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 14); 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 14: 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.

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 15.

Table 15: 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		N/A	ND

NDU/mL = RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not detected

Clinical performance evaluation – SARS-CoV-2

The clinical performance of the **cobas**® SARS-CoV-2 & Influenza A/B test for the detection of SARS-CoV-2 was separately evaluated using retrospective and prospective clinical samples from individuals suspected of respiratory viral infection consistent with COVID-19.

Clinical performance evaluation using retrospective clinical specimens

The clinical performance of the **cobas**® SARS-CoV-2 & Influenza A/B test for the detection of SARS-CoV-2 was evaluated using 56 known SARS-CoV-2 positive remnant nasopharyngeal clinical samples and 231 negative clinical samples collected prior to the COVID-19 pandemic (a mixture of nasopharyngeal and nasal swab samples) in UTM from patients with a suspected respiratory infection. Testing of retrospective clinical samples was performed with the **cobas**® SARS-CoV-2 & Influenza A/B test and a highly sensitive FDA-authorized laboratory-based RT-PCR SARS-CoV-2 EUA assay.

As shown in Table 16, all 56 SARS-CoV-2 positive samples tested positive with both the **cobas**® SARS-CoV-2 & Influenza A/B test on **cobas**® Liat System and the comparator assay.

As shown in Table 16, 229 valid negative samples tested negative for SARS-CoV-2 with both the **cobas**® SARS-CoV-2 & Influenza A/B test and the comparator assay. Five of the 231 negative clinical samples generated an initial invalid result with the **cobas**® SARS-CoV-2 & Influenza A/B test: 3 samples that generated valid results on repeat testing were included in the analysis and 2 samples that generated repeat invalid results were excluded from the analysis, yielding 229 valid negative samples. One negative sample tested positive for influenza A with the **cobas**® SARS-CoV-2 & Influenza A/B test; this result was confirmed with an FDA-cleared molecular influenza assay.

For retrospective specimens, the results of the clinical performance evaluation demonstrated 100% positive percent agreement and 100% negative percent agreement as compared to the comparator assay.

Table 16: Clinical performance comparison with a highly sensitive FDA-authorized RT-PCR SARS-CoV-2 EUA assay – Reetrospective specimens

		SARS-CoV-2 Comparator Assay	
		Positive	Negative
cobas® SARS-CoV-2 & Influenza A/B on **cobas**® Liat® System	Positive	56	0
	Negative	0	229*

* 2 repeated invalid samples were not included in the analysis

PPA	100% (95% CI: 93.6% - 100%)
NPA	100% (95% CI: 98.4% - 100%)

Clinical performance evaluation using prospective clinical samples

The clinical performance of the **cobas**® SARS-CoV-2 & Influenza A/B test for the detection of SARS-CoV-2 was evaluated using paired clinical nasopharyngeal swab (NPS) and nasal swab (NS) samples prospectively collected in UTM from patients with a suspected respiratory infection; NS samples were comprised of either healthcare provider-collected or self-collected swabs. Testing of prospective clinical samples was performed with the **cobas**® SARS-CoV-2 & Influenza A/B test and compared to results from NPS specimens using a highly sensitive FDA-authorized laboratory-based multiplexed RT-PCR EUA assay (reference method).

No coinfections with SARS-CoV-2 and influenza A/B were detected. No specimens tested in this performance evaluation were influenza A or influenza B positive by the **cobas**® SARS-CoV-2 & Influenza A/B test.

For prospective specimens, a total of 963 subjects were enrolled in this study. Of these, 2 subjects did not meet eligibility criteria. Additionally, 26 NPS specimens were excluded due to missing or invalid results from the Analyzer or the reference method. As such, a total of 935 NPS specimens were determined to be evaluable by both the **cobas**® SARS-CoV-2 & Influenza A/B test and the reference method, and these were included in the performance analysis. Furthermore, a total of 930 paired NS specimens were evaluable for testing with the **cobas**® SARS-CoV-2 & Influenza A/B test and the reference method.

As shown in Table 17 for prospective NPS specimens, the **cobas**® SARS-CoV-2 & Influenza A/B test demonstrated 95.2% positive percent agreement and 99.6% negative percent agreement compared to the reference method for SARS-CoV-2 detection.

Table 17: Clinical performance comparison with the reference method – Prospective NPS specimens

		Reference Method SARS-CoV-2 Result	
		Positive	Negative
cobas® SARS-CoV-2 & Influenza A/B on **cobas**® Liat® System **Nasopharyngeal Swab (NPS)**	Positive	79	3[a]
	Negative	4[a]	849

PPA	95.2% (95% CI: 88.3% - 98.1%)
NPA	99.6% (95% CI: 99.0% - 99.9%)

[a] Seven discordant results between NPS specimens tested with **cobas**® SARS-CoV-2 & Influenza A/B and the reference method showed late Ct values, which are indicative of samples from individuals with viral loads near or below the limit of detection of both assays.

As shown in Table 18 for prospective NS specimens, the **cobas**® SARS-CoV-2 & Influenza A/B test demonstrated 96.4% positive percent agreement and 99.5% negative percent agreement compared to paired NPS specimen results from the reference method for SARS-CoV-2 detection.

Table 18: Clinical performance comparison with the reference method – Prospective NS specimens

		Reference Method SARS-CoV-2 Result	
		Positive	Negative
cobas® SARS-CoV-2 & Influenza A/B on **cobas**® Liat® System **Nasal Swab (NS)**	Positive	80	4[a]
	Negative	3[a]	843

PPA	96.4% (95% CI: 89.9% - 98.8%)
NPA	99.5% (95% CI: 98.8% - 99.8%)

[a] Seven discordant results between paired NS and NPS specimens tested with **cobas**® SARS-CoV-2 & Influenza A/B and the reference method showed late Ct values, which are indicative of samples from individuals with viral loads near or below the limit of detection of both assays.

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: Analyical 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).

Reproducibility

Reproducibility study assesses the total variability of the assay in detecting Influenza A/B across operators, study sites, testing days, Analyzers, and assay tube lots. The reproducibility was evaluated at 3 sites. Two operators at each of the 3 sites tested a 10-member reproducibility panel in triplicate on 5 different days, for a total of ~900 runs (10 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 high negative, a low positive, and a moderate positive for each of Influenza A and Influenza B, in addition to a negative sample. For a given virus, the expected result for the true negative and the high 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, and Ct %CV for each site are shown in Table 20 and Table 21.

Table 20: Influenza A reproducibility

	Site 1			Site 2			Site 3			Total	
Sample	**Agreement w/ expected result**	**Avg Ct**	**Ct %CV**	**Agreement w/ expected result**	**Ct Avg**	**Ct %CV**	**Agreement w/ expected result**	**Ct Avg**	**Ct %CV**	**Agreement w/ expected result**	**95% CI**
Negative	30 / 30	-	-	31 / 31	-	-	30 / 30	-	-	91 / 91 (100.0%)	96.0% - 100.0%
Flu A High Negative*	29 / 30	37.0	-	30 / 30	-	-	29 / 30	35.7	-	88 / 90 (97.8%)	92.3% - 99.4%
Flu A Low Positive*	30 / 30	32.7	2.9%	30 / 30	32.1	1.6%	30 / 30	32.3	1.6%	90 / 90 (100.0%)	95.9% - 100.0%
Flu A Moderate Positive*	30 / 30	30.4	1.0%	30 / 30	30.0	1.2%	30 / 30	30.1	0.9%	90 / 90 (100.0%)	95.9% - 100.0%
Flu B High Negative*	30 / 30	-	-	31 / 31	-	-	30 / 30	-	-	91 / 91 (100.0%)	96.0% - 100.0%
Flu B Low Positive*	30 / 30	-	-	30 / 30	-	-	29 / 29[†]	-	-	89 / 89 (100.0%)	95.9% - 100.0%
Flu B Moderate Positive*	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
Total Agreement	209 / 210 (99.5%)			212 / 212 (100.0%)			208 / 209 (99.5%)			629 / 631 (99.7%)	98.9% - 100.0%

[†] One of 30 Influenza B Low Positive replicates yielded an "Assay Invalid. Repeat Assay" result, and was not repeated.

[*] Guidance for Industry and FDA Staff Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection or Detection and Differentiation of Influenza Viruses. Document issued on: July 15, 2011

Table 21: Influenza B reproducibility

	Site 1			Site 2			Site 3			Total	
Sample	**Agreement w/ expected result**	**Ct Avg**	**Ct %CV**	**Agreement w/ expected result**	**Ct Avg**	**Ct %CV**	**Agreement w/ expected result**	**Ct Avg**	**Ct %CV**	**Agreement w/ expected result**	**95% CI**
Negative	30 / 30	-	-	31 / 31	-	-	30 / 30	-	-	91 / 91 (100.0%)	96.0% - 100.0%
Flu A High Negative*	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
Flu A Low Positive*	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
Flu A Moderate Positive*	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
Flu B High Negative*	29 / 30	35.1	-	31 / 31	-	-	30 / 30	-	-	90 / 91 (98.9%)	94.0% - 99.8%
Flu B Low Positive*	30 / 30	31.9	1.8%	30 / 30	31.6	1.4%	29 / 29[†]	31.6	1.5%	89 / 89 (100.0%)	95.9% - 100.0%
Flu B Moderate Positive*	30 / 30	30.8	1.3%	30 / 30	30.4	1.4%	30 / 30	30.5	1.3%	90 / 90 (100.0%)	95.9% - 100.0%
Total Agreement	209 / 210 (99.5%)			212 / 212 (100.0%)			208 / 209 (99.5%)			629 / 631 (99.7%)	98.9% - 100.0%

[†] One of 30 Influenza B Low Positive replicates yielded an "Assay Invalid. Repeat Assay" result, and analysis was not repeated.

[*] Guidance for Industry and FDA Staff Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection or Detection and Differentiation of Influenza Viruses. Document issued on: July 15, 2011

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 22).

Table 22: Results of testing Influenza A and Influenza B strains

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

Virus Strain	Type / Subtype	Test Concentration	Inf A Result	Inf B Result
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 233).

Table 23: 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	8.0×104 $TCID_{50}$/mL	–	–
Corynebacterium sp	3.6×10^{6} CFU/mL	–	–
Escherichia coli	1.9×10^{6} CFU/mL	–	–
Haemophilus influenzae	2.3×10^{6} CFU/mL	–	–
Lactobacillus sp	1.9×10^{6} CFU/mL	–	–
Legionella pneumophila	6.7×10^{6} CFU/mL	–	–
Moraxella catarrhalis	2.5×10^{6} CFU/mL	–	–

Microorganism	Test Concentration	Inf A Result	Inf B Result
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 24).

Table 24: 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.

Interfering substances

Potentially interfering substances that may be encountered in respiratory specimens were evaluated. Medically and/or physiologically relevant concentrations of potential interferents were tested with 2 Influenza A strains and 2 Influenza B strains at ~3x LoD. As shown in Table 25, substances at the concentrations tested did not interfere in the detection of Influenza A and Influenza B.

Table 25: Influenza A/B interfering substances study results

Potential Interferent	Active Ingredient	Concentration
Mucin: bovine submaxillary gland, type I-S	Purified mucin protein	5 mg/mL
Blood	-	5% (v/v)
Nasal spray – Afrin	Oxymetazoline	5% (v/v)
Nasal corticosteroids – Veramyst	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

Matrix equivalency – UTM and Remel Media

Equivalence between UTM-RT and Remel Media (M4, M4RT, M5 and M6) was evaluated by spiking cultured viruses (Influenza A and Influenza B) at 3x LoD into five different transport media (M4, M4RT, M5, M6 and UTM). For each collection medium, five replicates of positive samples at 3x LoD and 5 negative samples were tested under various conditions. The results of the study showed that the assay was able to correctly detect the presence of the viral targets suspended in all five transport media (Table 26), and supported the recommended storage conditions of up to 4 hours at room temperature.

Table 26: Result comparison of UTM to Remel Media (M4, M4RT, M5 and M6)

Collection Media	Sample Concentration	Influenza A	Influenza B
		Detected	Detected
M4	3x LoD	5/5	5/5
	Negative	0/5	0/5
M4RT	3x LoD	5/5	5/5
	Negative	0/5	0/5
M5	3x LoD	5/5	5/5
	Negative	0/5	0/5
M6	3x LoD	5/5	5/5
	Negative	0/5	0/5
UTM	3x LoD	5/5	5/5
	Negative	0/5	0/5

Matrix equivalency – UTM and 0.9% physiological saline

Equivalence between UTM-RT and 0.9% physiological saline was evaluated by spiking cultured viruses (Influenza A, Influenza B and SARS-CoV-2) at 3x LoD into paired clinical negative nasopharyngeal swab specimens collected in UTM and 0.9% physiological saline using the **cobas**® SARS-CoV-2 & Influenza A/B Assay for use on **cobas**® Liat System. For each collection medium, 20 individual contrived low positive samples and 10 negative individual specimens were tested. As shown in Table 27, all low positive paired samples were positive in both sample matrices. All negative paired samples were negative in both sample matrices.

Table 27: Result comparison of UTM to 0.9% physiological saline

Specimen	Media	N	Influenza A	Influenza B	SARS-CoV-2
			% Positive	% Positive	% Positive
Positive	0.9% physiological Saline	20	100	100	100
	UTM	20	100	100	100
Negative	0.9% physiological Saline	10	0	0	0
	UTM	10	0	0	0

Clinical studies – Influenza A/B

The clinical performance of the assay was evaluated at 12 CLIA waived healthcare facilities. Prospective nasopharyngeal swab (NPS) specimens were collected from patients with signs and symptoms of respiratory infection in the US during the 2013-2014 and 2014-2015 flu seasons, and were tested prospectively at the study sites. Additionally, retrospective NPS specimens were obtained from 2 reference laboratories and were distributed to and tested at 3 of the 12 sites. The retrospective specimens were worked into the daily workload of those sites for testing.

Each patient's specimen was tested for Influenza A/B and an FDA-cleared laboratory-based multiplexed real-time reverse transcriptase PCR (RT-PCR) test (comparator test). The results for Influenza A/B were compared against the results from the comparator test. A total of 1,350 prospective NPS specimens and 292 retrospective NPS specimens were included in the performance analysis.

For prospective specimens, a total of 1,421 subjects were enrolled in this study. Of these, 41 specimens did not meet eligibility criteria. Additionally, 17 and 13 specimens were excluded due to invalid results from the Analyzer and the comparator tests, respectively. As such, a total of 1,350 prospective nasopharyngeal swab (NPS) specimens were included in the performance analysis (Table 28 and Table 29). Compared to the comparator test, the assay demonstrated positive agreement of 98.3% and 95.2% for Inf A and Inf B, respectively; and negative agreement of 96.0% and 99.4% for Inf A and Inf B, respectively.

Table 28: Clinical performance with prospective NPS specimens – Influenza A

		Comparator Test		
		Positive	**Negative**	**Total**
Liat	Positive	172	47[a]	219
	Negative	3	1128	1131
	Total	175	1175	1350

	%	**95% CI**
Positive Agreement	98.3%	(95.1% - 99.4%)
Negative Agreement	96.0%	(94.7% - 97.0%)

[a] Forty-one **cobas**® Influenza A/B positive, lab-based RT-PCR negative specimens were tested by PCR/sequencing. Of these, 18 were positive and 23 were negative by PCR/sequencing.

Table 29: Clinical performance with prospective NPS specimens – Influenza B

		Comparator Test		
		Positive	**Negative**	**Total**
Liat	Positive	40	8[a]	48
	Negative	2	1300	1302
	Total	42	1308	1350

	%	**95% CI**
Positive Agreement	95.2%	(84.2% - 98.7%)
Negative Agreement	99.4%	(98.8% - 99.7%)

[a] Six **cobas**® Influenza A/B positive, lab-based RT-PCR negative specimens were tested by PCR/sequencing. Of these, 5 were positive and 1 was negative by PCR/sequencing.

For retrospective specimens, a total of 300 specimens were tested at clinical sites. Of these, 5 and 3 specimens were excluded due to invalid results from the System and the comparator tests, respectively. As such, a total of 292 retrospective nasopharyngeal swab (NPS) specimens were included in the performance analysis (Table 30 and Table 31). Compared to the comparator test, Inf A and Inf B demonstrated positive agreement of 98.7% and 99.0% , respectively; and negative agreement of 99.1% and 99.5% for Inf A and Inf B, respectively.

Table 30: Clinical performance with retrospective NPS specimens – Influenza A

		Comparator Test		
		Positive	**Negative**	**Total**
Liat	Positive	76	2[a]	78
	Negative	1	213	214
	Total	77	215	292

	%	**95% CI**
Positive Agreement	98.7%	(93.0% - 99.8%)
Negative Agreement	99.1%	(96.7% - 99.7%)

[a] One **cobas®** Influenza A/B positive, lab-based RT-PCR negative specimen was tested by PCR/sequencing. This sample was negative by PCR/sequencing.

Table 31: Clinical performance with retrospective NPS specimens – Influenza B

		Comparator Test		
		Positive	**Negative**	**Total**
Liat	Positive	97	1	98
	Negative	1	193	194
	Total	98	194	292

	%	**95% CI**
Positive Agreement	99.0%	(94.4% - 99.8%)
Negative Agreement	99.5%	(97.1% - 99.9%)

During the clinical study testing of prospective and retrospective specimens, the assay initial invalid rate was 1.8% (29/1,656 specimens, 95% CI: 1.2% - 2.5%). Of these 29 specimens with initial invalid results, 5 specimens had 2 invalid or aborted runs, 16 specimens had 1 invalid run and were not repeated due to unavailability of residual samples, and 8 specimens had an initial invalid run and a repeat test per product instructions for use yielded a valid result.

Following addition of the SARS-CoV-2 assay targets, a study using banked retrospective clinical samples was conducted to demonstrate that the sensitivity and inclusivity of the existing influenza A and B targets was not altered. For this study, 11 nasopharyngeal swab specimens from patients with confirmed influenza A (n=5) or influenza B (n=6) infection were tested in parallel with both the **cobas®** SARS-CoV-2 & Influenza A/B script and the **cobas®** Influenza A/B Nucleic Acid Test script. The CDC 2019 Human Influenza Virus Panel, including two additional influenza A (H1N1 strain Brisbane/02/2018 and H3N2 strain Perth/16/2009) and two influenza B (Victoria lineage Colorado/06/2017 and Yamagata lineage Phuket/3073/2013) strains, were also tested in this study. Ct ranges of the sample included in the study ranged from 17.3 to 36.0. Agreement of both test scripts with expected results as 100% (15/15).

Failure codes

The result report may contain failure codes as described in Table 32, depending on potential run failures. For any questions, please contact your Roche Service representative.

Table 32: Failure codes and definitions

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

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

Additional information

Key test features

Sample type	Nasopharyngeal and Nasal swab samples collected in a Copan UTM System or the BD™ UVT System or Thermo Fischer™ Remel (M4®, M4RT®, M5®, M6®), and 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 33: 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	
As i /mL] Assigned Range (copies/mL)	Distributor *(Note: The applicable country/region may be designated beneath the symbol)*	SN Serial number
As i 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 UltraSen i Ultrasensitive Procedure
Collect Date Collect date	Manufacturer	U I Unique Device Identifier
Consult instructions for use	CONTROL − Negative control	Upper Limit of Assigned Range
Σ Contains sufficient for <*n*> tests	NON STERILE Non-sterile	II 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 co i /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 34: 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

This product is covered by US Patent Nos. 6727067, 6780617, 7799521, 6964862, 7935504, 8148116, 9662652, 7337072, 7718421, 8936933, 9708599, and 10443050, and foreign equivalent patents owned by Roche.

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See http://www.roche-diagnostics.us/patents

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Document revision

Document Revision Information	
Doc Rev. 2.0 12/2020	Updated to include 0.9% physiological saline solution as an additional media type Updated to include new Copan UTM-RT® and Thermo Fisher™ Scientific Remel™ part numbers acceptable for use with this test Added Made in statement Please contact your local Roche Representative if you have any questions.
Doc Rev. 3.0 03/2022	Updated to add **Clinical performance with prospective clinical samples** section, and to address additional conditions of EUA authorization set forth by FDA. Removed software version 3.2 reference. Updated Table 11 footnote to reflect alignment method used, Updated the harmonized symbol page. Updated to Economic Operators. Updated **Trademarks and patents** section. Please contact your local Roche Representative if you have any questions.

Quick Reference Instructions

cobas® SARS-CoV-2 & Influenza A/B

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

For use under the Emergency Use Authorization (EUA) only
IVD only
Rx only

This test has not been FDA cleared or approved in the United States; this test has been authorized by FDA under an EUA for use by CLIA Certified Moderate and High-Complexity laboratories and Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.

This test has been authorized only for the simultaneous qualitative detection and differentiation of nucleic acid from SARS-CoV-2, influenza A virus, and influenza B virus and not for any other viruses or pathogens.

This test is only authorized in the United States for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

Read the **cobas**® SARS-CoV-2 & Influenza A/B instructions for use from the Package Insert and the **cobas**® Liat® System User Guide for complete test procedure, result interpretation and further assay information before proceeding with test.

Specimen Collection into Transport Media

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 premeasured 3 mL 0.9% physiological saline. Part numbers for collection kits can be found in **cobas**® SARS-CoV-2 & Influenza A/B test Package Insert. This test is only for nasopharyngeal and nasal swab specimens.

cobas® SARS-CoV-2 & Influenza A/B test procedure for clinical specimens

Obtain the following materials:

□ 1 **cobas**® SARS-CoV-2 & Influenza A/B assay tube

□ 1 transfer pipette

□ 1 specimen in collection media

Step 1:

From the **Main** menu, choose **Run Assay** and choose the **Select** button. Then **Scan** the assay tube barcode.

Step 2:

Scan the sample ID barcode, or choose **Enter** to enter the ID manually.

Note: *Depending on analyzer configuration, if required to confirm the received patient information, choose the* ***Confirm*** *button.*

Step 3:

Firmly squeeze the bulb of the transfer pipette, lower it into the liquid and release the bulb to draw up the sample. Slowly transfer the sample into the assay tube, by squeezing the bulb and then recap the assay tube.

Note: Do not puncture the assay tube or the seal at the bottom of the sample compartment

Step 4:

Scan the assay tube barcode.

Step 5:

Turn and remove the assay tube sleeve and then insert the assay tube into the analyzer tube entry door. Processing begins automatically.

Step 6:

When the assay run is complete, remove and discard the assay tube.

Step 7:

Choose the **Report** button to view the result report.

To return to the **Main** menu, select **Back** and then choose the **Main** button.

Table 1: Interpretation of results of **cobas®** SARS-CoV-2 & Influenza A/B when running a sample

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 Detected	Positive test for SARS-CoV-2 (SARS-CoV-2 RNA present)
	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 Detected	Positive test for Influenza A (Influenza A RNA present)
	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 Detected	Positive test for Influenza B (Influenza B RNA present)
	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		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.
[Error]. Assay Aborted		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.

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.

Quality Control: Performing Lot Validation

External Controls must be run for each new lot of **cobas**® Liat® assay tubes.

Follow the Lot Validation procedure to validate assay tube lots on the **cobas**® Liat® Analyzer (see Package Insert for full procedure).

Obtain the following materials:

From **cobas**® SARS-CoV-2 & Influenza A/B assay tube Kit:	From **cobas**® SARS-CoV-2 & Influenza A/B Quality Control Kit:
□ 2 **cobas**® SARS-CoV-2 & Influenza A/B assay tubes	□ 1 Dilution UTM tube
□ 2 transfer pipettes	□ 1 **cobas**® SARS-CoV-2 Positive Control tube
□ Package Insert Barcode card	□ 1 **cobas**® Influenza A/B Positive Control tube
	□ Negative/Positive Control Barcode card

Add Lot Negative Control

Step 1:

From the **Main** menu, select **Assay Menu**. From the **Assay Menu**, select **[New Lot]**.

Step 2:

Select **Scan**, and scan the Package Insert barcode from the Package Insert Barcode Card.

Note: *You may be prompted to confirm that you have read the Package Insert, i.e., Instruction For Use.*

Step 3:

Check that the lot number on the Control Kit Barcode Card matches the control tube lot number.

Select **Scan** and scan the Negative Control barcode from the Control Kit Barcode Card

Step 4:

Firmly squeeze the bulb of the transfer pipette and draw up the control. Slowly transfer the control into the assay tube and then recap the assay tube.

Note: Do not puncture the assay tube or the seal at the bottom of the sample compartment.

Step 5:

Select **Scan,** and scan the assay tube barcode.

Step 6:

Turn and remove the assay tube sleeve and insert the assay tube into the analyzer tube entry door. Processing begins automatically.

Step 7:

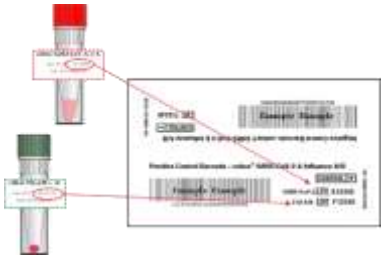
Once the Negative control result is accepted, choose **Confirm.** Then, remove and discard the assay tube. Select **Back** to continue with positive control run.

Add Lot Positive Control

Step 8:

Prepare the Positive control following the instructions provided in the Package Insert.

Prior to re-suspending the Positive control, check that the lot numbers on the Control Kit Barcode Card match the respective control tube lot numbers.



Step 9:

Resume with **Add Lot Positive Control** on the same instrument. Repeat steps 3–7.

Note: In Step 3, **Scan** the Positive Control barcode from the Control Kit Barcode Card.

When the positive control result is accepted, you can begin using the lot. Navigate **Back** to the **Main** menu.

Warnings and Precautions

Treat all biological specimens with universal precautions, including used **cobas**® Liat® tubes and pipettes.

Follow your institution's safety procedures for working with chemicals and handling biological samples.

Document Information

Software version 3.3 or higher

P/N: 09341536001

Technical support

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

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Rx Only

IVD

Emergency Use Authorization (EUA)

CE

Document Revision

Document Revision Information	
Doc Rev. 1.0 09/2020	First Publishing.
Doc Rev. 2.0 12/2020	Added 0.9% physiological saline solution, added examples for flocked swab with a synthetic tip, corrected a typo and updated formatting. Added Made in statement. Please contact your local Roche Representative if you have any questions.

cobas® SARS-CoV-2 & Influenza A/B

IVD

Rx Only

Emergency Use Authorization/ Zulassung für die Anwendung in Notfallsituationen/ Autorisation d'utilisation d'urgence/ Autorizzazione all'uso per emergenza/ Autorización de uso de emergencia/ Autorização para utilização de emergência/ Godkendelse til brug i nødsituationer/ Behörighet för användning i nödsituationer/ Goedkeuring voor gebruik in noodsituaties

This card is for Add Lot purposes. Do not throw away./ Diese Karte wird benötigt, wenn Sie Chargen hinzufügen möchten. Nicht entsorgen./ Cette carte est destinée à l'ajout de lots. Ne pas jeter./ Questa scheda è richiesta durante l'aggiunta di un lotto. Non gettarla via./ Esta tarjeta se utiliza en el procedimiento para añadir lotes. No la tire./ Este cartão é para efeitos de adição de lotes. Não deitar fora./ Dette kort er til brug ved tilføjelse af lot. Det må ikke bortskaffes./ Du behöver det här kortet när du registrerar nya loter. Kasta inte bort det./ Deze kaart is bedoeld ten behoeve van Partij toevoegen. Niet weggooien.

PLACE PACKAGE INSERT BARCODE HERE

USA:

website: http://e-labdoc.roche.com

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Non-USA:

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