TRAINING UPDATE

Lab Location:	GEC	**Date Distributed:**	1/19/2021
Department:	Core Lab	**Due Date:**	2/19/2021

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

SARS-CoV-2 RNA (COVID-19) and Influenza A and B, Rapid NAAT by cobas® LIAT GEC.E103 v2

Liat SARS-CoV-2 / FLU A&B PCR Quality Control Log AG.F566.3

Description of change(s):

The changes are intended to make the QC process better align with the instrument printout.

SOP:

Section	Reason
6.4	Added notes to clarify external QC results

Form:

- Documentation of external QC changed to 'valid' or 'invalid'
- Corrected instructions to delete running ext. QC each day of testing
- Changed instruction to reflect Run Status (instead of internal QC)

The revised SOP and Log will be implemented on January 27, 2021

Document your compliance with this training update by taking the quiz in the MTS system.

Liat SARS-CoV-2 / FLU A&B PCR Quality Control Log

Germantown Emergency Center

Last external QC performed (date): _____ **Next external QC is due = *Month*** _____ *Circle day below*

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31

1. **External Positive and Negative Controls** are tested and documented ~~each day of testing, and~~ each new kit lot or shipment or every 31 days, whichever is more frequent.
2. ~~**Internal controls**~~ **Run Status** must be documented each time the test is performed.
3. If QC results are not acceptable, document corrective action. Do not accept patient results before reviewing QC results for proper reactions.

Date	Patient Name / MR#	Kit	Patient Results DET / NTD	Run Status	External Pos Control Valid = Detected / Positive		External Neg Control Valid = Not detected / Neg		Tech Code
		Lot # / Expire		OK / Not OK	Lot # / Expire	Valid / Invalid	Lot # / Expire	Valid / Invalid	
			CoV-2 Flu A Flu B						
			CoV-2 Flu A Flu B						
			CoV-2 Flu A Flu B						
			CoV-2 Flu A Flu B						
			CoV-2 Flu A Flu B						
			CoV-2 Flu A Flu B						
			CoV-2 Flu A Flu B						
			CoV-2 Flu A Flu B						

Weekly review:	Weekly review:	Weekly review:
Weekly review:	Weekly review:	Monthly review:

Technical SOP

Title	**SARS-CoV-2 RNA (COVID-19) and Influenza A and B, Rapid NAAT by cobas® LIAT**
Prepared by	Ron Master Date: 12/10/2020
Owner	Ron Master Date: 12/10/2020

Laboratory Approval	**Local Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
CoV2 (COVID-19) and FLU A/B, Rapid, Qualitative	Real-time reverse transcriptase polymerase chain reaction (RT-PCR)	IFCVD

Synonyms/Abbreviations
SARS-CoV-2 and Flu A/B PCR, SARS CoV-2 RNA (COVID-19) and Influenza A and B, Rapid Qualitative NAAT

Department
Core Lab

2. ANALYTICAL PRINCIPLE

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.

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.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	Follow manufacturer's instructions. Avoid nasal sprays, ointments, gels and antibiotics prior to collection. Avoid use of throat lozenges.

Component	Special Notations
	1. Insert the collected swab into a UTM/VTM tube. Place the swab head at least ½ inch below the surface of the media and swirl the swab in the media. 2. Break the swab shaft and leave the swab in the UTM tube (i.e. sample tube) 3. Attach the cap securely. Store at room temperature (up to 4 hours) or refrigerate (up to 72 hours)
Special Collection	N/A
Other	N/A

3.2 Specimen Type & Handling

Criteria		
Type -Preferred **-Other Acceptable**	Sterile nasopharyngeal swabs, in approved UTM/VTM (Viral Transport media). See list of acceptable swabs/VTM kits below	
Approved Collection Container	**Specimen Collection Kit** Nasopharyngeal Swab Collection Kits Flexible minitip FLOQSwab™ with Universal Transport Medium™ (UTM) from Copan Diagnostics; P/N 305C OR BD Universal Viral Transport (UVT) 3-mL collection kit with a flocked flexible minitip swab; P/N 220531 ThermoFisher Scientific Remel™ MART; R12565,R12566,R12567 ThermoFisher Scientific Remel™ M4; R12550 ThermoFisher Scientific Remel™ M5; R12555 ThermoFisher Scientific Remel™ M6; R12563,R12568,R12569	
Volume - Optimum **- Minimum**	3.0 mL 1.0 mL	
Transport Container & Temperature	Sample collection kit at room temperature	
Stability & Storage Requirements	Room Temperature:	0-4 hours
	Refrigerated (2-8°C)	0-72 hours
	Frozen: (-70°C) or colder	Indefinitely (≥ 72 hours) (and transport on dry ice)
Timing Considerations	N/A	
Unacceptable Specimens & Actions to Take	• Specimens containing calcium alginate swabs, cotton swabs with wooden shaft. • Dry Swabs, Swabs transported without UTM/VTM • Swabs not listed on the Approved Collection List • Specimens exceeding stability • Quantity Not Sufficient • Specimens from leaking, uncapped or broken container	

Criteria	
Compromising Physical Characteristics	None
Other Considerations	None

NOTE: Labeling requirements for all reagents, calibrators and controls include: (1) Open date, (2) Substance name, (3) Lot number, (4) Date of preparation, (5) Expiration date, (6) Initials of tech, and (7) Any special storage instructions. Check all for visible signs of degradation.

4. REAGENTS

The package insert for a new lot of kits or reagents must be reviewed for any changes before the kit is used.

4.1 Reagent Summary

Reagents / Kits	Supplier & Catalog Number
SARS-CoV-2 & Influenza A&B, 20/Kits EA	Roche Supplier #09211128190 PS # 209349

4.2 Reagent Preparation and Storage

Reagents in the cobas® SARS-CoV-2 & Influenza A/B Reagent Tube	
Reagent 1	cobas® Liat® SARS-CoV-2 & Influenza A/B Master Mix-1
Reagent 2	cobas® Liat® SARS-CoV-2 & Influenza A/B Master Mix-2
Reagent 3	cobas® Liat® SARS-CoV-2 & Influenza A/B Master Mix-3
Reagent 4	cobas® Liat® Internal Process Control
Reagent 5	Proteinase K
Reagent 6	cobas® Liat® Magnetic Glass Particles
Reagent 7	cobas® Liat® Lysis Buffer
Reagent 8	cobas® Liat® Wash Buffer
Reagent 8	cobas® Liat® Elution Buffer
Storage	Store at 2-8°C
Stability	Before use, reagent is stable until the expiration date indicated on label. Once opened, the reagent tube should be used immediately (patient or QC material added). The Reagent tube with the Patient/QC sample added may be stored up to 4 hours at Room Temperature before testing.

5. CALIBRATORS/STANDARDS

Not applicable

6. QUALITY CONTROL

6.1 Controls Used

Quality Control	Supplier & Catalog Number
CTRL, SARS-COV-2, INFLUENZA A&B ASSAY, (3 sets of Positive & Negative Controls)	Roche, Sup # 09211128190 PS# 209349

6.2 Control Preparation and Storage

Control Kit Components:	
Control -Pos	cobas® SARS-CoV-2 & Influenza A/B Positive Control for SARS-CoV-2 (+) C
Control -Pos	cobas® SARS-CoV-2 & Influenza A/B Positive Control for FLU A/B (+) C
control -Neg	cobas® Dilution UTM Dilution UTM (-) C
Storage	2–8°C
Stability	Before use, stable until the expiration date indicated on label.
Preparation	Ready to use (liquid in tubes)

6.3 Number and Frequency

	QC Frequency
1	**Internal Control:** An Internal Process Control (IPC) is included in every assay. It verifies the adequate processing of the sample. Results of the IPC are not reported, but failure of the IPC will result in an Invalid test result.
2	**External Control:** External QC (both positive and negative controls) must be run: 1. At new instrument Set-up 2. For each new lot number (see Lot Validation) 3. For each new shipment to a site. 4. Every 31 Days **Note**: QC run for Lot Validation or New shipments can fulfill the 31-Day QC requirement as well. The frequency for the 31-Day QC requirement re-starts with the completion of the Lot Validation or New shipment QC.

6.4 Tolerance Limits and Criteria for Acceptable QC

A. Tolerance Limits

Tolerance Limits	
1.	Internal Process Control is performed on each test. Failure to yield a valid Internal Process Control indicates the test is invalid, and patient results cannot be resulted. If the Internal Process Control is acceptable, the Run Status will be listed as "OK."
2.	External Control runs must produce the expected result (positive or negative) prior to running patient samples. **Notes**: • For controls run during new lot testing, the report will display result as 'valid' or 'invalid' • For controls run to satisfy the 31-day frequency, the report will display individual results for all 3 targets. • For either QC scenario, results are documented on the QC log as 'valid' or 'invalid'

B. Criteria for Acceptable QC
- Each test must produce a valid Internal Process Control result.
- The patient test is valid if no flags appear for both controls, which include one negative control and one positive control: [SARS-CoV-2(+) C] and [BUF (-) C].
- If the negative control or the positive controls are flagged as Invalid, then the patient result is invalid and must be repeated.
- Controls and patient data must be reviewed for acceptability and for atypical or unexpected results or trends prior to reporting patient results.
- DO NOT release results from runs with unacceptable controls or with unusual patterns, trends or distribution in patient values.

C. Corrective Action
- All rejected runs must be effectively addressed and include the following documentation:
  - Control(s) that failed (e.g., positive control with negative result or invalid controls) and/or atypical or unexpected patient results
  - Actions taken
  - Statement of what was done with the patient samples from the affected run/batch,
  - Date and initials of the person recording the information.
- Patient samples in failed analytical runs must be reanalyzed.

NOTE: The laboratory director or designee may override rejection of partial or complete runs. Justification for the override must be documented in detail.

6.5 Documentation

- Record all Quality Control results (failed and successful) manually or electronically.

- Quality control records are reviewed daily at the bench, weekly by the Group Lead or designee, and monthly by the Supervisor/Manager or designee.
- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.
- Refer to Quest Diagnostics Records Management Program for Quality Control record retention requirements.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

- cobas® Liat® Analyzer

7.2 Equipment

N/A

7.3 Supplies

- VTM/UTM tubes (from collection kit)
- cobas® SARS-CoV-2 & Influenza A/B Assay tubes
- cobas® SARS-CoV-2 & Influenza A/B Transfer pipettes
- Rack

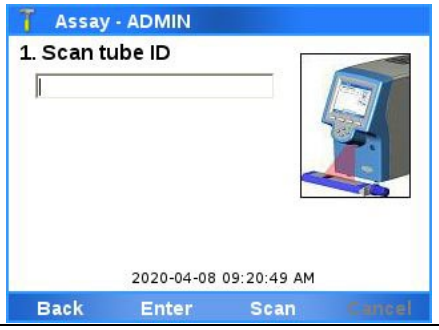
8. PROCEDURE

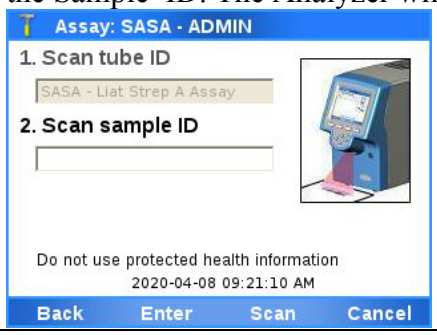
NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

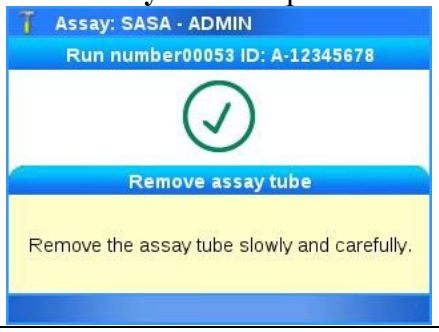
8.1	Instrument Set-up Protocol
1.	For initial set-up, refer to pages 33-38 of the cobas® Liat® System User Guide, and other sections as needed. 1. Ensure that the **cobas**® Liat® Analyzer is powered on. 2. Select **"Login"** on the screen of the **cobas**® Liat® Analyzer. 3. Enter user name when prompted, 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 or Operator's Manual).* 5. From the Main Menu, select "**Run Assay**".

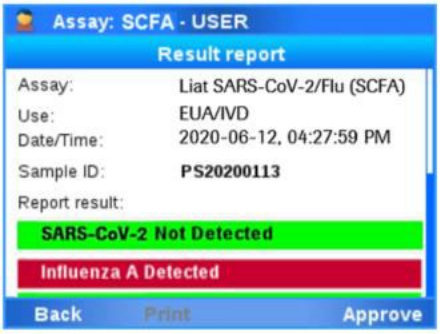
8.1	Instrument Set-up Protocol
2.	**<u>For set-up or New LOT # of REAGENT</u>:** At Set-up or before using a new lot of **cobas®** SARS-CoV-2 & Influenza A/B assay tubes, a Lot Validation procedure must be performed on the **cobas®** Liat® Analyzer to validate the **cobas®** SARS-CoV-2 & Influenza A/B assay tube lot. The procedure includes running a Negative Control sample and a Positive Control sample. **Note:** Refer to **Addendum 1** for detailed instructions (or see the **cobas®** Liat® System User Guide

8.2	Running QC (30-Day/New Shipment)
1.	Hold a tube of Control upright and lightly tap on a flat surface to collect liquid at the bottom of the tube. Visually check that the Dilution UTM-RT® has pooled at the bottom of the tube.
2.	Open up a **cobas®** SARS-CoV-2 & Influenza A/B assay tube foil pouch (from the lot to be added) and remove the contents.
3.	Use the transfer pipette provided in the pouch to add the 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.***
4.	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.
5.	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 assay tube and the transfer pipette, and restart the testing procedure with a new cobas® assay tube and pipette.***
6.	Screw the cap back onto the **cobas®** SARS-CoV-2 & Influenza A/B assay tube. Dispose of the transfer pipette as biohazardous material.
7.	Select **Scan** and scan the ***cobas®*** *Liat® tube barcode* on the Tube sleeve by placing the **cobas®** Liat® tube on the table and sliding the tube towards the Analyzer until the red scan light is over the entire barcode. The assay is determined by the barcode scanned.
8.	For New shipment: Select **Scan** again and scan the *QC barcode (provided in the QC Kit).* The Analyzer will prompt **"Add UTM sample & re-scan tube ID**
9.	Continue with steps 5-18 in section 8.4
10.	Refer to section 10.1 for interpreting the QC results.
11.	QC results must be documented in the Quality Control Documentation form

8.3	Test Runs
1.	Minimum of 200 µL sample volume is required for the cobas® Liat® SARS-CoV2 and FLU A/B assay. The test procedure is described in detail in the cobas® Liat® PCR System Operator's Manual, pages 77- 92. The procedure is summarized below. Quality Control material for the 30-day requirement or Competency/training is run in the same manner as patient samples. (see section 8.2)
2.	**Prepare analyzer:** Press the **Power** button. At **logon,** the analyzer prompts you for your user ID and password, for your badge barcode and password, or for your badge barcode only. Select **Run Assay** on the main menu using the touch screen or function button. And press the Select button.  
3.	Select **Scan** and scan the ***cobas®*** *Liat® tube barcode* on the Tube sleeve by placing the **cobas®** Liat® tube on the table and sliding the tube towards the Analyzer until the red scan light is over the entire barcode. The assay is determined by the barcode scanned. 

8.3	Test Runs
4.	Select **Scan** again and scan the *Patient or Sample barcode*, or select **Enter** and type in the Sample ID. The Analyzer will prompt **"Add UTM sample & re-scan tube ID** 
5.	Unscrew the UTM sample tube cap. Lift the cap and any attached swab to allow a pipette to be inserted into the sample tube. Avoid lifting the swab completely out of the sample tube.
6.	Obtain the transfer pipette from the **cobas®** Liat® tube pouch. Firmly squeeze the bulb of the pipette until the bulb is fully flat.
7.	While holding the pipette bulb fully flat, insert the pipette tip into the sample below the liquid surface.
8.	Slowly release the bulb while keeping the pipette tip below the liquid surface, drawing up ~200 µL of sample. After releasing the bulb completely, withdraw the pipette from the sample.
9.	Unscrew the cap from the **cobas®** Liat® tube.
10.	While watching through the viewing window in the sleeve, carefully insert the pipette into the **cobas®** Liat® tube. Place the pipette tip near the bottom of the sample compartment. 
11.	Slowly squeeze the bulb to empty the contents of the pipette into the **cobas®** Liat® tube. Do not release the pipette bulb. **Note:** Do not puncture the **cobas®** Liat® tube or the seal at the bottom of the sample compartment. If either of these is damaged, discard both the **cobas®** Liat Tube and the transfer pipette, and restart the testing procedure with new assay components
12.	While still holding the pipette bulb, withdraw the pipette from the tube. Screw the cap back on the **cobas®** Liat® tube. Dispose of the transfer pipette. **Note:** Start the **cobas®** Liat® assay run as soon as possible, but no later than 4 hours after adding the sample to the **cobas®** Liat® tube.

8.3	Test Runs
13.	Select **Scan** and re-scan the **cobas®** Liat® tube barcode. The tube entry door on top of the Analyzer will open automatically. 
14.	Remove the **cobas®** Liat® tube sleeve, and immediately insert the tube into the Analyzer until the Tube clicks into place. The Tube only fits in one way. **Note:** If the **cobas®** Liat® tube is not inserted by the time the door closes, re-scan the Tube barcode (step 3a) and insert the Tube again. 
15.	Once the **cobas®** Liat® tube is properly inserted, the Analyzer will close the door automatically and begin the test. (During the test, the Analyzer displays the running status and estimated time remaining)
16.	Once the test is complete, the Analyzer displays the message, **"Please remove the tube slowly…"** and opens the tube entry door automatically. 

8.3	Test Runs
17.	Lift the Tube out of the Analyzer 
18.	Select **Report** to see the Result Report.  Review the results on screen (use the down arrow key to scroll to see all results.
19.	To approve results: Select "**Approve"**, then select "**Release"**, then "**Yes"**.
20.	Print results by selecting "**Print"**, then select "**Confirm"**.
21.	Record the results in Sunquest
22.	Select "**Back"**, and then "**Main"** to return to the main menu for the next test.

NOTE: In the event that the test system becomes inoperable, notify supervision or designee for further direction. Patient specimens must be stored in a manner that maintains the integrity of the specimen.

9. CALCULATIONS

None

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

Interpreting QC Material:

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.

Interpreting Patient Results

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.

Result Report		Interpretation
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.

10.2 Units of Measure

N/A

10.3 Analytical Measurement Range (AMR)

N/A

10.4 Review Patient Data

- Review patient results for unusual patterns, trends or distribution.
- Report atypical or unexpected results or trends for this test to appropriate supervisory personnel, prior to releasing results.

10.5 Repeat Criteria and Resulting

- **see Addendum 2 for Sunquest reporting instructions**

IF the result is …	THEN…
Not detected	Result as **NTD** (Not Detected)
Detected	Result as **DET** (Detected)

IF the result is …	THEN…
Invalid / Error	**Repeat the assay with the same UTM sample and a new *cobas*® *Liat® tube*:** If valid results are obtained on the repeat run, record on the QC Log and indicate the original Invalid/Error was repeated and that valid results were obtained upon repeat. If the second result remains Invalid/Error (or if the original specimen was QNS for repeat), obtain a new nasal/nasopharyngeal specimen from the patient and repeat the test. If the patient is not available for re-collection OR the recollected specimen still results as Invalid/Error, indicate on the QC Log that the test was confirmed as INVALID. Resulting repeat INVALID results: Report as **INVD** Add comment **INVLT** - this code translates to: *Unable to report. Repeat analysis of this specimen yielded invalid, inconsistent or unacceptable results.*"

11. EXPECTED VALUES

11.1 Reference Intervals

Not Detected

11.2 Critical Values

None

11.3 Standard Required Messages

GLIAT - Performed on Cobas Liat

Please review the "Fact Sheets" and FDA authorized labeling available for healthcare providers and patients using the following websites:
https://www.fda.gov/media/142191/download
https://www.fda.gov/media/136048/download

This test has been authorized by the FDA under an Emergency Use Authorization(EUA) for use by authorized laboratories.

12. CLINICAL SIGNIFICANCE

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.

13. PROCEDURE NOTES

- **FDA Status:** Emergency Use Authorization (EUA)
- **Validated Test Modifications:** None

14. LIMITATIONS OF METHOD

- 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-RT System (UTM-RT) or BD™ Universal Viral Transport System (UVT) or Thermo Fisher™ Scientific Remel™ media. Testing of other sample types may lead to inaccurate results.
- Results from analytical studies show potential for competitive inhibition of lower titer influenza in specimens with higher titer SARS-CoV-2 also present. Consider further investigation of negative influenza results if a co-infection is suspected and detection of influenza would change clinical management.
- 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 software version 3.2, refer to Operator's Manual). If spills occur on the **cobas**® Liat® Analyzer, follow the appropriate instructions in the **cobas**® Liat® System User Guide (if software version 3.2, refer to Operator's Manual) to clean.
- 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.
- Detection of SARS-CoV-2 RNA may be affected by sample collection methods, patient factors (e.g., presence of symptoms), and/or stage of infection.
- As with other tests, negative results do not preclude SARS-CoV-2, Influenza A or Influenza B, infection and should not be used as the sole basis for treatment or other patient management decisions.
- 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® Liat® SARS-CoV-2 to help identify the specimens containing substances that may interfere with nucleic acid isolation and PCR amplification. These samples will result as Invalid.

14.1 Precision

Refer to package insert

14.2 Interfering Substances

Refer to package insert

14.3 Clinical Sensitivity/Specificity/Predictive Values

Refer to Package Insert

15. SAFETY

Refer to your local and corporate safety manuals and Safety Data Sheet (SDS) for detailed information on safety practices and procedures and a complete description of hazards.

16. RELATED DOCUMENTS

- Biological Safety Cabinet, Micro procedure
- Laboratory Quality Control Program

- Laboratory Safety Manual
- Safety Data Sheets (SDS)
- cobas® Liat® Systems User Guide Version 9.0 Software Version 3.3, rev Oct 2019
- cobas® Liat® PCR System Operator's Manual, Publication version 9.0
- cobas® SARS CoV-2 & Influenza A/B IFU, Rev 1.0
- Liat SARS-CoV-2 / FLU A&B PCR Quality Control Log (AG.F566)

17. REFERENCES

1. Current package insert for cobas® Liat® SARS-CoV-2 & Influenza A/B Test for use on the cobas® Liat® Systems.
2. QDMD738 – Molecular Best Practices.
3. Clinical and Laboratory Standards Institute (CLSI) MM19-A. Establishing molecular testing in clinical laboratory environments; approved guideline. CLSI Document MM-19A: Wayne, PA; CLSI 2011.
4. Center for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories, 5th ed. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention. National Institutes of Health HHS Publication No. (CDC) 21-1112, revised December 2009.
5. Clinical and Laboratory Standards Institute (CLSI). Protection of laboratory workers from occupationally acquired infections. Approved Guideline-Fourth Edition. CLSI Document M29-A4: Wayne, PA; CLSI, 2014

18. DOCUMENT HISTORY

Version	Date	Section	Revision	Revised By	Approved By
1	1/11/21	6.4	Added notes to clarify ext. QC results	L Barrett	R Master

19. ADDENDA

Addendum	Title
1	Lot Validation Procedure
2	Reporting Results in Sunquest
3	Instrument Maintenance

Addendum 1

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:

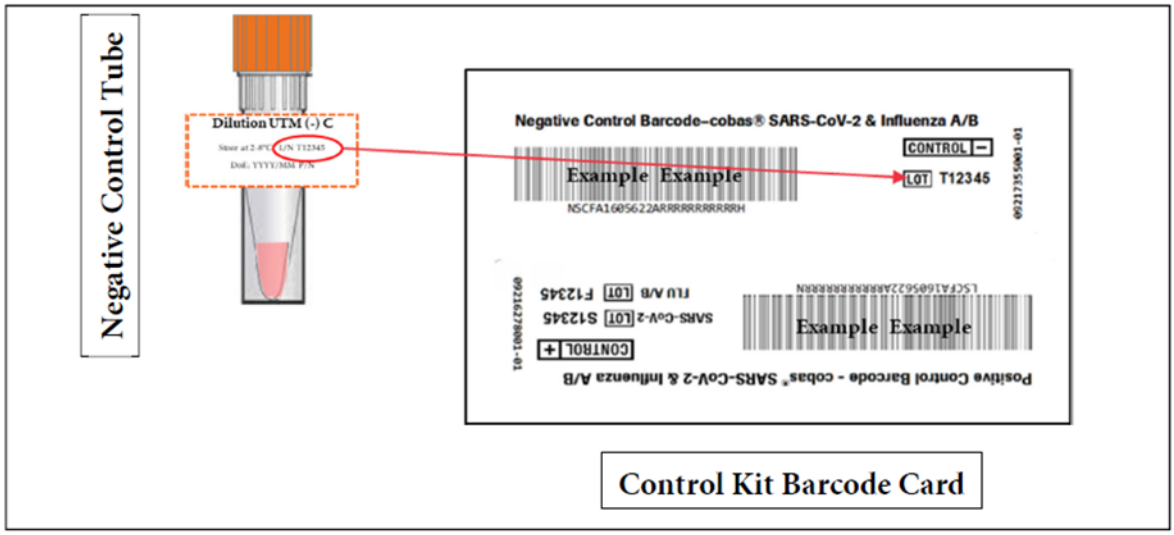
- Negative Control: Negative Control Barcode (see Control Kit Barcode Card), 1 Dilution UTM-RT® tube (used as the negative control sample)
- Positive Control: Positive Control Barcode (see Control Kit Barcode Card), 1 **cobas**® SARS-CoV-2 Positive Control tube, 1 **cobas**® Influenza A/B Positive Control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

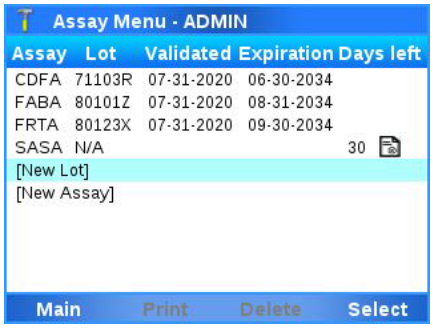


Assay tube Lot Validation workflow

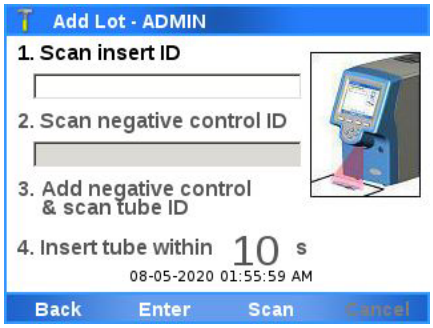
1. Press the power on/off button to start the **cobas**® Liat® Analyzer.
2. Select **"Login"** on the screen of the **cobas**® Liat® Analyzer.
3. Enter user name when prompted, select "**OK**".
4. Enter user password when prompted, select "**OK**".

   Note: You may be prompted to confirm you have read the User Manual (i.e., cobas® Liat® System User Guide or Operator's Manual).
5. Select "**Assay Menu**" on the main menu of a **cobas®** Liat® Analyzer.

6. Select "**New Lot**" at the bottom of the list.

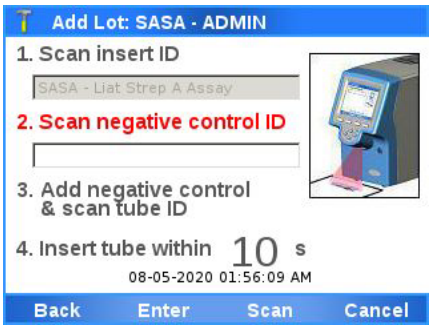


7. When prompted to **Scan the Insert ID,** select "**Scan**" and scan the **cobas®** SARS-CoV-2 & Influenza A/B Package Insert ID Barcode card. Ensure that the red scan light is over the entire barcode.

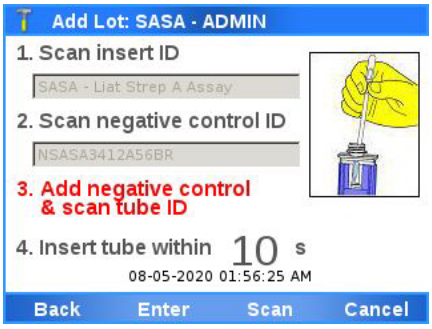


Note: You may be prompted to confirm you have read Instructions For Use.

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-RT® 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.

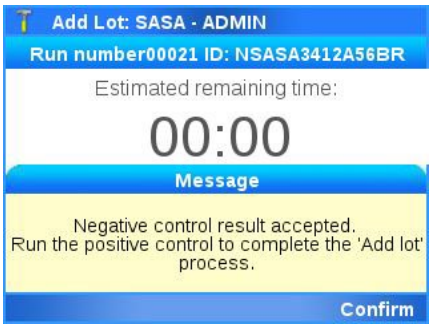
16. Remove the **cobas®** SARS-CoV-2 & Influenza A/B assay tube sleeve and immediately insert the **cobas**® SARS-CoV-2 & Influenza A/B assay tube into the **cobas®** Liat® Analyzer until the tube clicks into place.

    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 software version 3.2, select **"OK")**. 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.



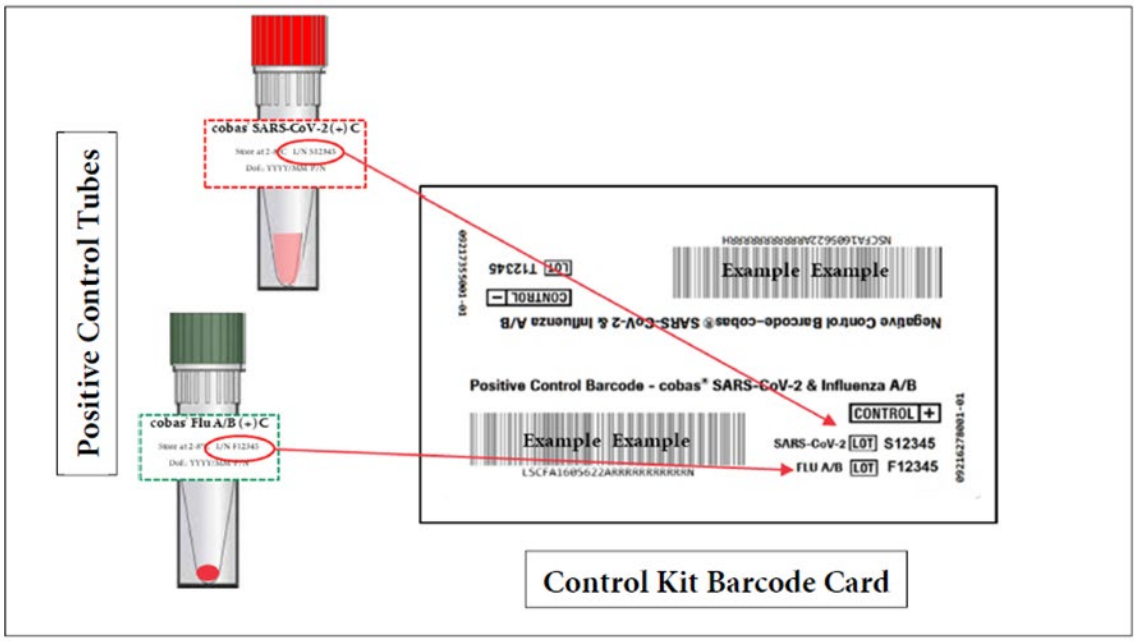
20. Select **"Confirm"** to proceed with the **cobas®** SARS-CoV-2 & Influenza A/B Positive Control test on the same instrument.
21. Prepare positive control sample as follows.

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

Materials needed:

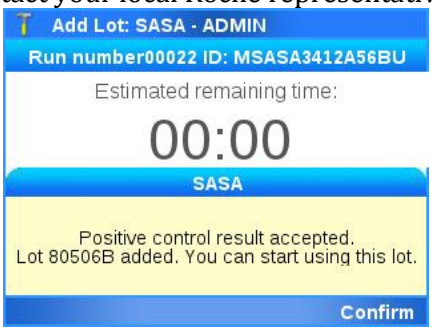
- 1 transfer pipette (Use only transfer pipettes contained in the **cobas®** SARS-CoV-2 & Influenza A/B Quality Control Kit)
- 1 **cobas®** SARS-CoV-2 Positive Control
- 1 **cobas®** Influenza A/B Positive Control (pellet comprising dried positive control material at bottom of tube)

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( ) 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.

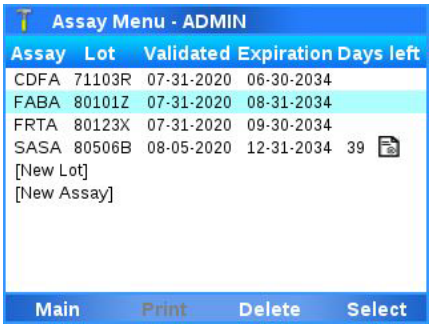


1. After opening **cobas**® Influenza A/B Positive Control pouch, discard desiccant packet.
2. After opening **cobas**® SARS-CoV-2 Positive Control pouch, hold the tube upright and lightly tap on a flat surface to collect liquid at the bottom of the vial. Visually check that the liquid has pooled at 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 <u>is not visible</u> 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.



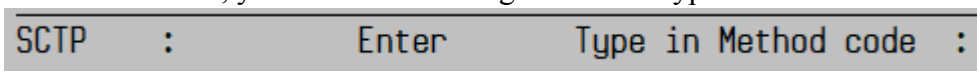
8. Select "**Assay Menu**" to verify that the new lot has been added.



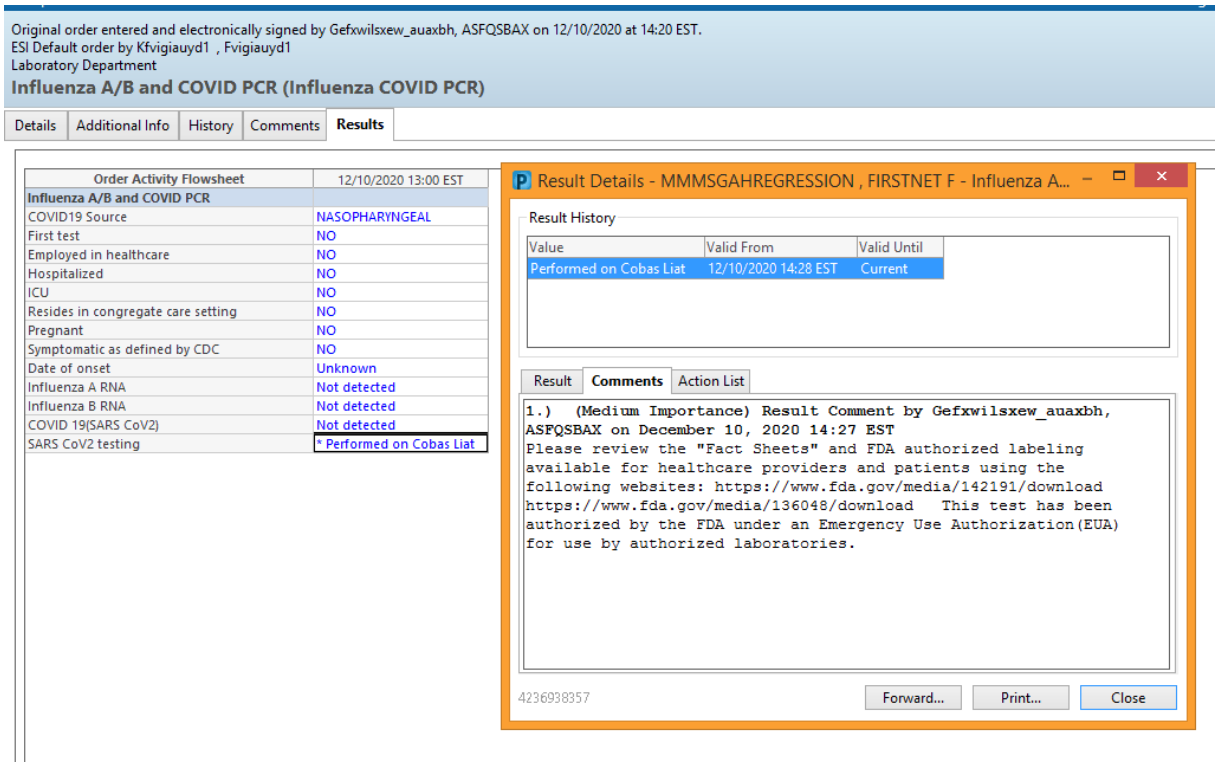
Addendum 2

Resulting Cobas Liat in Sunquest:

1. Smarterm
   a. Function: MEM
   b. Worksheet: GIM2
   c. TEST-1: IFCVD
   d. ACC. NO.: Type in the Accession number
   e. Type in results for
      INFUA
      INFUB
      COVNT
   f. At the SCTP test, you will see a message "Enter Type in Method code:"



   g. Type in the Cobas Liat method code **GLIAT.** This will add the EUA documentation to the results.
   h. Review results with instrument print out. If results on printout match Sunquest, then accept the results in Sunquest. Results will now transmit to Cerner.
   Examples
      Cerner:



      Sunquest Inquiry:

H1407 COLL: 12/10/2020 13:00 REC: 12/10/2020 14:20 PHYS: Znaicisyhp6,Nai

Influenza COVID PCR
COVID 19 SOURCE NASOPHARYNGEAL
First Test NO
Employee in Healthcare NO
Hospitalized NO
ICU NO
Resident in congregate ca NO
Pregnant NO
Symptomatic as defined by NO
Date of onset Unknown
Influenza A RNA Not detected
[NTD]
Influenza B RNA Not detected
[NTD]
COVID 19 Not detected
[NTD]
SARS CoV2 testing Performed on Cobas Liat
Please review the "Fact Sheets" and FDA authorized labeling available for healthcare providers and patients using the following websites:
https://www.fda.gov/media/142191/download
https://www.fda.gov/media/136048/download

This test has been authorized by the FDA under an Emergency Use Authorization (EUA) for use by authorized laboratories.

Addendum 3

cobas Liat Instrument Maintenance

Cleaning the analyzer

NOTICE!
Using unsuitable cleaning materials can damage the touch screen and other surfaces.
• Do not use harsh, abrasive cleaners or wipes.
• **Do not use isopropanol or bleach solution to clean the touch screen.**

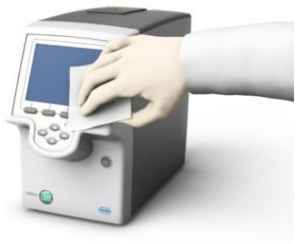
• Monthly check the rear vent and bottom of the analyzer for excessive dust or debris.

• When prompted by the message "Use cleaning tool" on the screen, use the provided cleaning tool following the instructions included with the cleaning tool kit.
• **Only use the supplied cleaning tool when prompted by the message**. Do not attempt to clean the tube chamber of the analyzer with anything other than the cleaning tool kit.

Cleaning the outside of the analyzer

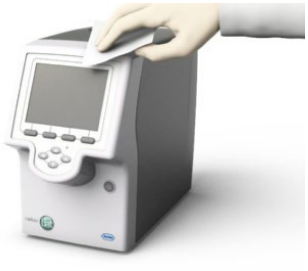
To clean the touch screen

Keep the touch screen clean from excessive fingerprints and moisture by gently wiping it with a soft, lint-free cloth.
• **Do not use isopropanol or bleach solution to clean the touch screen.**



To clean the front buttons and the exterior of the analyzer

The exterior of the analyzer and front buttons can be cleaned using a soft lint-free cloth moistened with 70% isopropanol.
1. Moisten a lint-free cloth with either 70% isopropanol
2. Gently wipe the surfaces as required.



Cleaning spillages or leakages from an assay tube

In the unlikely event of a spillage or leak of an assay tube, special precautions apply because the tube contains various potentially biohazardous materials and hazardous chemicals.

CAUTION!

Contamination of cleaning tool

Do not use the cleaning tool for biohazardous contamination. The cleaning tool does not decontaminate the analyzer but becomes contaminated itself.

1. Dispose of the assay tube in accordance with the disposal policy of your institution and according to local regulations.
2. If the leak happened inside the analyzer, stop using the analyzer and immediately contact your Roche representative for further instructions.
3. Follow laboratory best practices.
   - Follow Good Laboratory Practice for working with biohazardous materials and hazardous chemicals.
   - Refer to the appropriate assay tube Safety Data Sheet and package insert or the Instructions for Use for assay-specific information.