**PROCEDURE: Cobas SARS-CoV-2 Assay Procedure**

1. **PRINCIPLE:**
   1. **cobas**® SARS-CoV-2 for use on the **cobas**® 6800/8800 Systems is a real-time RT-PCR test intended for the qualitative detection of nucleic acids from clinician-collected nasopharyngeal swab samples from patients who meet COVID-19 clinical and/or epidemiological criteria. **cobas**® SARS-CoV-2 is for use only under Emergency Use Authorization (EUA) in U.S. laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform moderate complexity tests, and in U.S. laboratories certified under CLIA to perform high complexity tests, by clinical laboratory personnel who have received specific training on the use of the **cobas**® 6800/8800 Systems.
   2. Results are for the detection of SARS-CoV-2 RNA that are detectable in nasopharyngeal swab samples during infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses.
   3. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.
   4. **cobas®** SARS-CoV-2 is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. Automated data management is performed by the **cobas®** 6800/8800 software, which assigns test results for all tests. Results can be reviewed directly on the system screen, and printed as a report.
   5. Nucleic acid from patient samples and added internal control RNA (RNA IC) molecules are simultaneously extracted. Nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris and potential PCR inhibitors, are removed with subsequent wash steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature. External controls (positive and negative) are processed in the same way with each **cobas®** SARS-CoV-2 run.
   6. Selective amplification of target nucleic acid from the sample is achieved by the use of target-specific forward and reverse primers for ORF1 a/b non-structural region that is unique to SARS-CoV-2. Additionally, a conserved region in the structural protein envelope E-gene were chosen for pan-Sarbecovirus detection. The pan-Sarbecovirus detection sets will also detect SARS-CoV-2 virus.
   7. Selective amplification of RNA Internal Control is achieved by the use of non-competitive sequence specific forward and reverse primers which have no homology with the coronavirus genome. A thermostable DNA polymerase enzyme is used for amplification.
   8. The **cobas**® SARS-CoV-2 master mix contains detection probes which are specific for the coronavirus type SARS-CoV-2, members of the Sarbecovirus subgenus, and the RNA Internal Control nucleic acid. The coronavirus and RNA Internal Control detection probes are each labeled with unique fluorescent dyes that act as a reporter. Each probe also has a second dye which acts as a quencher. When not bound to the target sequence, the fluorescent signals of the intact probes are suppressed by the quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5' to 3' exonuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly. Each reporter dye is measured at defined wavelengths, which enables simultaneous detection and discrimination of the amplified coronavirus target and the RNA Internal Control. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon). Any contaminating amplicons from previous PCR runs are destroyed by the AmpErase enzyme [uracil-N-glycosylase], which is included in the PCR mix, when heated in the first thermal cycling step. However, newly formed amplicons are not destroyed since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.
2. **TEST CODE:**
   1. MCV19
   2. SNCOV
3. **SPECIMENS:**
   1. Collect nasopharyngeal specimens according to standard collection technique using flocked or polyester-tipped swabs and immediately place in 3 mL of Copan Universal Transport Medium (UTM-RT), BD™ Universal Viral Transport (UVT).
   2. After collection, the specimen should be stored at 2-25°C and processed within 48 hours.
   3. If processing will exceed 48 hours, specimens should be frozen at -70°C.
4. **REAGENTS:**
   1. cobas® SARS-CoV-2 -192 Test Kit, store at 2-8°C
   2. cobas® SARS-CoV-2 Control Kit, store at 2-8°C
   3. cobas® Buffer Negative Control Kit, store at 2-8°C
   4. cobas omni MGP Reagent, store at 2-8°C
   5. cobas omni Specimen Diluent, store at 2-8°C
   6. cobas omni Lysis Reagent, store at 2-8°C
   7. cobas omni Wash Reagent, store at 15–30°C
5. **CONTROLS:**
   1. cobas® Buffer Negative Control
   2. cobas® SARS-CoV-2 Positive Control
6. **REAGENT AND CONTROL EXPIRY CONDITIONS:**

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| **Reagent** | **Kit expiration date** | **Open-kit stability** | **Number of runs for which this kit can be used** | **On-board stability (cumulative time on board outside refrigerator)** |
| **cobas®** SARS-CoV-2 – 192 | Date not passed† | 90 days from first usage\*,† | Max 40 runs† | Max 40 hours† |
| **cobas®** SARS-CoV-2 Control Kit | Date not passed† | Not applicable | Not applicable | Max 8 hours† |
| **cobas®** Buffer Negative Control Kit | Date not passed | Not applicable | Not applicable | Max 10 hours |
| **cobas omni Lysis Reagent** | Date not passed | 30 days from loading\* | Not applicable | Not applicable |
| **cobas omni MGP Reagent** | Date not passed | 30 days from loading\* | Not applicable | Not applicable |
| **cobas omni Specimen Diluent** | Date not passed | 30 days from loading\* | Not applicable | Not applicable |
| **cobas omni Wash Reagent** | Date not passed | 30 days from loading\* | Not applicable | Not applicable |

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| \*Time is measured from the first time that reagent is loaded onto the **cobas**® 6800/8800 Systems.  †The performance has not been established for suggested use cycles and time, but is based on similar reagents used on the same system. |

1. **TEST PROCEDURE:**
   1. Procedure Notes
      1. Refer to Cobas 6800 Systems Operator Manual on M drive/Molecular Folder/ Roche 6800.
      2. Refer to Coro Molecular Micro Cobas 6800 Operating Procedure for detailed procedure
      3. Clean benchtops with 10% bleach followed by 70% alcohol pre and post running the assay.
   2. Remove samples from the refrigerator and allow them to reach room temperature.
   3. Go to the 6800 Monitor Tab and check the taskbar and messages at the top left on the monitor screen
   4. Address any issues or maintenance due.
   5. Refill reagents and consumables as prompted by the system:
      1. Load wash reagent, lysis reagent and diluent.
      2. Load tip racks, processing plates and amplification plates.
      3. Load Magnetic Glass Particles.
      4. Load test specific reagents.
      5. Load control cassettes.
      6. Replace rack for clotted tips.
   6. Set the system to “Ready”.
      1. In the task overview, ensure that there is no maintenance overdue.
      2. On the **Monitoring** tab, choose the **Start** button.
      3. The system changes to **Preparing** status.
      4. Wait for the system to change to **Ready** status before you start loading. This may take 15 minutes.
   7. Organize the CoV-2 runs for the day and make Tasklists.
   8. Rack Based ordering will be used.
      1. Racks numbered 61-100 are designated for CoV-2 samples and are labeled with orange tape.
      2. For any samples without LIS barcodes, ie. Environmental samples, the sample ID must be entered in the Manual Barcode Entry tab.
   9. Bring Racks and samples to the hood for loading racks with samples.
   10. Vortex primary sample tube.
       1. Vortex all sample tubes at once prior to transferring to allow settling of fluid.
   11. Unscrew the primary sample tube cap.
       1. Use caution to prevent cross contamination of specimens. During vortexing sample may reach the top of the tube. Change gloves as necessary if they become contaminated.
   12. Lift the cap and any attached swab to allow a pipette to be inserted into the sample tube.
   13. Transfer 0.6 mL into the prepared barcoded secondary tube.
   14. Transfer secondary tube to a rack. Close the primary sample tube cap.
   15. When rack is full:
       1. Cover tubes with Wypall.
       2. Change your gloves and place face shield over your face.
       3. Place racks on cart and bring racks to instrument.
       4. Load trays with sample racks onto the Sample Supply module and remove Wypall.
   16. Go to the **Batches** tab.
       1. Monitor the error lane.
          1. To safely remove samples from the error lane.
             1. Change gloves and put on face shield
             2. Remove rack carefully and cover with a wypall.
             3. Resolve any issues, ie. add sample ID in Manual Barcode Entry.
             4. Load rack via stat lane holding the wypall over the tube until inserted.
       2. After the sample barcodes are read it will make the “batch” and list the number of tests in the batch.
          1. Make sure that number matches the expected number of tests.
          2. Resolve any discrepancies.
   17. Hit the “**Start Manually**” button to begin processing.
       1. At this point you may go to the Routine Tab> Test Order Status to see the finish time.
   18. Monitor the instrument during processing in the Transfer Module.
       1. Address any errors or issues.
       2. **Do not walk away from instrument until samples are moved to the Processing module.**
   19. Place face shield over face and unload racks and samples when finished pipetting by covering with Wypall.
       1. Bring tubes to the hood and discard into a biohazard bag lined with a Wypall.
       2. After run is finished move Positive samples to the Positive Save rack in the freezer. Negative samples will be saved for 1 week in the refrigerator.
   20. Unload consumables at the end of processing:
       1. Remove amplification plates from the analytic module.
       2. Unload empty control cassettes.
       3. Empty solid waste.
       4. Empty liquid waste.
2. **RESULT INTERPRETATION:**
   1. Results
      1. From the **Routine Tab > Control Batch**
      2. Choose the control batch for your run
      3. From the drop-down list choose Print from both right and left side of screen.
         1. The printout from the left has lot #s of reagents and controls
         2. The printout from the right has the actual results of controls.
         3. View results
      4. Reporting Results- Refer to Appendix A for LIS resulting instructions.
   2. The cobas® 6800/8800 Systems automatically detects the SARS-CoV-2 , for each individually processed sample and control, displaying individual target results for samples as well as test validity and overall results for controls.
   3. One cobas® Buffer Negative Control [(-) Ctrl] and one [SARS-CoV-2 (+)C] are processed with each batch. In the cobas® 6800/8800 software and/or report, check for flags and their associated results to ensure the batch validity. All flags are described in the cobas® 6800/8800 Systems User Guide. The batch is valid if no flags appear for any controls. If the batch is invalid, repeat testing of the entire batch.
   4. Validation of results is performed automatically by the cobas® 6800/8800 software based on negative and positive control performance.
   5. A valid batch may include both valid and invalid sample results. Invalid results for one or more target combinations are possible and are reported out specifically for each channel.
   6. Results and their corresponding interpretation for detecting SARS-CoV-2 are shown below:
      1. Target 1 is the ORF1a/b non-structural region that is unique to SARS-CoV-2.
      2. Target 2 is a conserved region in the structural protein envelope E-gene for pan-Surbecovirus detection. The pan-Surbecovirus detection sets will also detect SARS-CoV-2 virus.

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| **Target 1** | **Target 2** | **Interpretation** |
| Positive | Positive | Detected |
| Positive | Negative | Detected |
| Negative | Positive | Detected |
| Negative | Negative | Not Detected |
| Positive | Invalid | Detected |
| Invalid | Positive | Invalid |
| Negative | Invalid | Not Detected |
| Invalid | Negative | Invalid |
| Invalid | Invalid | Invalid |

* 1. Release Results
     1. Select all test results to be released
     2. Choose the **Release button**
     3. Test results are sent to the SOFT Instrument Menu for posting.
  2. Invalid Test Results
     1. Check invalid flags in the result report. If invalid due to QNS do not release results. Add 1 ml UTM and repeat on the next run.
     2. Invalid results due to result interpretations.
        1. Result as Invalid and add a statement that test is being repeated.
        2. Reorder at “No Charge” and change collection time by 10 minutes.
        3. Run the test at Coro. Add 1 ml of UTM if there is not enough sample.

1. **LIMITATIONS:**
   1. Reliable results depend on proper sample collection, storage and handling procedures.
   2. This test is intended to be used for the detection of SARS-CoV-2 RNA in nasopharyngeal swab samples collected in a Copan UTM-RT System (UTM-RT), BD™ Universal Viral Transport System (UVT). Testing of other sample types with cobas® SARS-CoV-2 may result in inaccurate results.
   3. 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.
   4. As with any molecular test, mutations within the target regions of cobas® SARS-CoV-2 could affect primer and/or probe binding resulting in failure to detect the presence of virus.
   5. False negative or invalid results may occur due to interference. The Internal Control is included in cobas® SARS-CoV-2 to help identify the specimens containing substances that may interfere with nucleic acid isolation and PCR amplification.
2. **TECHNICAL ASSISTANCE:**
   1. Roche Support Network Customer Support Center at 1-800-526-1247.
3. **REFERENCES:**
   1. cobas® SARS-CoV-2 Package Insert

Appendix A SCC Soft Resulting

**Cobas SARS-CoV-2 SCC Soft Resulting**

Test ID: **MCV19/ SNCOV**

Template: **RCH19**

Workstation: **RMOLM**

1. Create a Tasklist
   1. Follow procedure for “Creating a Tasklist” in Soft Manual under TASKLIST
      1. Template= RCH19
   2. Print a second barcode label for each sample
   3. Number the specimens and secondary tubes according to the tasklist beginning with #1 and ending with #94. Delete any controls that populate in the tasklist.
   4. Print the worklist and check it against the samples to verify both are in the same order.
2. Posting Results using LIS Interface
   1. View and Review results from the cobas 6800.
   2. Check any error flags
   3. Click on all results to be released and click the **RELEASE** button.
      1. Do not release any invalids due to QNS.
   4. Results will transfer to the Soft Instrument Menu
   5. From SoftLab, go to “Interfaces”, and “Instrument Menu”
   6. Select Cobas 6800 from the Instrument Menu
   7. Select “Loadlist and Today’s Results”, “Not Posted”, “By Sequence”
   8. Post results.
   9. Follow the most recent algorithm for calling/faxing of results.
   10. For any result that needs to be called: Call up the Resulted Tasklist.
       1. From Resulting Worklist > Select Tests by Tasklist > Enter Tasklist number
       2. Highlight the order number from the instrument menu
       3. Call the unit and give a verbal report
       4. Open “Comment” box for line CCV19 or SNCOV and add comment, i.e. @CALM, to the box and enter phone report. OK and Save
   11. For patient results that need to be faxed.
       1. From Resulting Worklist > Select Tests by Tasklist > Enter Tasklist number
       2. Highlight the order number from the instrument menu
       3. Open “Comment” box for line CCV19 or SNCOV and add comment, i.e. @Fax to the box and enter the date and time. OK and Save
       4. Go back to the report and print the Instant Report
       5. Instant reports can be manually faxed.
       6. The QA Nurse can be faxed through Soft. After adding the Fax comment to the report, highlight the order, go to Instant Report > Fax > Remote > enter ID 11a, highlight QA Nurse> OK. The QA Nurse fax number is 444-5541
   12. Invalid results due to result interpretations.
       1. Result as Invalid and add a statement that test is being repeated.
       2. Reorder at “No Charge” and change collection time by 10 minutes.
       3. Run the test at Coro. Add 1 ml of UTM if there is not enough sample.
   13. Check Results
       1. Go to “Resulting Worklist” by Tasklist
       2. Choose Tasklist
       3. Enter Tasklist ID
       4. Review Worklist to verify that all results have posted. They should all have “\*” next to them.
       5. Print a new pending worklist by Tests, SNCOR and MCV19, and check on any outstanding orders.