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| **SUBJECT: SARS-Cov-2 & Influenza A/B Roche Liat (FDA-510K)** |

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| **1. PRINCIPLE:** |
| 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. |

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| **2. 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.  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. |

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| **3. SPECIMEN:**  **A. COLLECTION AND PROCESSING:** |

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

**Patient samples may be collected using a nasal swab OR a nasopharyngeal swab.**

**Nasopharyngeal Sample Collection Procedure:**

**Important:  Wear a mask and eye protection when performing this procedure. An appropriately placed splash shield may be used alternatively to eye protection.**

1. Gather the following items that you need to collect your nasopharyngeal sample:

a. Gloves

b. Tube containing 3 mL Universal Transport Media (3 mL) or 3ml 0.9% physiological saline

c. Appropriate sterile swab

d. Nasal tissues

2. Identify the patient according to the established protocols.

3. Explain the purpose and steps of the procedure to the patient.

4. Wash hands and don gloves.

5. Ask the patient to blow his/her nose into a tissue.

6. Place the patient in a seated position with head against a fixed object (e.g., a wall) to prevent patient from pulling away during the sample collection procedure.

7. Tilt the patient’s head back at 70-degree angle.

8. Open the sterile swab, insert it into one nostril and aim straight back (not upwards). Continue along the floor of the nasal passage for several centimeters until reaching the nasopharynx region (resistance will be met). Do not force the swab. If an obstruction is met, before reaching the nasopharynx, remove the swab and try the other nostril.

Note: The distance from the nose to the ear gives an estimate of the distance the swab should be inserted.

9. Gently rotate the swab for 5-10 seconds to loosen epithelial cells.

10. Remove the swab and immediately insert the swab into the transport media tube, placing the swab head at least ½ inch below the surface of the media.

11. Break the swab shaft and leave the swab in the tube. Attach the cap securely.

12. Label the specimen according to established policies.

13. Remove and discard gloves and wash hands according to established policies and procedures.

**Nasal Sample Collection Procedure:**

**Important: Wear a mask and eye protection when performing this procedure.**

1. Using a flocked or spun polyester swab, insert the entire absorbent tip of the swab (usually ½ to ¾ of an inch (1 to 1.5 cm) inside the nostril and firmly sample the nasal wall by rotating the swab in a circular path against the nasal wall at least 4 times.

2. Take approximately 15 seconds to collect the sample.

3. Be sure to collect any nasal drainage that may be present on the swab.

4. Sample both nostrils with same swab.

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| **B. REJECTION:**  The following are rejection criteria for nasopharyngeal samples:   * Improper transport media * Leaky transport tube * Improper storage of sample * Incorrect swab used for sample collection * Mislabeled sample * Improper sample collection |

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| **C. STORAGE AND PRESERVATION:** |

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| The following are guidelines for storage and handling of nasopharyngeal samples:  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.  All applicable transport regulations should be met regarding transportation of etiologic agents. |

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| **4. REAGENTS, STANDARDS, AND CONTROLS:**  **A. REAGENTS:**  1. cobas® Liat® assay Quality Control Kit  2. Lot-specific Negative Control Barcode Card from the QC kit  3. Lot-specific Positive Control Barcode Card from the QC kit  4. Two cobas® Liat® assay tubes with sleeves on from the test kit you are using (leave the sleeve on the tube until you are ready to insert the tube into the analyzer tube slot)  5. 200 µL pipettes from the QC kit and the cobas® Liat® assay tube pouch  6. One sample dilution medium tube from the QC kit (UTM).  7. One positive control tube containing a pellet of dried chemically-inactivated Influenza A/B and one positive control tube containing liquid SARS-Cov-2 control.  8. Operational cobas® Liat® Analyzer |

**Do not mix components from one kit with another.**

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| **B. CONTROL PROCEDURE:** |

External Quality Control Testing Procedure

**Prepare a negative control sample**.

1. Take a UTM dilution medium tube from the QC kit.  The dilution tube serves as the negative control sample.

2. Hold the dilution tube by the tube cap and shake down the liquid using a quick, sharp, downward wrist motion (as if shaking down a mercury thermometer). Visually check that all of the liquid is at the bottom of the tube. If not, repeat the shakedown procedure.

**Prepare a positive control sample**

1. Take a UTM dilution tube from the QC kit.

2. Hold the dilution tube by the tube cap and shake down the liquid using a quick, sharp, downward wrist motion (as if shaking down a mercury thermometer) to pool the liquid at the bottom of the tube. Visually check that all of the liquid is at the bottom of the tube. If not, repeat the shakedown procedure.

3. Take a Positive FLU A/B Control tube and a Positive SARS-Cov-2 Control tube from the QC kit:

4. Using a transfer pipette, gently transfer all the liquid from the SARS-Cov-2 tube into the bottom of the Positive Flu A/B Control Tube. Avoid creating bubbles in the sample.

5. Recap the Positive Control Tube containing the mixture of FLU A/B and SARS-Cov-2.

6. Holding the tube by the cap, shake down the liquid in the tube using a quick, sharp, downward wrist motion.

7. Allow the Positive Control Tube to sit for 5 minutes as the dried material within the tube dissolves.

8. After the positive control has sat for 5 minutes, use another transfer pipette from the cobas® Liat® assay tube pouch, slowly pipette the sample up and down 10 times to dissolve and mix the positive control sample.

**Test External Control Samples**

1. Select Run Assay from the Main Menu.

2. Select Scan and scan the cobas® Liat® assay tube barcode on the tube sleeve by placing the tube on the table and sliding the tube towards the analyzer until the red scan light is over the entire barcode.

3. Select Scan again and scan the appropriate negative or positive control barcode on the control kit barcode card (match the lot number of the sample tube label to the lot number of the control barcode label on the Control Kit Barcode Card).

Example: 

4. Obtain a transfer pipette from the cobas® Liat® assay tube pouch or QC kit. Firmly squeeze the bulb of the pipette until it is fully flat.

5. While holding the pipette bulb fully flat, insert the pipette tip into the sample below the liquid surface.

6. Slowly release the bulb while keeping the pipette tip below the liquid surface, drawing up the sample. After releasing the bulb completely, withdraw the pipette from the sample.

7. Unscrew the cap from the cobas® Liat® assay tube.

8. While watching through the viewing window in the sleeve, carefully insert the pipette into the tube placing the pipette tip near the bottom of the sample compartment.

9. Slowly squeeze the bulb to empty the contents of the pipette into the sample compartment of the tube. Do not release the pipette bulb.  Note: Do not puncture the tube or the seal at the bottom of the sample compartment. If either of these is damaged, discard both the tube and the transfer pipette and restart the testing with new assay components.

10. While still holding the pipette bulb flat, withdraw the pipette from the tube. Screw the cap back on the tube.

11. Scan the tube sleeve barcode again. The tube entry door on top of the analyzer will automatically open.

12. Remove the cobas® Liat® assay tube sleeve and immediately insert the tube into the tube entry slot until it clicks into place. The tube only fits in one way. If the tube is not inserted by the time the door closes, re-scan the cobas® Liat® assay tube barcode and insert the cobas® Liat® assay tube again. Once the tube is inserted the door will close automatically and the assay will begin.

13. When the test is complete, the cobas® Liat® Analyzer screen displays the message “Please remove the tube slowly.”

14. Lift the tube out of the cobas® Liat® Analyzer and discard following all applicable biohazard safety precautions.

15. Touch or click Report after completion of the assay.

**Negative Control Results:** The Report Result must be SARS-Cov-2 and Influenza A and B not detected for the negative control to pass.

**Positive Control Results:** The Report Result must be SARS-Cov-2 and Influenza A and B detected for the positive control to pass.

16. Select Back.

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| **5. PROCEDURE:**  **PERFORMANCE:**  1. Before testing, the following items are required as part of a modified PPE Practice  a. Testing cartridges should be loaded in a Biocontainment Hood.  Standard PPE (Non-permeable laboratory coat, gloves)  b. Alternatively, when a Biocontainment Hood is not used, operator should additionally utilize eye covering, either a face shield or approved safety goggles.  Gather the following items:  a. cobas® Liat® SARS-Cov-2 & Influenza A/B assay tube (from a lot that has been validated and entered into the system)  b. cobas® Liat® Transfer pipette (in cobas® Liat® reagent box with tubes)  c. Appropriately collected patient sample  d. Biohazard container  e. Operational cobas® Liat® Analyzer  2. Scan the barcode:  a. Tear open the foil packaging of the cobas® Liat® Tube and remove the tube and the transfer pipette.  **Note:** Do not use a damaged cobas® Liat® assay tube. Do not open individual tube packaging until you are ready to perform testing. Each single-use cobas® Liat® assay tube is used to process one test. Do not attempt to reuse a spent cobas® Liat® assay tube.  b. Select Run Assay on the Main Menu.  c. Select Scan and scan the cobas® Liat® assay tube barcode on the tube sleeve by placing the tube on the table and sliding the tube towards the analyzer until the red scan light is over the entire barcode.  d. Select Scan again and scan the Patient or Sample barcode, or select Enter and type in a Sample or Patient ID. The screen will then prompt you to add the sample to the assay tube and rescan the tube.  3. Add the sample to the assay tube:  a. Unscrew the cap from the cobas® Liat® assay tube. Do not discard the cap.  b. Hold the swab sample tube on its ends with the thumb and forefinger; vigorously shake the tube for 5 seconds to evenly disperse the patient specimen in the liquid medium.  c. Unscrew the sample tube cap. Hold the tube between the thumb and ring finger and hold the cap between the index finger and middle finger. Lift the cap and any attached swab to allow a pipette to be inserted into the tube. Avoid lifting the swab completely out of the tube.  d. Firmly squeeze the bulb of the pipette until the bulb is fully flat.  e. While holding the pipette bulb fully flat, insert the pipette tip into the sample just below the liquid surface.  f. Slowly release the bulb while keeping the pipette tip below the liquid surface. You will see the liquid sample rising into the pipette. After releasing the bulb completely, withdraw the pipette from the sample. This will deliver ~200 μL of sample into the tube.  **Note:** You do not need to measure the sample volume; the cobas® Liat® Analyzer will adjust the sample volume if more sample was loaded or output an error if not enough sample was loaded.  g. Insert the pipette into the cobas® Liat® assay tube.  h. Place the pipette tip near the bottom of the sample compartment. Do not puncture the tube or the seal at the bottom.  i. Slowly squeeze the bulb to empty the contents of the pipette into the cobas® Liat® assay tube.  **Note:** Avoid creating bubbles in the sample. Do not release the pipette bulb.  j. While still squeezing the pipette bulb, withdraw the pipette from the tube. Screw the cap back on the tube.  4. Insert the cobas® Liat® assay tube into the analyzer and run test.  a. Select Scan and re-scan the cobas® Liat® assay tube barcode. The tube entry door on top of the analyzer will open automatically.  b. Remove the cobas® Liat® assay tube sleeve.  c. Immediately insert the cobas® Liat®assay tube into the analyzer until the tube clicks into place. The cobas® Liat® assay tube only fits in one way. If the tube is not inserted by the time the door closes, re-scan the cobas® Liat® assay tube barcode and insert the cobas® Liat® assay tube again. Once the cobas® Liat® assay tube is properly inserted, the cobas® Liat® Analyzer will close the door automatically and begin the test.  d. During the test, the analyzer screen displays the running status and estimated time remaining. Once the test is complete, the analyzer displays the message, “Please remove the tube slowly…” and opens the tube entry door automatically.  e. Gently lift the cobas® Liat® assay tube out of the analyzer and discard the tube according to established procedure for disposing of biohazardous materials.  5. View the result.  a. Select Report to see the Result Report.  b. Select Print to print the report (if applicable).  c. Select Back, and then Main to return to the main menu for the next test.  6. Interpret the results.  The cobas® Liat® System reports result as “Detected”, “Not Detected”, “Indeterminate”, “Assay Invalid” or an error message.  See table below for guidance on how to interpret and follow up on various results: |
| **6. INTERPRETATION OF RESULTS:**  A. Results are reported through the cobas® Infinity POC Interface.   |  |  |  | | --- | --- | --- | | Influenza A | Influenza A Not Detected  Influenza A Detected  Influenza A Invalid. Repeat Assay. | Negative test for Influenza A (no Influenza A RNA detected)  Positive test for Influenza A (Influenza A RNA present)  Presence or absence of Influenza A cannot be determined. Repeat assay with same sample or, if possible, new sample. Do not report indeterminate. Cancel test, notify physician and send out an alternative method. | | Influenza B | Influenza B Not Detected  Influenza B Detected  Influenza B Invalid. Repeat Assay. | Negative test for Influenza B (no Influenza B RNA detected)  Positive test for Influenza B (Influenza B RNA present)  Presence or absence of Influenza B cannot be determined. Repeat assay. Do not report indeterminate. Cancel test, notify physician and send out an alternative method with same sample or, if possible, new sample. | | SARS-COV2 | SARS-COV2 Not Detected  SARS-COV2 Detected  SARS-COV2 Invalid. Repeat Assay. | Negative test for SARS-COV2 (no SARS-COV2 RNA detected)  Asymptomatic: A negative result from an asymptomatic individual is presumptive. A negative result obtained with a nasal swab collected from an asymptomatic patient should be followed up by testing at least twice over three days with at least 48 hours between tests. Negative results do not preclude SARS-CoV-2 infection.  This applies to Nasal swab collections ONLY. Nasopharyngeal specimens do not require the same follow-up process.  Positive test for Influenza B (SARS-COV2 RNA present)  Presence or absence of SARS-COV2 cannot be determined. Repeat assay. Do not report indeterminate. Cancel test, notify physician and send out an alternative method with same sample or, if possible, new sample. | | Assay Invalid. Repeat Assay. |  | Presence or absence of Influenza A, and Influenza B cannot be determined. Repeat assay with same sample or, if possible, new sample. Do not report invalid. Cancel test, notify physician and send out an alternative method. | | [Error]. Assay Abort |  | Presence or absence of target viruses cannot be determined. Repeat assay. |   D. 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. | | |

**7. QC**

**A. PERFORMANCE**

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| Internal Processing  Control (IPC) | With each control or patient test | IPC is integrated into each cobas® Liat® SARS-Cov-2 & Influenza A/B Tube. |
| External positive and  negative controls | Validation of every new cobas® SARS-Cov-2 & Influenza A/B reagent that is added to the system. | The cobas® Liat® System requires the validation of new tube lots as part of its “Add Lot” function and will not allow testing with a new tube lot without successful completion of positive and negative external control testing. |

Internal Process Control (IPC) is included in every assay tube. It verifies the adequate processing of the biological sample. The IPC passes if sample purification and target amplification meet validated acceptance criteria. This process occurs automatically with each test and requires no operator processing. Like the SARS-Cov-2 & Influenza A/B assay target, the IPC target is also evaluated in every assay run. In the case that the assay target is not detected, the IPC target must be detected for the result to be called “Not Detected”; if the IPC is also not detected or if the IPC PCR curve is abnormal, the result is called “Assay Invalid. Repeat Assay.” In some cases, high concentration of the assay target may inhibit the amplification of IPC; as such, IPC is not taken into consideration when the assay target is detected.

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| **B. FAILURE/REMEDIAL ACTION:** | |
| Troubleshooting Error Messages or Unexpected Results  1. The cobas® Liat® Analyzer monitors its operation and logs abnormal events. Based on the severity, the cobas® Liat® Analyzer tries to recover or fix errors while running. If the error is unrecoverable, the cobas® Liat® Analyzer stops. If repeating the test as suggested in the results table above does not yield a clear positive or negative result. Call the Roche Support Network Customer Support Center cobas® Liat® hotline at 1-800-800-5973.  2. Event Logs: Certain error messages are recorded in the Event Log file. To view the log, select Tools, then Event Logs. Large Event Logs might take some time to load. Viewing the Event Log requires Supervisor or higher security level. If needed, select Print to print the Event Log. See the cobas® Liat® User’s Manual for a full list of error messages and their meanings.  3. Real Time Error Messages: See the cobas® Liat® System User’s Manual for a table that lists the real-time messages and suggested troubleshooting. Certain error messages are accompanied by a unique alphanumeric error code displayed after the error messages. Please provide this code when contacting Roche Support Network Customer Support Center cobas® Liat® hotline at 1-800-800-5973.  4. Warning! Only a Roche-qualified service representative can service the system or perform preventative maintenance. Never attempt to repair or adjust the system yourself. Disassembly could result in electrical hazards. Contact the Roche Support Network Customer Support Center cobas® Liat® hotline at 1-800-800-5973 if the cobas® Liat® System fails to operate properly. | |

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| **8. EXPECTED RESULTS:**  **A. REPORTABLE RANGE:**  N/A | | | | | | | | | |
| **B. REFERENCE RANGE:**  ‘NOT DETECTED’ | | | | | | |
| **C. CRITICALVALUES:**  None | | | | |
| **9. PROCEDURAL NOTES:**  **A. BACKUP FOR INOPERABLE SYSTEM** | | | | | | | |
| Refer to St. Rita’s Medical Lab. Any specimen transported must be packaged in compliance with NVML regulations covering the transportation of etiologic agents. | | | | | | |
| **10. LIMITATIONS AND INTERFERRING SUBSTANCES:** | | | |
| When both Influenza A and Influenza B are Not Detected, these results should be considered presumptive in samples that have a positive SAR-CoV-2 result. Consider retesting with an FDA approved test if influenza detection would alter clinical management. | | | | |
| **11. METHOD VALIDATION:**  **November 2020** | | |

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| **12. REFERENCES:**  Roche Diagnostics  Engineering Operations Department  9115 Hague Road  P.O. Box 50457  Indianapolis, IN  46250-0457  USA |

**Policy Review: Deny Morlino, MT**

**Immunohematology Lead Technologist**

**Date: 6/26/2024**

**Policy Approval: Dr. Patrick C Feasel, MD**

**Laboratory Medical Director**

**Date: 6/28/2024**