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| Purpose | This procedure provides instructions for testing **Cobas**® Influenza A/B using the instrument **Cobas**® Liat System. |

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| Policy | The test will be ordered in KP HealthConnect as **INFLUENZA TYPE A AND B RNA, QL, PCR [87502A].** For results reported as “Indeterminate” or “Invalid”, the laboratory will contact the ordering provider to place an order for confirmatory testing as **RESPIRATORY PATHOGEN PANEL [17 VIRUSES, 3 BACTERIA], MULTIPLEX PCR, INPATIENT [242326].**  This will be performed on the previously collected specimen. |

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| Principle | The technology of the **cobas**® Liat System is Roche’s proprietary lab-in-a-tube platform. The **cobas**® Liat Assay Tube uses a flexible tube as a sample processing vessel. It contains all assay reagents pre-packed in tube segments separated by breakable seals. Multiple sample processing actuators in the **cobas**® Liat compress the **cobas**® Liat Assay Tube to selectively release reagents, move the sample from one segment to another, and control reaction conditions. A detection module monitors the reaction in real-time, while an on-board computer analyzes the collected data and outputs an interpreted result.  The **cobas**® Liat Influenza A/B Assay is a rapid, automated *in vitro* diagnostic test for qualitative detection and differentiation of Influenza type A and type B viral RNA. The assay is performed on the **cobas**® Liat. The **cobas**® Liat automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence in biological samples using real-time RT-PCR Nucleic acid test for use on the **cobas**® Liat System. |

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| Scope | This procedure is intended for performing the **cobas**® Influenza A/B nucleic acid test for use on the **cobas**® Liat System (**cobas**® Liat Influenza A/B). The **cobas**® Liat Influenza A/B is an automated multiplex real-time RT-PCR assay for the rapid *in vitro* qualitative detection and discrimination of Influenza A virus and Influenza B virus RNA in nasopharyngeal swab specimens from patients with signs and symptoms of respiratory infection in conjunction with clinical and epidemiological risk factors. The test is intended for use as an aid in the differential diagnosis of Influenza A and Influenza B in humans and is not intended to detect Influenza C.  Negative results do not preclude Influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions. Conversely, positive results do not rule-out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. |

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| Specimen sources | * nasopharyngeal swab specimens |

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| Specimen collection | * Sterile flexible minitip flocked swab with a synthetic tip (e.g. Dacron, nylon, or rayon) and an aluminum or plastic shaft. DO NOT use cotton or calcium alginate swabs, or swabs with wood shafts. * Tube containing 3 mL Universal Transport Media (UTM)   *Procedure:*  1. Instruct the patient to blow their nose.  2. Place the patient in a seated position with head against a fixed object (e.g. a wall) to prevent the patient from pulling away during this procedure.  3. Tilt the patient’s head back at a 70 degree angle.  4. Insert the swab into one nostril straight back (not upwards) and continue along the floor of the nasal passage for several centimeters until reaching the nasopharynx (resistance will be met).   1. The distance from the nose to the ear gives an estimate of the distance the swab should be inserted. 2. Do not force the swab, if obstruction is encountered before reaching the nasopharynx, remove the swab and try the other nostril.   5. Rotate the swab gently for 5-10 seconds to loosen the epithelial cells.  6. 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. Break the swab shaft and leave the swab in the tube. Attach the cap securely. |
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| Specimen transport | * Specimen should be sent to the laboratory immediately after collection. |

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| Specimen storage | * Specimens not tested immediately would have to be refrigerated (2-8°C) and are stable for testing for up to 72 hours. * For long duration transport or storage, specimens should be frozen at -70°C or colder and transported on dry ice. Storage at -20°C is less satisfactory than storage at 4°C or -70°C. Ensure that all applicable regulations for the transport of etiologic agents are met. |

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| Specimen rejection | Common rejected samples: Nasal wash & nasal swabs |

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| Reagents and/or Media | **Description** | **Vendor** | **Storage** |
| **cobas**® Liat Influenza A/B Pack   * Influenza A/B Assay Tube * Transfer pipette * Package Insert Barcode Card(lot specific | Roche –  Cat # 07341890190 | 2 – 8ºC |
|  | **cobas**® Liat Influenza A/B Quality Control Kit   * **cobas**® Influenza A/B Positive Control * **cobas**® Influenza A/B Dilution UTM * Transfer Pipette, * Control Kit Barcode Card (Negative & Positive) | Roche –  Cat# 07402660190 | 2 – 8ºC |

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| Materials and supplies | * Acceptable collection kits include Universal Transport Medium (UTM), Swab Collection Kits (BD Cat # 220531 or Copan Cat # 305C), each kit containing a Collection Swab and a tube containing 3 mL of UTM, or equivalent. |

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| Equipment | * Roche Cobas® Liat |

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| Safety or Special Safety Precautions | To avoid error and sample cross contamination, change gloves between samples and work on one sample at a time. DO NOT add multiple samples into multiple **cobas**® Liat Influenza A/B tubes at the same time.  ***Refer to the safety manual for general safety requirements.*** |

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| QualityControl | 1. Verify that External Quality Control (**cobas**® Liat Influenza A/B Quality Control Kit) is acceptable before testing sample patients. ***External Quality Control is only performed every new lot or new shipment.*** If the controls do not perform as expected, do not test patient specimens 2. In addition to External QC, LIAT uses an Internal Process Control (IPC). IPC is an encapsulated RNA that is included in each **cobas**® Liat Influenza A/B Tube to verify adequate processing of target viruses. The IPC verifies that sample purification of the target viruses has occurred and verifies that the specimen processing is adequate. Additionally, this control detects specimen-associated inhibition of the RT-PCR reactions.   ***Note:*** ***IPC will not show in test result report but instead will give an “Assay Invalid” result in Influenza A/B if criteria was not met during the run.*** |

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| Before you begin | * Verify that Quality Controls were performed and are acceptable before testing sample patients. * Verify that **Cobas**® Liat System is properly set up for testing and all maintenance are up to date. * For system set up consult the *C****obas****® Liat User Manual*. |

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| Verifying QC | Follow steps below to verify if External QC are acceptable   |  |  | | --- | --- | | **Step** | **Action** | |  | Select Assay Menu | |  | Select Test/Lot of tube assay being used | |  | This will display validity of QC performed for test assay on Negative and Positive Control. | | |
| External Quality Control + Add Influenza A/B Tube lot | Follow steps below to perform **adding a new Cobas® Liat Influenza A/B Tube Lot** and running **External Quality Controls(cobas® Liat Influenza A/B Quality Control Kit).**  Before using a new lot of **cobas**® Liat Influenza A/B Tubes, the Add Lot procedure must be performed on the **cobas**® Liat to validate the **cobas**® Liat Influenza A/B Tube lot at your site.  The **cobas**® Liat will prompt you to add the lot if you try to run an assay from a new un-validated lot.  **4 barcodes** are needed for this procedure. Make sure to scan the right barcode when prompted by the **cobas**® Liat.   1. · **Package Insert Barcode**: On the Package Insert Barcode Card contained in the **cobas**® Liat Influenza A/B Assay Tube Pack. This barcode is lot-specific; match the lot number next to the barcode with the lot number on the **cobas**® Liat Influenza A/B Tubes. 2. · **cobas® Liat Influenza A/B Tube Barcode**: on the **cobas**® Liat Influenza A/B Tube sleeve. 3. · **Negative Control Barcode**: on the Control Kit Barcode Card contained in the QC Kit. Match the lot number next to the barcode with the lot number on the Dilution UTM tube. 4. · **Positive Control Barcode**: on the Control Kit Barcode Card contained in the QC Kit. Match the lot number next to the barcode with the lot number on the Positive Control tube. | |
| External Quality Control + Add Influenza A/B Tube lot | |
| **Step** | **Action** |
|  | Adding a **Cobas® Liat Influenza A/B Tube Lot**   1. Select **Assay Menu** on the main menu of a **cobas**® Liat. 2. Select **New Lot** at the bottom of the list. 3. Select **Scan** and scan the Package Insert Barcode on the Package Insert Barcode Card from the **cobas**® Liat Influenza A/B Assay Tube Pack. |
|  | Prepare and test Negative Control sample   1. Select **Scan** and scan the Negative Control Barcode on the Control Kit Barcode Card. The **cobas**® Liat will prompt “*Add Negative Control & scan Liat Tube ID*.” 2. Take a Dilution UTM tube from the QC Kit; this is used as the negative control sample. Hold the Dilution UTM tube by the tube cap and shake down the liquid in the tube using a quick, sharp, downward wrist motion. Visually check that the Dilution UTM has pooled at the bottom of the tube. If not, repeat the shake down procedure. 3. Using the Dilution UTM as sample, run the assay following the Running **cobas**® Liat Influenza A/B Assay procedure, step B.2.b-h (Add Sample) and B.3 (Insert **cobas**® Liat Influenza A/B Tube). 4. If *“Negative Control Result Accepted”* is displayed at the end of the run, select **OK**. If the result is rejected, repeat the negative control run (step 1.a.). 5. Select **Back**. |
|  | Prepare Positive Control sample   1. Hold the Dilution UTM tube by the tube cap and shake down the liquid in the tube using a quick, sharp, downward wrist motion. Visually check that the liquid has pooled at the bottom of the tube. If not, repeat the shake down procedure. 2. Using a transfer pipette, transfer all the liquid from the Dilution UTM tube into the Positive Control tube: 3. Squeeze the bulb of pipette 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 Dilution UTM tube. 4. Slowly release the bulb completely while keeping the pipette tip below the liquid surface. You will see the liquid UTM rising into the pipette. After releasing the bulb completely, withdraw the pipette from the Dilution UTM tube. 5. Insert pipette into the Positive Control tube until the pipette tip is at the bottom of the tube. 6. Slowly squeeze the bulb to empty the contents of pipette. Avoid creating bubbles in the sample. Do not release the pipette bulb. 7. While still squeezing the pipette bulb, withdraw the pipette from the tube. 8. Cap the Positive Control tube. Hold the Positive Control tube by the cap and shake down the liquid in the tube using a quick, sharp, downward wrist motion. 9. Let the Positive Control tube sit for 5 minutes. |
|  | Test Positive Control sample   1. On the **cobas**® Liat used for the Negative Control test, select **Scan** and scan the Positive Control Barcode on the Control Kit Barcode Card. The **cobas**® Liat will prompt “*Add Positive Control & scan Liat Tube ID*”. 2. After the Positive Control tube from Step A.2 has set for 5 minutes, use the transfer pipette from the **cobas**® Liat Influenza A/B Tube pouch to slowly pipette the sample up and down 10 times to dissolve and mix the positive control sample. 3. Using the Positive Control as a sample, run the assay following the Running **cobas**® Liat Influenza A/B Assay procedure, step B.2.b-h (Add Sample) and B.3 (Insert **cobas**® Liat Influenza A/B Tube). 4. If “*Positive Control Result Accepted. Lot … added*” is displayed at the end of the run, select **OK** to return to the assay menu. If the result is rejected, repeat the positive control run (steps 2.a and 3.a). |

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| Sample Analysis | Follow the steps below to run **Cobas**® Liat Influenza A/B Assay | |
| Sample Analysis | |
| **Step** | **Action** |
|  | Scan Barcode  Tear open the foil packaging of the **cobas**® Liat Influenza A/B Tube and remove the tube and the transfer pipette.   1. Select **Run Assay** on the main menu using the touch screen or function button.      1. Select **Scan** and scan the **cobas**® Liat Influenza A/B 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.      1. Select **Scan** again and scan the Patient or Sample barcode, or select **Enter** and type in a Sample or Patient ID. The Analyzer will prompt *“Add UTM sample & re-scan tube ID.”* |
|  | Add Sample  Unscrew the cap from the **cobas**® Liat Influenza A/B Tube. Do not throw away the cap. Use the transfer pipette to load ~200 μL of the sample into the **cobas**® Liat Influenza A/B Tube. You do not need to measure the sample volume; the **cobas**® Liat will adjust the sample volume if more sample was loaded, or output an error if not enough sample was loaded.   1. 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. 2. Firmly squeeze the bulb of the pipette until the bulb is fully flat. 3. While holding the pipette bulb fully flat, insert the pipette tip into the sample just below the liquid surface. 4. 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. 5. Insert the pipette into the **cobas**® Liat Influenza A/B Tube. 6. Place the pipette tip near the bottom of the sample compartment. Do not puncture the tube or the seal at the bottom. 7. Slowly squeeze the bulb to empty the contents of the pipette into the **cobas**® Liat Influenza A/B Tube. Avoid creating bubbles in the sample. Do not release the pipette bulb. 8. While still squeezing the pipette bulb, withdraw the pipette from the tube. Screw the cap back on the **cobas**® Liat Influenza A/B Tube. |
|  | Insert **cobas**® Liat Influenza A/B Tube   1. Select **Scan** and re-scan the **cobas**® Liat Influenza A/B Tube barcode. The tube entry door on top of the Analyzer will open automatically. 2. Remove the **cobas**® Liat Influenza A/B Tube sleeve. 3. Immediately insert the **cobas**® Liat Influenza A/B Tube into the **cobas**® Liat until the tube clicks into place. The **cobas**® Liat Influenza A/B Tube only fits in one way. If the tube is not inserted by the time the door closes, re-scan the **cobas**® Liat Influenza A/B Tube barcode (step 3a) and insert the **cobas**® Liat Influenza A/B Tube again. Once the **cobas**® Liat Influenza A/B Tube is properly inserted, the **cobas**® Liat will close the door automatically and begin the test. ***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.*** |
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|  | View Result  During the test, the system displays the running status and estimated time remaining.    Once the test is complete, the **cobas**® Liat displays the message, “Please remove the tube slowly…” and opens the tube entry door automatically.   1. Now, gently lift the **cobas**® Liat Influenza A/B Tube out of the Analyzer. 2. Select **Report** to see the Result Report. 3. Log test result with accession number on **LIAT System –Influenza A/B test log form**. *See attachment L1*   Select **Back**, and then **Main** to return to the main menu for the next test. |

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| Reasons to Repeat the Assay   * If the test result is “Indeterminate” for a target virus or “Invalid”, repeat the assay with the same patient specimen. * If an “Error” is reported by the **cobas**® Liat and/or the assay is aborted, repeat the test on a different instrument. Contact your Roche Service Representative if repeat “Errors” are reported by the instrument. |

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| Expected Values | The **cobas**® Liat reports results as “Detected”, “Not Detected”, or “Indeterminate” for each of Influenza A and Influenza B, or “Assay Invalid”. |

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| Interpretation / Results / Alert Values | |  |  |  |  | | --- | --- | --- | --- | | **Instrument Result Report** | | **Interpretation** | **Cerner Reporting** | | Influenza A | Influenza A  Not Detected | Negative test for Influenza A  (no Influenza A RNA detected) | **Not Detected** | | Influenza A  Detected | Positive test for Influenza A  (Influenza A RNA present) | **Detected** | | Influenza A Indeterminate | Presence or absence of Influenza A cannot be determined. ***Repeat assay*** once with original sample, and only report “Indeterminate” if such persist. | **Indeterminate**  \*Send out specimen for confirmatory test | | Assay. Invalid | Presence or absence of Influenza A and Influenza B cannot be determined. ***Repeat assay*** once with original sample, and only report “Invalid” if such persist. | **Invalid**  \*Send out specimen for confirmatory test | | Influenza B | Influenza B  Not Detected | Negative test for Influenza B  (no Influenza B RNA detected) | **Not Detected** | | Influenza B  Detected | Positive test for Influenza B  (Influenza B RNA present) | **Detected** | | Influenza B Indeterminate | Presence or absence of Influenza B cannot be determined. ***Repeat assay*** once with original sample, and only report “Indeterminate” if such persist. | **Indeterminate**  \*Send out specimen for confirmatory test | | Assay. Invalid | Presence or absence of Influenza A and Influenza B cannot be determined. ***Repeat assay*** once with original sample, and only report “Invalid” if such persist. | **Invalid**  \*Send out specimen for confirmatory test | | **\*Results reported as Indeterminate or Invalid** | | Contact ordering provider to order **Respiratory pathogen panel (242326)**. Previous collected sample to be send out to SWL. | | | [Error]. Assay Aborted | | Presence or absence of target viruses cannot be determined. Repeat assay once on another instrument.  If instrument “Error” persist:   1. Cancel test order as **Instrument error, test not performed** 2. Contact ordering provider to order **Respiratory pathogen panel (242326**). Previous collected sample to be send out to SWL. 3. Contact Roche Service Representative to resolve Error issue. | | |

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| Maintenance and Calibration | **Self-check**  The **cobas**® Liat performs self-diagnostics during power-on startup (initialization) and utilizes an advanced error diagnostics system to monitor the **cobas**® Liat’s performance during an assay. An operator is not required to perform any maintenance, other than touch screen calibration. Under normal operation, the **cobas**® Liat alerts the operator in the event that a malfunction or error is detected.  **Auto calibration**  The **cobas**® Liat periodically performs automatic recalibration. During auto calibration, “(AutoCal)” is displayed on the title bar. If the user selects Run Assay at this time, a message “AutoCal started. This could take up to a minute.” is displayed. Select **OK** to close this message window. Wait until (AutoCal) is no longer in Title Bar.  **Clean the cobas® Liat**   * Keep the touch screen clean from excessive fingerprints and moisture by gently wiping it with a soft, lint free cloth. * The exterior of the **cobas**® Liat and front buttons can also be cleaned using a soft lint free cloth moistened with either 70% isopropanol or 5-10% bleach solution. If bleach is used, it must be wiped twice using 70% isopropanol to remove all bleach residues. * Periodically check the rear vent and bottom of the **cobas**® Liat for excessive dust or debris. * When prompted by the message “Use cleaning tool” on the **cobas**® Liat screen, use the provided cleaning tool following the instructions included with the Cleaning tool kit. |
| Limitations | * + The performance of the **cobas**® Liat Influenza A/B Assay was evaluated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test.   + As with other tests, negative results do not preclude Influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions. Results from the **cobas**® Liat Influenza A/B Assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician.   + Analyte targets (viral nucleic acid) may persist in vivo, independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious, or are the causative agents for clinical symptoms.   + This test has been evaluated for use with human specimen material only.   + False negative results may occur if a specimen is improperly collected, transported or handled. False negative results may occur if inadequate numbers of organisms are present in the specimen.   + If the virus mutates in the target regions, Influenza viruses A or B may not be detected or may be detected less predictably.   + This test has not been evaluated for patients without signs and symptoms of Influenza infection.   + This test is a qualitative test and does not provide the quantitative value of detected organism present.   + Cross-reactivity with respiratory tract organisms others that those tested can lead to erroneous results.   + This assay has not been evaluated for patients receiving intranasal administered Influenza vaccine.   + This assay has not been evaluated for immunocompromised individuals.   + This test cannot rule out diseases caused by other bacterial or viral pathogens. |

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| Non-Controlled Documents | The following non-controlled documents support this procedure.   * Cobas® Liat User Manual * Cobas® Influenza A/B Package insert |

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| Controlled Documents | The following controlled documents support this procedure.   * LIAT System –Influenza A/B test log form |

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| Author(s) | Alvin Castillo, CLS |

