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COVID-19
STANDARD OPERATING PROCEDURE FOR SAMPLE STORAGE @ -80˚C FREEZER AND PREPARATION OF RNA

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COVID-19 - STANDARD OPERATING PROCEDURE FOR SAMPLE STORAGE @ -80˚C FREEZER AND PREPARATION OF RNA

Appendix

1. Description of Facility:

The facility consists of fourteen rooms: 1st room (Registration room); 2nd room (vestibule); 3rd room (transition room); 4th room (general lab), 5th room (data-documentation), quarantine containing rooms (Access room (6th) four individual rooms identified as A, B, C and D (7 to 10th); 11th room (exchange material room). A second wing is former by three additional rooms: Entrance Room – 12th, General Laboratory (13th) and the incubation room (14th).

1st Room (Registration room)

The 1st room has two doors. The Door #1 is an entrance/exit to the quarantine facility and connected to the interior of Biology bldg. The door is labeled on the outside: **"Center for Excellence in Quarantine & Invasive Species, Limited Entry".** The visitor's logbook located in this room. Also, the room has a black light trap operating at all time. The door #2 connected with 2nd room (vestibule).

2nd Room (vestibule)

The 2nd room (vestibule) has three doors. The door #2 mentioned before connecting with Registration room; Door # 8, gives access to the 7th room (exchange material room) and the door #3 connected with the 3rd room (transition room). The 2nd room has a coat rack on the right with clean lab coats to be put on when entering the facility. There is an emergency exit light in front of the door to the vestibule.

3rd Room (Transition room)

The 3rd room has three doors connecting to different rooms. Door # 4 is the entrance to the 5th room (data-documentation, office). Door #5 gives access to the 4th room (general lab). Door # 7 connected with 6th room (quarantine containing rooms). The door #5 labeled on the outside: **"Entrance Only"** and the door #7 labeled **"Exit Only".**

4th Room (General lab. I)

Inside the 4th room is door # 6, which connects to the 6th room (quarantine containing rooms).

5th Room (data-documentation)

Door # 4 is the entrance for this room. It is equipped with a computer, printer, scanner and telephone. The standard operating procedure manual and the detailed building operation manual are also kept in this office. The quarantine officer and quarantine officer assistant use this room as office space and data-entry room. All permits and documentation regarding the quarantine facility will be located in this room.

6th Room (Quarantine containing room)

The quarantine containing room is the room immediately after the general lab. Door # 6 is the sole entrance to this room. Inside there are four divided and individual rooms (A, B, C and D – 7th to 10th). There is an automatic autoclave in this room, which is used to sterilize materials, trash, and instruments leaving the quarantine containing facility. The door #7 is an exit only and is labeled as **Emergency Exit**.

11th Room (exchange material room)

This room has a roll-up pass-through and pass-through chamber are the only entrances to the material inside the quarantine facility. The roll-up pass-through don't is an emergency exit.

12th Room (exchange material room)

Connecting 11th room with the additional wing. Connects to Rooms 13 and 14.

13th Room (General Laboratory II)

Facilities with working benches and storage space. Workstations with HEPA certified air-filtering and UV sterilization system.

14th Room (Incubation room)

Isolated area connected from room 12th.

2. Purpose

This SOP describes the procedures for sample storage at a Thermo Fisher 700 Series -80˚C freezer and processing of RNA for preparation for full genome sequencing at the Center for Excellence in Quarantine & Invasive Species (CEQIS) BL-2 facilities, University of Puerto Rico (UPR), Agricultural Experimental Station, Jardín Botánico Sur, 1193 Calle Guayacán, San Juan, Puerto Rico 00926-1128 under the supervision of Dr. Jose Carlos Verle Rodrigues.

3. Scope

This SOP applies to every person or research group interested in storing samples and sample remnants associated with COVID-19 testing or researchers interested in projects involving COVID-19 samples. This SOP applies to researchers, public health officials and laboratory staff at public or private clinical laboratories. The RNA extractions will be ONLY performed in the designated room (#13) that will not be processing any other sample or material during the work. A PCR workstation will be dedicated to virus RNA extraction, and not used for any other purpose.

3. Background

As of May 5, 2020, the COVID-19 pandemic has already claimed over 255,000 lives with over 3.6 million confirmed cases, worldwide. Its causative agent, the *Severe acute respiratory syndrome-related coronavirus* (SARS-CoV-2), has spread with tremendous speed to over 200 countries and regions. Coronaviruses are a large family of viruses that are common in people and many different species of animals, including camels, cattle, cats, and bats.

So far, there is no approved vaccine for use in humans against this or any other coronavirus. Potential circulation among domesticated animals add an additional challenging to public health. Clearly, there is an urgent need for research aimed at understanding various aspects of this new virus, behavior in the environment and the broad spectrum of clinical manifestations associated with it.

To fulfill this need for research, we established a Covid-19 Sample Storage Unit at UPR-CEQIS-AES BL-2 Facilities, Botanical Garden South. The samples stored in this facility will be available for coronavirus-related research projects.

A -80˚C freezer (F-06) located at the CEQIS Facility room #4, has recently been made available in whole for the purpose of storing the samples. The purpose of this freezer is to serve as a long-term storage unit for samples collected as part of the Covid-19 testing protocols in place at many hospitals, clinical laboratories and other medical

facilities. The freezer is connected to a constant electrical power supply with a robust back-up generator facility within a BSL-2 lab facility with the appropriate air quality system. The freezer is a Model 756 with serial number 818813 – 25. The Unit will only be accessed by approved personnel.

The protocols for the collection, storage and disposal of Covid-19 samples will be evaluated by Institutional Review Board (IRB) used by the specific investigator.

An RNA virology laboratory is established at a brand-new wing of the quarantine. The space (Room) is designated **only** for this purpose.

4. Conduct near the storage freezer facilities

Due to the valuable nature of the samples stored in the Storage Unit and the current already standards at the facility, is required a highest level of care and the display of appropriate conduct for all personnel that enter the Facility. This is to ensure the integrity and appropriate use of the stored samples.

- Every person allowed into the facility must be accompanied or authorized after properly training and familiarity with SOPs by Dr. José Carlos Verle Rodrigues
- The designated number of the freezer is F-06.
- Do not open the freezer if not authorized or unaccompanied by the officers in charge of the storage freezer. The freezer will be locked key.
- Make sure that freezer space is assigned to the samples before the samples are deposited.
- Report any freezer alarms to Dr. Verle Rodrigues, via email and/or text message, immediately.
- Samples should be transported only in sample carriers with a secondary container. Sample carriers may contain ice or dry ice.
- Follow the appropriate hygiene protocols which include wearing gloves, face shields and gown on entering the facility, and using 70% ethanol to spray on sample boxes upon removing the from the sample carrier.
- Samples will only be moved into or out of the storage freezer. No processing, fractionation, dilution, dissolution or handling will take place in the room (#4) where the freezer is located.
- Samples will be directly shipped to cooperators or processed for RNA extraction at separated designated room (#13) under the additional protection of the PCR workstation and the person performing the extraction will be wearing all individual protective equipment (masks, double gloves, single use protective coat)

5. Freezer allocations

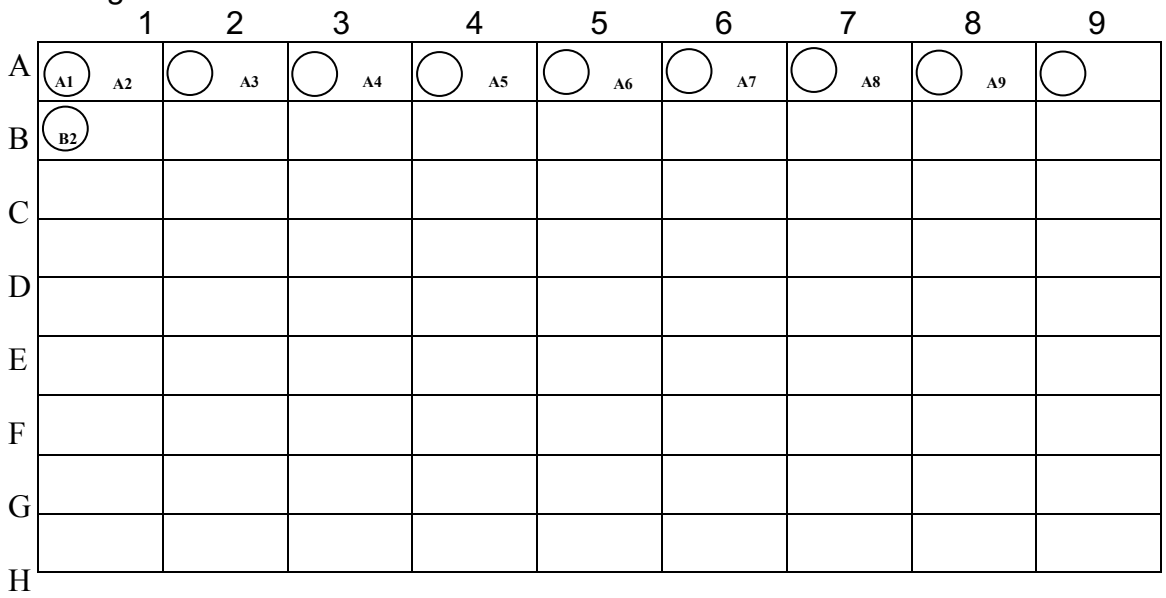
Prior to bringing the sample to the storage facility, the submitting laboratory must send via email a spreadsheet detailing the content of each of the boxes that will be stored. A spreadsheet entry needs to be filled for every sample transferred to the storage facility. Based on the data entered into the spreadsheet, freezer space will be assigned. Once the data for the samples is entered into the system, the samples will be stored in

assigned freezer racks and a sample locator legend will be available in hard copy next to the freezer. Freezer arrangements may be updated, and/or modified.

6. Receiving samples, storage and processing RNA

- The following guidelines for sample storage will be in place the Covid-19 Sample Storage Facility. Samples will be transported from Hospital or Clinical Laboratory to the Storage Facility.
- Written consent will be obtained before samples are taken. Patient information gathered will include symptoms, underlying medical conditions, age, gender and recent travel history.
- The nasopharyngeal swab will then be placed in a 15 ml molecular biology grade sterile plastic tube filled virus inactivation solution and stored in a 4°C refrigerator. The samples will be identified, and named according to the CDC recommended SARS-CoV-2 naming convention (github.com/CDCgov/SARS-CoV-2_Sequencing#sequencing).
- The samples will be placed in a sealed cooler filled with ice and transported to the CEQIS by Dr Serrano for storage at –80 °C, in a BSL-2 freezer.
- Sample delivery must be arranged beforehand between the Clinical/Hospital Laboratory and the Facility Director.
- Packages must enter quarantine through the pass-through chute located in Room 11. Once the package enters quarantine, it must be immediately taken to the Receiving Room (Room #4) and placed in the freezer.
- Sample identifier and associated data must be included in a spreadsheet and submitted to the Storage Facility before the samples are sent.
- The samples must be in the appropriate cryovials and/or cryotubes fully capped.
- The samples must be labeled with the appropriate code given the originator Clinical Laboratory.
- Ideally, the samples will be sent contained within a card board storage box labeled on all four sides with the LID of the sample in position number A1 (samples are stored by coordinates in a 9 X 9 or 10X10 box with alphabetic rows and numeric columns, being filled from right to left, top to bottom of the box. The top left-hand corner is identified by A1 or 1. and the bottom right hand corner is I9 as in figure 1) using an alcohol resistant marker.
- The CEQIS will provide boxes to the Clinical Laboratories to be filled with sample remnants.

Figure 1: Filling order of a 9 X 9 box



- The appropriate labeling of samples within a box will be done by the Clinical Laboratory.
- The boxes will be label with the Date of Submission and the name of the Clinical Laboratory. This information will be contained in a sticker label with a numeric as well as a QR code for identification.
- Associated with the QR code, there will be a Cryobox Content Log Form that will be filled by the Clinical Laboratory.
- RNA samples will be processed in a designated room (#13).
- Samples will be processed inside Workstation operating with HEPA filter system and UV for pre an post use at Room #13. A Qiagen RNeasy kit will be used for extraction following the protocol.
- The RNA samples will be quantified and verified for quality using a nanodrop. The RNA samples will be shipped blind to Dr. Scot Dowd of MrDNA (www.mrdnalab.com).
- Complete sanitation and sterilization will follow with ozone, ETOH in the working surfaces, hypochlorite in the floors and higher temperature vapor system in both places (SARS-CoV-2 is inactivated with 56 ^{o}C or higher temperatures).

7. Sample retrieval

- The retrieval of samples from the storage freezer will be a controlled process similar to the deposition of samples. Researchers wishing to carry out a project or to answer specific scientific questions, will have access to the stored samples. There will be three requirements for the retrieval of samples:

1) A sample request form must be filled to state the name of the researchers in the team, the nature and objectives of the research project.

2) An IRB application must be submitted to the appropriate IRB committee from the researcher's institution. The description of the study protocol must be available for evaluation and for the permanent record.

3) An appropriate power calculation must also be submitted in order to justify the number of samples requested.

- Based on the retrieval request documentation, a set of samples will be agreed upon, located among the boxes and handed over to the researchers. The use of pass through chambers (double-window or double door) available in the facility will be used to provide the samples between the storage room #4 and the processing RNA samples #room 13.

8. Temperature monitoring devices

Freezer temperatures are monitored by an automated system composed of a computer, thermocouples connected to the freezers and an interface between the freezers and the computer. The thermocouples relay an electrical signal from the freezers to the interface, which converts the signal into a digitally displayed temperature in degrees Celsius. The temperature readings are automatically imported into the computer at one-minute interval and saved into an excel file. The temperature history for a freezer can be obtained by accessing an excel file for each freezer.

The temperature monitoring system is connected to an alarm system monitored 24 hours a day by an external security company. If a freezer temperature goes out of the minimum and/or maximum set temperature range the computer sends out an e-mail to the laboratory director, and to freezer room technical staff. If the freezer has failed all the samples will be quickly transferred into an operational freezer set within the same temperature ranges as the failing freezer. This secondary freezer is available at the Virology Laboratory, EEA and can be available in case of additional emergency and an extra contingency.

8. Alarms

The alarm system relays are de-activated in the normal position and will be activated during alarm. Therefore, an alarm will be generated during **total power failure**. An alarm can also be generated by:

1. One of the temperature sensors defects.
2. Temperature out of minimum- maximum range.
3. Maximum time door open.

<u>In case of total power failure and sensor defect – please contact the Scientific Director immediately. (Contact details posted in the freezer room and at the Facility Entrance).</u>

9. Maintenance of freezer and equipment

The freezers will be checked and qualified for sample storage use by qualified personnel in the following manner.

Every time the freezer is opened:

- Minimize the time in which you perform activities with the freezer door opened to a minute or less.

- Ensure that all internal doors are shut properly and then shut the main freezer door.

- If any user experiences difficulty closing the freezer door, the most likely reason is frost build-up. If you encounter this situation, scrapping of the frost in the door is necessary immediately; perform the scrapping rapidly according to the maintenance section of the freezer manual and record the action in the cleaning logbook.

Periodical maintenance:

- Every month the door seals will be cleaned with a soft cloth and excess frost on the inside surfaces will be wiped rapidly (no more than 1 minute) by Facility Personnel and this action will be recorded in the cleaning logbook.

- Every 3 months the condenser filter will be cleaned by Facility Personnel according to the maintenance section of the freezer manual and this action will be recorded in the cleaning logbook. At this time the condenser coils will be visually inspected for accumulation of dust and debris, record the findings in the comment section of the cleaning logbook and consult the freezer manual for recommended action if necessary.

Processing equipment:

- All individual equipment used will be disinfected under UV light for 30min (Pipettes, tubes);
- Ethanol 70% will be spray before and post processing in micro-centrifuge body, lids and interior.
- All equipment will be designated ONLY for this purpose and will remain in the designated room for the period of the work.

10. Actions to take in case of occurrence of emergency events

1. In case of power outage – a diesel power plant is available and connected to the laboratory power grid;
2. In case of localized fire, use the available fire extinguisher units and isolate the area (room);
3. The laboratory is not located in areas susceptible to flood, tornados.
4. The building is steady to support strong winds, hurricanes, windstorms and earthquakes

12. People to contact in case of emergencies

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jose_carlos@mac.com

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Horacio Serrano
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I have received and reviewed Standard Operating Procedures. I agree to abide by all requirements and procedures while performing my assigned duties in the Center for Excellence in Quarantine & Invasive Species facility (CEQIS) at University of Puerto Rico located in San Juan, PR.

Date: _____ Name (Printed): _____