iMM Covid19 Diagnostic

Standard Operating Procedure
and
Risk Assessment

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Approved by iMM Safety & Compliance and Board of Directors

Contents

A. Overall Description

The present document was prepared following a Risk Analysis carried out on the basis of current knowledge about Covid-19 infection, which indicates that the virus is transmitted mostly by large droplet spread.

This document may be shared with other research institutions (see details below), with the sole purpose of supporting these institutions in designing their own SOPs for similar purposes. Under no circumstances is iMM liable for any decisions regarding the procedures implemented by those institutions.

iMM will assist on the testing for COVID-19. The samples consist of specimens such as nasopharyngeal or oropharyngeal swabs from suspected and/or confirmed cases for COVID-19. iMM board of directors designated a team of 3 people to lead this initiative, and required that iMM affiliated staff with required expertise/set of skills were available to integrate this action. All iMM affiliated staff designated by iMM direction must follow these Standard Operations Procedures and Risk Control Measures listed below.

!!! **Important** !!! This is an evolving document being constantly updated. Every page is dated based on when it was made available.

For the more recent version please go to:

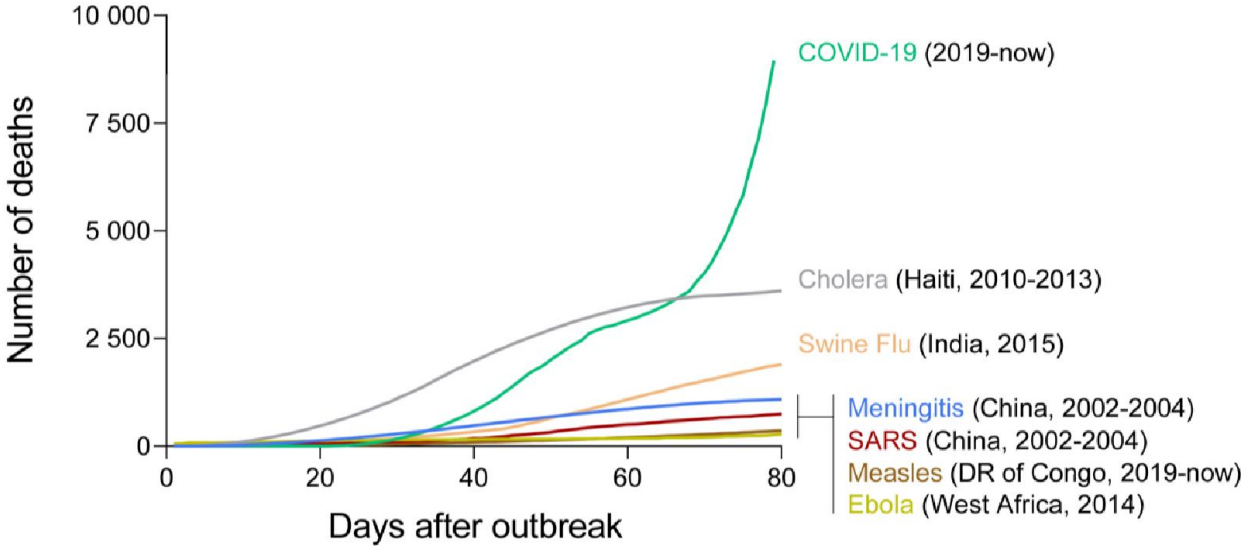
bit.ly/iMMCovid19SOP

B. Important notes and abbreviations

Hoods	BSC = Biosafety cabinets
Bleach Wipes	paper towels soaked in 15% Bleach
Dates	use dd_mm_yyyy format
Hours	Use am-pm format
Benchcoat	linen/pad to protect surfaces
HSM	Hospital Santa Maria
AES	Ana Espada de Sousa
EG	Edgar Gomes
JB	João Barata
CF	Claudio Franco
SOP	Standard Operating Procedure
PPE	Personal Protective Equipment
Virus Inactivation Room	AES Cell Culture = Room P3-C-52
EG Cell Culture	RNA extraction Room
JB/CF Cell culture	RT-PCR Room
iMM internal sample labeling	1, 2, 3, ...
RNA Stock Box labeling	RNA Covid Stock Box 1, RNA Covid Stock Box 2, ...
RNA Work Box labeling	RNA Work Box 1, RNA Work Box 2, ...
Original Swab Box labeling	Original Swab Box 1, Original Swab Box 2, ...,

Overall Debrief

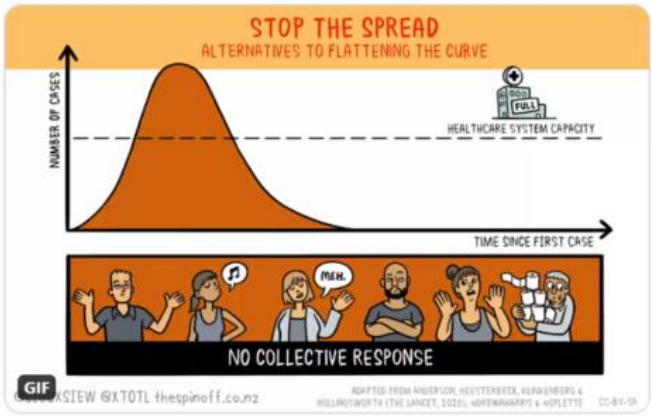
For an overview about the Novel coronavirus (2019-nCoV), watch this video on the World Health Organization (WHO) Youtube channel.

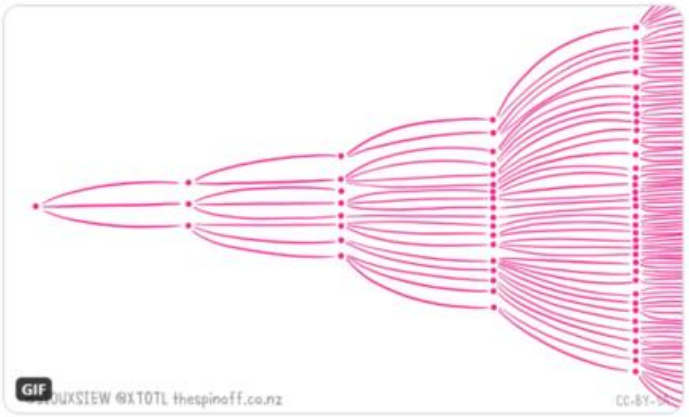


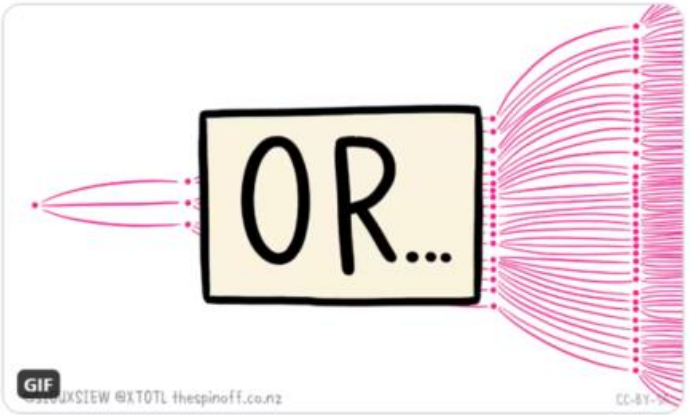
COVID-19 is the deadliest epidemic in the last two decades

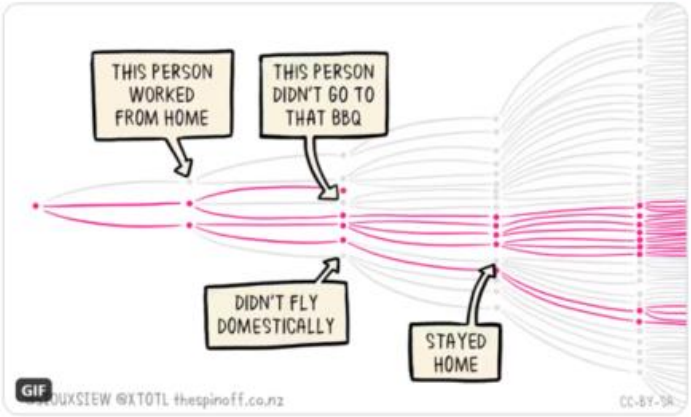
(evolution of the number of deaths during the first 80 days of the 10 most relevant epidemics since 2000 adapted by João Vieira from https://twitter.com/jeremiascrf/status/1242844267384582148

It is important to bear in mind that, when coming to iMM to work on the COVID-19 iMM Task force (as well as when working at the different stations for COVID-19 diagnosis), you must follow all the guidelines to prevent the spread of the virus. For instance, the importance of social distance is illustrated in the figures below.

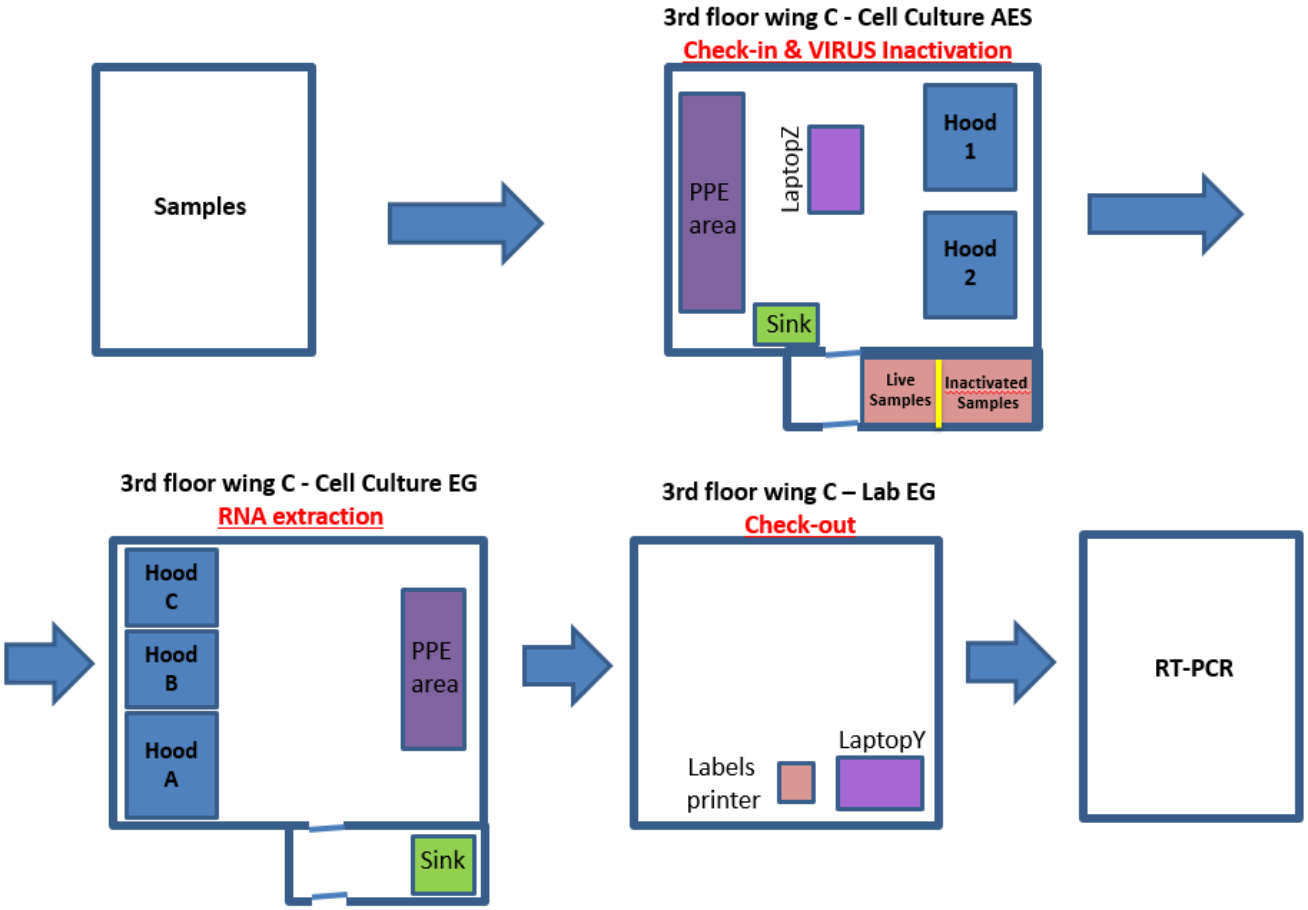








C. Graphical representation of the workflow



D. Personal Belongings

- ➢ All staff will receive a list of items to bring with them to IMM when called, such as, spare clothes, hair band, etc.
- ➢ Upon arrival the staff must go to the meeting room, register in the login sheet, and choose a table to leave their personal items. All personal belongings must stay in this room. Cell phones are exceptionally allowed, only if an urgent call is expected. In such cases, the phone must be placed in a ziplock bag and left by the Laptop Y.

E. Detailed Standard Operating Procedure (SOP)

● Transversal information to all stations

- ➢ Use of the **surgical mask is mandatory**.
  If you are experiencing any symptoms, such as cough or sneezing, please inform the coordinators.
- ➢ **NO SPRAYs** can be used, as these will create aerosols; bleach wipes will be used
- ➢ Full cover clothes. No open shoes or shorts/skirts.
- ➢ Hair always tied up and no long beards.
- ➢ No jewelry or watches of any kind.
- ➢ Run UV 30 min in all Hoods before and after use
- ➢ Bleach wipes and Wastes have to be made fresh every 24h!
- ➢ Check all Personal Protection Equipment, PPE stocks
- ➢ Check all pipette filter tip and reagents stocks
- ➢ Ensure there is only the minimum essential material inside Hoods to follow the SOP

● Sample Transport HSM

Receive samples at hospital and transport to iMM

1. Personal Protection Equipment:

- ➢ Wear regular lab coat over your clothes
- ➢ Put hair up and restrain it
- ➢ Wear disposable Wrist Length Gloves (remove one glove when entering iMM)
- ➢ Wear surgical mask (optional)

2. Go to Microbiology Laboratory, 4th floor, HSM building.

3. Samples are transported in decontaminated containers provided by HSM. (Each sample vial exterior is also decontaminated before placed into the container.)

4. Transport samples to iMM-Virus Inactivation Room. Leave them in the designated area in ante-chamber.

● Sample Transport from Red Cross

Red Cross ensures sample transport to IMM.

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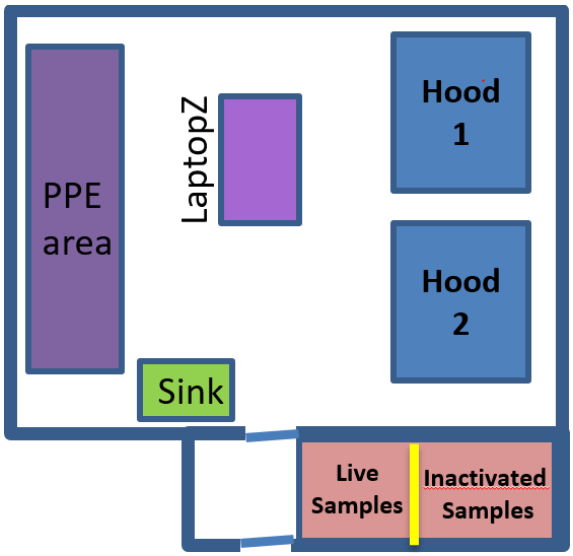
- Virus Inactivation ROOM



➢ In this station, we have **2 Hoods**. Each with one **person working (operator) on the BSC** and **one person overseeing (overseer)** the work at all times. The overseer must be standing behind the operator during the procedure. The overseer will ensure the operator follows all the procedures and will be ready to help in any emergency. **One person sitting away** from the Hoods is responsible **to check-in the samples** on Laptop Z **(check in person)**. One extra person might come to the ante chamber temporarily to drop or pick-up samples, but never enter the virus room.
Maximum capacity with 2 Hoods running: **5 people**
Minimum capacity 1 Hood working: 3 people

➢ Samples will arrive in 15 mL falcons containing a swab in 1 mL of transport medium. From here, 200 µL are transferred to Eppendorfs. 400 µL of Lysis Buffer are added to the 200 µL of sample to inactivate the virus. The falcons are stored at -80 in boxes labelled “Original Swab Box #X” and the inactivated virus proceeds to the RNA Extraction Room. Detailed protocol below, short protocol attached.

➢ Everyone in this room must wear the same personal protection equipment (only exception is the person in the Laptop, who does not need to wear elbow long gloves because this person will never touch the samples under any circumstance)

➢ The person in Laptop Z, inside the Virus Inactivation Room is responsible for checking in all the samples, and anotatting all the details related to the samples for this room. (document attached)

- ➢ The person handling Laptop Y, in EG Lab (room P3-C-48), must wear a regular Lab Coat and NO GLOVES. They will check the live document being filled in the Virus Inactivation Room.

- ➢ SPILL KIT - in case of a spill, **cover the spill with BLEACH WIPES** (or Biocidal). **DO NOT throw anything directly on the spill.**

- ➢ **Before you enter this Room, Be sure to do a Bathroom Run.** You will be there for 4 hours using PPE. We cannot waste PPE for bathroom runs.

- ➢ **Maximum of 18 samples** will be processed in any inactivation round.

DETAILED PROCEDURE

This procedure applies to the operator, unless specifically noted.
If you receive the samples already as 200 µL eppendorfs, start on step 30.

1. Put **Lab coat and gloves on**.
2. **Follow the checklist for the Virus Inactivation Room** (document attached) to ensure all reagents and materials are available and placed correctly.
3. **Wash Hands in the sink** with soap.
4. Put **PPE ON** (next image and documents attached):

* Check MASK Flow Chart

5. **Set up Biosafety cabinet (BSC)** with all essential reagents and materials:
    a. Benchcoat,
    b. Vortex,
    c. Solid waste,
    d. 1L Bleach waste,
    e. 1000 µL pipette tip Box,
    f. 1000 µL pipette,
    g. Disposable/sterile pasteur pipette,
    h. **Bleach wipes @15% bleach,**
    i. Bleach squirt bottle,
    j. Ethanol squirt bottle,
    k. Rack 1 and Rack 2 for eppendorfs,
    l. Rack A and Rack B for falcon tubes,
    m. Lysis Buffer (prepared by RNA Extraction Room Overseer),
    n. Eppendorf labels,

o. Parafilm strips,
p. Rack 3 and Rack C or Storage Box on the table next to spill Kit.

6. Collect samples from antechamber and move the **Sample Container into the working area** (on the benchcoat).
7. Open container (if bagged, remove container from the bag and open it). Discard bag in solid waste container.
8. **Remove Falcon tubes**. (If individually bagged, remove from bag). Discard bag in solid waste container.
9. **Wipe Falcon tubes with Bleach Wipes, check that the lid is tightly screwed on,** and move it to Rack A (if Falcon tubes come labelled with numbers) or Rack B (if Falcon tubes come labelled with barcode).
10. Repeat 8-9 for all samples inside the container.
11. **If Falcon tubes come labelled with numbers**, "sing" the numbers to the Check-In person and simultaneously label the Falcon tube with a numbered square green or red (for priority samples) sticker.
12. **If Falcon tubes come labelled with barcode**, move Falcon tubes from **Rack B to Rack C, being held by the overseer outside of the BSC. Overseer** takes **Rack C to the check-in area to scan bar codes. For all samples (one by one):**
    a. **Overseer labels Falcon tubes** with internal iMM number (1-xxxx) placing green labels on the tube side of the falcon (most important).
    b. **Flagged Falcon tubes** (red sticker) should be labelled with numbered square red labels
    c. Check-in person Scans barcode (each tube should be removed from Rack to be scanned individually, to avoid misreadings).
13. **IN THE MEANTIME**
    a. Inside the BSC, open the flask with **Eppendorf tubes with lock mechanism** and place opened them in Rack 1. (place the number of tubes necessary to match samples to inactivate).
    b. **Close Eppendorf tubes** in Rack 1, **label each tube with white circle labels**, and move Rack 1 to the back of the benchcoat area.
    c. **Close the flask** of Eppendorf tubes and **move it** to the side.
14. Overseer brings **Rack C to BSC**.
15. Transfer Falcon tubes from **Rack C to Rack B**.
16. **Vortex** each Falcon tube for **10 seconds** and return to **Rack A**. Move Rack A to the back.
17. **While waiting for aerosols to settle in Falcon tubes in Rack A**:
    a. Bring **Rack 1** forward
    b. Open tips box and Inactivation Buffer tube
    c. One by one, **open Eppendorf tube in Rack 1**, **pipette 400 ul Inactivation Buffer** into **Eppendorf tube**, and **close Eppendorf tube**
    d. Close tips box and Inactivation Buffer Tube. Move tips box, Inactivation Buffer rack, pipette and Rack 1 to the back.
18. Bring **Rack A** forward

19. **One by one, remove Parafilm** from Falcon tube and **place green or red square label on the lid**, with the number matching that on the tube.
20. Ensure **liquid waste bottle** is **on the pad**
21. For all samples **one-by-one**:
    a. **Move Falcon tube** to empty space on Rack A
    b. **Loosen lid** of Falcon tube but leave it on the tube
    c. **Open Eppendorf tube** (number 1-xxxx) and leave it on Rack 1,
    d. Pick-up plastic **Pasteur pipette**. Unwrap and discard wrap in solid waste,
    e. **Open Falcon tube** (number 1-xxxx),
    f. **Collect 200 µL** of the sample from Falcon tube with a plastic Pasteur pipette, bring Eppendorf tube close to Falcon tube, and transfer the sample into the Eppendorf tube. NOTE: the lysis buffer inactivates viruses and bacteria,
    g. **Discard plastic Pasteur pipette** in liquid waste container,
    h. **Close Eppendorf tube,**
    i. Close Falcon tube.
    j. Return Falcon tube to its intended position in **Rack A**,
22. **Move Rack A to the back of the BSC**, but leave it in the working area.
23. **Close** liquid waste bottle.
24. **Move Rack 1 forward** in the working area
25. **Vortex each Eppendorf tube for 10 seconds** and return to **Rack 1**.
26. **Wipe Eppendorf tubes, one-by-one, with Bleach Wipe and move to Rack 2.**
27. **Move Eppendorfs** tubes from **Rack 2 to Rack 3, being held by the overseer outside of the BSC.**
28. Overseer **checks volumes in Eppendorf tubes** for unexpected inconsistencies.
29. Overseer places **Rack 3 in the antechamber** in the clean zone, for RNA extraction.
30. **Wipe Falcon tubes, one-by-one, with Bleach Wipe and move to Rack B.**
31. **Put Parafilm on Falcon tubes.**
32. **Move Falcons** tubes from **Rack B to Rack C or Storage Box, being held by the overseer outside of the BSC.**
33. Overseer places **Rack C or Storage Box in the antechamber** in the clean zone, for storage.
34. Person in the laptop calls RNA Room to announce samples are ready for pick up.
35. **Disinfect** the inside and outside of the **Samples Container** with Bleach Wipes and move it outside of the working area for collection.

36. **IF swapping positions with the overseer:**
37. Wipe Rack A with Bleach Wipes.
38. Remove outer gloves, exit the BSC, and put on a new pair of outer gloves.
39. The person overtaking the work at the BSC should check that all necessary materials are in place and then proceed from Step 6.

40. **WHEN closing for the day OR for the next team, proceed with full decontamination procedure:**

41. **Wipe all items OUTSIDE the working area** with Bleach Wipes (squirt bottles, box of bleach wipes, solid waste container, liquid waste container, samples container, parafilm box, Rack 2 and Rack B).
42. **Wipe all items INSIDE the working area** with Bleach Wipes (rack A, Tips Box, Pipette, Buffer, Buffer Rack) and move out of pad as they are wiped.
43. Carefully **close the pad** of the working area and **trash it in the solid waste bag**.
44. **Wipe all the surface of BSC with Bleach Wipes**.
45. Wipe the **surface of BSC and the P1000 Pipette with 70% ethanol** to remove traces of bleach.
46. Discard all wipes in the solid waste bag.
47. Close the **solid waste bag** inside the BSC, twist it tight, fold it down and hold it in place using rubber bands.
48. Wipe the outer surface of the solid waste bag with **Bleach wipes**.
49. Collect **solid waste bag and liquid waste flask**, exit the biosafety cabinet and **place in Big Red Bin.**
50. **Dispose of outer gloves** and **used bleach wipes** in the same Big Red Bin.
51. **Leave all wiped materials in the BSC for UV decontamination.**
52. **Overseer** closes the sash, turns off light and air flow, and turns on the UVs. **Overseer** must come back to **turn off the UVs after approximately 30 min**.
53. Continue **removing PPE**:

* Check MASK Flow Chart

- Emergency action in case of a spill:

In considering the response to any incident, DO NOT PANIC.
In the event of a spill inside the BSC, always leave the BSC ON.

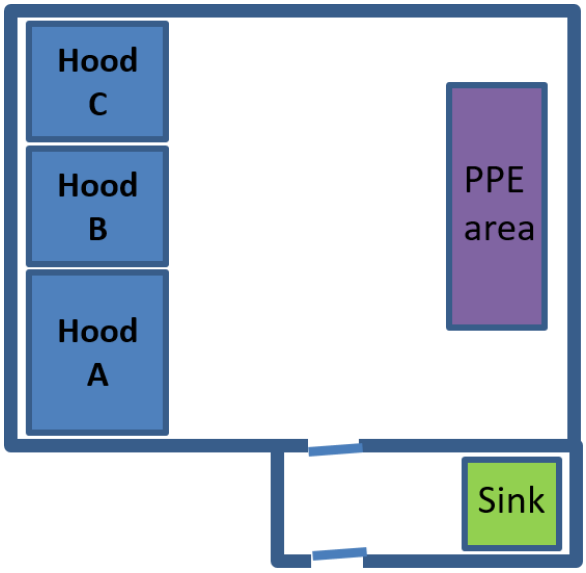
- ➢ Small spills (of less than 1ml) will probably be contained by the benchcoat on the workspace. Immediately cover with Bleach Wipes, allow it to act for 20 minutes, and discard the benchcoat into the primary solid waste bag. Close the bag with a rubber band, wipe it and place in the solid container for disposal. Replace the external gloves. Continue work, if necessary (using a fresh benchcoat sheet) after wiping the area clean with bleach wipes followed by wiping with 70% ethanol.
- ➢ If the spill is not on the benchcoat, cover with Bleach Wipes while leaving the BSC running. Immediately cover with Bleach Wipes, allow them to act for 20 minutes. Thoroughly wipe the area and discard the waste into the primary solid waste bag. Close the bag with a rubber band, wipe it and place in the solid container for disposal. Replace the external gloves. Continue work, if necessary (using a fresh benchcoat sheet) after wiping the area clean with bleach wipes followed by wiping with 70% ethanol.
- ➢ If a spill occurs outside the Biosafety Cabinet, all personnel should exit the room immediately. Once outside the room, remove PPE following the regular procedure and place all disposable items in a red container. Wait 1 hour before re-entering the room, to allow time for aerosols to settle. Put on new PPE before entering the room and proceed to clean the spill. Cover with Bleach Wipes, allow it to act for 20 minutes. Thoroughly wipe the area and discard the waste into a primary solid waste bag. Close the bag with a rubber band, wipe it and place in the solid container for disposal. Replace the external gloves. Continue work, if necessary.

If your overall is contaminated, remove any contaminated clothing / PPE, bag it, and discard in the solid waste container.

In the unlikely event that a spill may reach your clothes, emergency clothing is available and will be provided.

- ➢ Inform coordinators for further assistance.

- RNA Extraction Room



➢ In this station we have **3 hoods**. Each hood is one RNA Extraction station. Stations can work simultaneously.
One person will be working per hood (**operator**),
One person will oversee the entire room standing (**overseer**),
Maximum capacity, 3 Hoods running: **4 people,**
Minimum capacity, 1 Hood working: **2 people**.

➢ The team members of each shift will be added to the Samples log form in Laptop Y, in EG Lab (room P3-C-48).

➢ The overseer will prepare the Lysis Buffer (Buffer NR w/ 1% of 2-mercaptoethanol) in the fume hood. and deliver it to the antechamber of the Virus Inactivation Room

➢ The overseer will pick up the Rack 3 with samples from the antechamber of the Virus Inactivation Room once ready.

➢ Everyone working in the RNA room will wear PPE: Lab coat; Wrist length gloves; Surgical mask.

➢ The person in Laptop Y, will print the labels according to the information received from the Virus Inactivation Room and bring them to the RNA Extraction Room.

Before starting the protocol Operator and Overseer should go through the detailed checklist (attached) and make sure that all the following reagents and materials are in place:

Overseer will check materials in **Fume hood**:

a. 2-Mercaptoethanol,
b. 50 mL Falcon Tubes, Pippetes
c. Buffer NR.

Operator will check material in **Biosafety Cabinet**:

a. Waste bottle with 15% bleach (not provided by the kit),
b. 70% EtOH, in RNAse free water (not provided by the kit),
c. Buffer NI,
d. Buffer NWR1,
e. Buffer NWR2, with EtOH added,
f. RNase-free Water,
g. Collection tubes (5x the number of samples).

Notes:

- ➢ We will be using the NZY Total RNA isolation kit from NZYTech.
- ➢ All centrifugations must be performed inside the BSL2 safety cabinet.
- ➢ After each centrifugation step transfer the column to a new collection tube and discard the previous collection tube with the flow through.

DETAILED PROCEDURE

Overseer in EG lab Fume hood:

1. Prepare the Lysis Buffer for the Virus inactivation Room. Pipette the necessary volume (+ 10% excess) of NR Buffer, into a 50 mL Falcon tube, and add 1% of 2-Mercaptoethanol.
2. Place it in the antechamber of the Virus Inactivation Room.
3. Go back to the RNA Extraction Room to oversee the extraction protocol.

Operator, in the hood in the RNA Extraction Room:

3. Label the NZYSpin Binding column (blue ring) with the internal iMM number (1-xxxx).
4. Prepare the 3 racks according to the rack organization attached.
5. Label 2 sets of 1.5mL RNAse-free Eppendorf tubes for RNA elution with the internal iMM number (1-xxxx) written on the lid.

After receiving the samples brought by overseer from the Virus inactivation room in Rack 3:

6. **Centrifuge** the tubes at **11,000 *g* for 1 min** to remove drops from the lid. Check for precipitates. In case there are precipitates transfer the supernatant to a new eppendorf and proceed to step 7.
7. One by one, add **600 µL of 70% EtOH**. Mix immediately by pipetting up and down at least 2 times.
8. Pipette **600 µL of the lysate** onto the column. **Centrifuge** at **11,000 *g* for 1 min.** Transfer the column into a new collection tube, and discard the previous collection tube with the flow-through in the waste bottle.
9. Pipette the remaining **600 µL of the lysate** onto the column. **Centrifuge at 11,000 *g* for 1 min**.

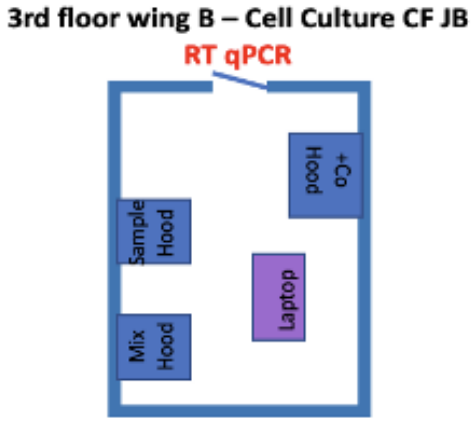
   Transfer the column into a new collection tube, and discard the previous collection tube with the flow-through into the waste bottle.
10. Pipette **350 µL of Buffer NI** into each column. **Centrifuge at 11,000 *g* for 1 min.** Transfer the column into a new collection tube, and discard the previous collection tube with the flow-through into the waste bottle.
11. Add **200 µL of Buffer NWR1** into each column. **Centrifuge at 11,000 *g* for 1 min**. Transfer the column into a new collection tube, and discard the previous collection tube with the flow-through into the waste bottle.
12. Add **600 µL of Buffer NWR2** into each column. **Centrifuge at 11,000 *g* for 1 min**. Transfer the column into a new collection tube, and discard the previous collection tube with the flow-through into the waste bottle.
13. Add **200 µL of Buffer NWR2** into each column. **Centrifuge at 11,000 *g* for 1 min**.
14. **Do not remove the samples from the centrifuge**. Centrifuge again at 11,000 *g* for 1 min (to dry the membrane).
15. **Place the column in a 1.5 mL eppendorf tube**. Add **50 µL RNase-free water** directly to the column membrane (without touching in the membrane). Discard the collection tube into the waste bottle.
16. **Incubate for 1 minute**. **Centrifuge at 11,000 *g* for 2 min** to elute the RNA. Discard the column into the waste bottle.
17. Transfer **25 µL of the RNA** into the second eppendorf tube.
18. Label each RNA with the **labels** brought to the room by the Laptop Y person.
19. Store one RNA aliquot in the **RNA Stock Box (1- XX)** at -20ºC and take another RNA aliquot to the PCR freezer to be processed for RT-PCR.

WHEN closing for the day proceed with cleaning procedure:

20. Close the full Waste Bottle.
21. Turn OFF centrifuge.
22. Remove all buffers and solutions.
23. Wipe the surface of BSC with 70% ethanol
24. Wipe all the surface of BSC with RNAse OUT wipes..
25. Turn ON UV (30min).

Between shifs clean the BSC surface with RNAse OUT wipes.

- RT-qPCR Room (This section of the document is still being updated)



- ➢ In this station we have 3 hoods: Mix / NTC hood; Samples / Negative Control hood and Positive Control (+Co) hood. **Workflow in the laboratory should proceed in a unidirectional manner.**
- ➢ One person will be preparing the plate (**operator**).
- ➢ One person will oversee the work standing, and register the team members of each shift in the Samples log form using the computer in this room (**overseer**).
  Maximum capacity: 2 people.

IMPORTANT NOTES:

- ➢ The RT-qPCR protocol for the detection of Sars-CoV-2 described below is a changing document that may be updated in the future as the RNA sequence of the virus evolves. Thus, the primers and probes used may need to be optimized in the future, by aligning their sequences with the available viral sequences that are circulating in the population at the time of diagnosis.
- ➢ The RT-qPCR Room will be physically separated from Virus Inactivation and RNA Extraction Rooms. Moreover, in this room, work is performed with extracted nucleic acids. Thus, samples do not pose any danger to operators.
- ➢ To avoid sample and reagent contamination with genetic material from the operator (and given that contact with other people involved in the work will be required), standard protective equipment will be mandatory in the room: everyone is expected to wear a mask, room designated lab coat and gloves.
- ➢ Amplification technologies, such as PCR, are sensitive to accidental introduction of PCR products from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the real-time PCR reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon).

Other considerations:

- ➢ Maintain separate areas for assay setup (Mix hood) and handling of nucleic acids (Sample hood).
- ➢ Maintain **separate areas** as well as **separate, dedicated equipment** (e.g., pipettes, microcentrifuges) **and supplies** (e.g., microcentrifuge tubes, pipette tips) for assay setup (Mix hood) and handling of nucleic acids (Sample hood).
- ➢ **Do not substitute or mix reagents** from different kit lots or from other manufacturers.
- ➢ **Change aerosol barrier pipette tips** between all manual liquid transfers.
- ➢ During preparation of samples, compliance with good laboratory techniques is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of nucleases into samples during and after the extraction procedure. Proper aseptic techniques should always be used when working with nucleic acids.
- ➢ Maintain separate, dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g., microcentrifuge tubes, pipette tips) for assay setup and handling of extracted nucleic acids.
- ➢ Wear a clean lab coat and powder-free disposable gloves (not previously worn) when setting up assays.
- ➢ Change gloves whenever contamination is suspected.
- ➢ Keep reagents and reaction tubes, in boxes, capped or covered as much as possible.
- ➢ Primers, probes (including aliquots), and enzyme master mixes must be thawed and maintained on cold block at all times during preparation and use.
- ➢ Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products such as 10% bleach, “DNAZap™” or “RNase AWAY®” to minimize risk of nucleic acid contamination.
- ➢ RNA should be maintained on cold block, or on ice, during preparation and use, to ensure stability.

Reagents and Supplies

a. rRT-PCR primer/probe sets,
b. Positive template controls,
c. Nuclease-free PCR grade 1.5ml tubes,
d. One-step NZYSpeedy RT-qPCR Probe kit, ROX,
e. Molecular grade nuclease-free water,
f. Disposable powder-free gloves,
g. **In Mix Hood:** one full set of micropipettes (P10 to P1000) + one P100 dispenser pipette,
h. **In Sample Hood:** one P10 pipette + one 8-channel P10 pipette,
i. **In +Co Hood:** one P10 pipette,
j. P2/P10, P20, P100, P300 and P1000 aerosol barrier tips will be distributed in each BSC and **are never exchanged between BSCs**.
k. PCR Machines: ABI 7500 Fast Real Time PCR system with ABI Quant Studio 5 software,
l. 96-well real-time PCR reaction plates and optical seals,

m. Acceptable surface decontaminants:
- RNase Cleaner (NZYtech)
- 70% Ethanol

Primers and probes

Primers and probes used in this diagnostic were published by CDC.
(available on https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html)

These include:

a. 2 primers + probe sets for 2 different virus gene regions (N1 and N2)
b. 1 human gene primer + probe set (RNAse P)

All probes are to be read in the FAM fluorescence and each gene detection (N1, N2 and RP) requires one individual PCR reaction.

Table 1 -2019-Novel Coronavirus (2019-nCoV) Real-time rRT-PCR Panel Primers and Probes

2019-Novel Coronavirus (2019-nCoV) Real-time rRT-PCR Panel Primers and Probes			
Name	**Description**	**Oligonucleotide Sequence (5'>3')**	**Label1**
2019-nCoV_N1-F	2019-nCoV_N1 Forward Primer	5'-GAC CCC AAA ATC AGC GAA AT-3'	None
2019-nCoV_N1-R	2019-nCoV_N1 Reverse Primer	5'-TCT GGT TAC TGC CAG TTG AAT CTG-3'	None
2019-nCoV_N1-P	2019-nCoV_N1 Probe	5'-FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1-3'	FAM, BHQ-1
2019-nCoV_N2-F	2019-nCoV_N2 Forward Primer	5'-TTA CAA ACA TTG GCC GCA AA-3'	None
2019-nCoV_N2-R	2019-nCoV_N2 Reverse Primer	5'-GCG CGA CAT TCC GAA GAA-3'	None
2019-nCoV_N2-P	2019-nCoV_N2 Probe	5'-FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1-3'	FAM, BHQ-1
RP-F	RNAseP Forward Primer	5'-AGA TTT GGA CCT GCG AGC G-3'	None
RP-R	RNAseP Reverse Primer	5'-GAG CGG CTG TCT CCA CAA GT-3'	None
RP-P	RNAseP Probe	5'-FAM – TTC TGA CCT GAA GGC TCT GCG CG – BHQ-1-3'	FAM, BHQ-1

[1]TaqMan® probes are labelled at the 5'-end with the reporter molecule 6-carboxyfluorescein (FAM) and with the quencher, Black Hole Quencher 1 (BHQ-1) at the 3'-end (Eurofins Genomics

Primer and probe stock solutions preparation upon arrival:

1. When primers and probes arrive they should be taken to the **Mix Hood** in **the RT-qPCR Room**.

IMPORTANT NOTE: It is mandatory that these never go to the sample preparation area (Virus Inactivation room) or to the Sample Hood in the RT-qPCR Room. Primers and probes could become contaminated with viral nucleic acids.

2. Both primers and probes should be resuspended with RNAse-free water to 100 µM (stock concentration).
3. Primer and probe stocks should be aliquoted and stored at -20^{o}C:

a. Probes are aliquoted as 5 µL aliquots of 100 µM solution,
b. Primers are aliquoted as 20 µL aliquots of 100 µM solution.

4. Thaw an aliquot of each primer and one for the probe. Prepare a combined primer/probe mix (**Combined PP**) for each set (N1, N2, RP) by mixing:

a. 405 µL RNAse free water,
b. 5 µL probe stock solution,
c. 20 µL Fw primer stock solution,
d. 20 µL Rev primer stock solution,
e. Aliquot the Combined PP in 75 µL aliquots and store at -20^{o}C until use.

5. One **Combined PP** mix aliquot should be thawed, kept at 4^{o}C at all times and discarded after a single use.

Reaction Master Mix and Plate Set-Up (Mix Hood)

Notes:

Each RT-qPCR reaction preparation involves two team members: one operator handling the reagents and samples and one overseer double checking everything, registering all samples and setting info in the computer and providing any required support to the operator.

Clean and decontaminate all work surfaces, pipettes, centrifuges and other equipment prior to use, using RNase Away® followed by 70% EtOH.

Plate set-up configuration can vary with the number of specimens and work day.
Include always N1, N2 and RP primers/probe sets for each sample.

Additionally include NTCs and +Co+ samples for each mix in each run.

1. Make plate design considering the number of samples to be included.
2. Determine the number of reactions to set up per assay. It is necessary to make excess reaction mix for NTC, positive control (+Co) reactions.
3. For each primers/probe set, calculate the amount of each reagent to be added for each reaction mixture. Calculate always the amounts needed +15% in order to account for loss during pipetting. An excel worksheet will be prepared for each RT-qPCR run and is shown as an example in annexes.

Table 2 - Master Mix Composition

Reagent	Vol. of Reagent Added per Reaction (µL)
Nuclease-free Water	3.2 µL
Combined PP	1,8 µL
NZYSpeedy RT qPCR Probe master mix (2x)	10.0 µL

4. Thaw NZYSpeedy RT-qPCR Probe master mix and Combined PP prior to use.
5. In the Mix Hood, place NZYSpeedy qPCR Probe master mix and Combined PP on cold-block. Keep cold during preparation and use.
6. Label one 1.5 mL eppendorf for each primers/probe set.
8. Dispense reagents into each respective labelled 1.5 mL microcentrifuge tube. After addition of the reagents, mix reaction mixtures by pipetting up and down. ***Do not vortex***.
9. Order of addition is Nuclease-free Water; One-step NZYSpeedy RT-qPCR Probe master mix (2x), Combined PP.
10. Centrifuge for 5 seconds to collect contents at the bottom of the tube, and then place the tube in a cold rack.
11. Set up a reaction plate in a 96-well rack.
12. Dispense 15µL of each master mix into the appropriate wells using the dispenser pipette.
12. Prior to moving to the Sample Hood, prepare the NTC reactions in the Mix Hood.
13. Pipette 5 µL of nuclease-free water into the NTC sample wells. Cap NTC wells before proceeding.
14. Cover the entire reaction plate and move the reaction plate to the specimen nucleic acid handling area (Sample Hood).

Template Addition (Sample Hood)

15. Spin down nucleic acid sample tubes for approximately 10 seconds.
16. After centrifugation, place extracted nucleic acid sample tubes in the cold rack.
17. Transfer 18ul of RNA samples to 8 tube PCR strips to transfer to the plate with a multichannel pipette.
18. Samples should be added to the specific assay that is being tested. Using the 8-channel pipette. CAREFULLY pipette 5.0 µL each sample into the respective wells labelled for that sample.*Change tips after each addition.*
19. Change gloves often and when necessary to avoid contamination.

20. Cover the entire reaction plate and move the reaction plate to the positive template control handling area (Positive Control Hood).

Assay Controls

a. Assay controls should be run concurrently with all test samples.
b. +Co – positive template control with an expected Ct value range – (EDX SARS-CoV-2 Standard, ExactDiagnostics) (Positive Control Hood)
c. NTC – negative template control added during RT-PCR reaction set-up – no template addition.

RP – all clinical samples should be tested for human RNAse P (RP) gene to assess specimen quality.

Assay Control Addition

21. Pipette 5 µL of +Co to the appropriate sample wells (Positive Control Hood)
22. Carefully remove NTC caps
23. Seal plate with appropriate optical seal
24. Centrifuge plates for 30 seconds at 500 x g, 4°C.

NOTES:

Calculations for 29 samples See TEMPLATE MIXES NZYSpeedy qPCR annexe

Vol final	20	ul
Number of Samples+Controls	31	
Excess	15	%

	Volume per tube (ml)	Vol per Mix	Vol per Mix + excess
(Template)	5		
3. Combined PP	1.8	54.0	62.1
2. RT + qPCR master mix	10.0	300.0	345
1. H2O	3.2	96.0	110.4
TOTAL		450.0	517.5

Plate arrangement for 29 Samples:

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC1	NTC1	NTC1	S8	S8	S8	S16	S16	S16	S24	S24	S24
B	S1	S1	S1	S9	S9	S9	S17	S17	S17	S25	S25	S25
C	S2	S2	S2	S10	S10	S10	S18	S18	S18	S26	S26	S26

D	S3	S3	S3	S11	S11	S11	S19	S19	S19	S27	S27	S27
E	S4	S4	S4	S12	S12	S12	S20	S20	S20	S28	S28	S28
F	S5	S5	S5	S13	S13	S13	S21	S21	S21	S29	S29	S29
G	S6	S6	S6	S14	S14	S14	S22	S22	S22	NTC2	NTC2	NTC2
H	S7	S7	S7	S15	S15	S15	S23	S23	S23	+Co	+Co	+Co

Equipment preparation

25. Turn on the qPCR machine.
26. Perform plate set up and select cycling protocol on the instrument.
27. Instrument Settings:
    a. Detector (FAM),
    b. Quencher (None),
    c. Passive Reference: (None),
    d. Run Mode: (Standard),
    e. Sample Volume (20 µL).

Table 3 - RT-qPCR protocol

4. Equipment preparation			
Step	**Cycles**	**Temp**	**Time**
RT incubation	1	50°C	20 min
Enzyme activation	1	95°C	5 min
Amplification *	45	95°C	3 sec
		55°C	30 sec *

* Fluorescence data (FAM) should be collected during the 55°C incubation steps.

PCR Running and results

28. Put the plate on the PCR machine.
29. Name the Run according to the flowing codding: (1-XX)_PCR_YYYY_MM_DD
30. Register iMM Sample numbers on corresponding wells.
31. Run the PCR according to the above setup.
32. When PCR run is finished, adjust the threshold above the noise and to fall on the exponential part of the curves. Use preferentially automatic setup of threshold.
33. Export data has an excel file with indication of CT values for each sample and each probe. Excel files should be coded has: (1-XX)_PCR_YYYY_MM_DD
34. Save raw data .eds file to the server.
35. Fill in the table below for each sample

36. PCR ID: (1-XX)_PCR_YYYY_MM_DD

Table 4 - Analysis of RT-qPCR and Covid Diagnostic

	N1	N2	RP	Conclusion
Sample ID	**CT or Undeterm.**	**CT or Undeterm.**	**CT or Undeterm.**	**Detected or Undetected or Inconclusive**
NTC	**CT or Undeterm.**	**CT or Undeterm.**	**CT or Undeterm.**	
+Control	**CT or Undeterm.**	**CT or Undeterm.**	**CT or Undeterm.**	

Conclusions:

Detected : +;+;+ and NTC -;-;- and +C +;+;+

Undetected -;-;+ and NTC -;-;- and +C +;+;+

Inconclusive: one of the viral probes gives + and other neg, if + after CT35 and curves are not as expected, if neg for viral probes and neg for RP; if NTC is positive; if neg but +Co is also neg.

Room cleaning and preparation

After the plate is running go back to the RT-qPCR room and prepare room for next plate setup:

37. Using new gloves restore reagents in the Mix Hood to proper storage
38. Discard all tubes and used materials in the Mix Hood
39. Clean the Mix Hood with RNAse Cleaner followed by 70% EtOH
40. Close the Hood and put on the UVs for 15 minutes
41. Restore samples in Sample Hood to proper storage or discard them if no longer required.
42. Repeat steps 2-4 for Sample Hood
43. Restore +Co in +Co Hood to proper storage or discard it if no longer required.
44. Repeat steps 2-4 for +Co Hood

F. Risk Assessment and Risk Control Measures

The risk assessment was made taking into account the contributions of the Hospital de Santa Maria (HSM), the orientations of the World Health Organization presented in the document "Laboratory Biosafety Guidance related to coronavirus disease 2019 (COVID-19), Interim Guidance, 12 february 2020" and the UK Guidance "COVID-19: Safe Handling and Processing for samples in laboratories, 12 march 2020".

During your presence in the Egas Moniz Building (EMB) and through all the procedures, do not forget to follow the COVID-19's prevention rules: social distance (specially if someone is sick - more than 1 metre distance); respiratory hygiene, wash your hands frequently or disinfect them and do not touch touch eyes, mouth and nose with unclean hands. If you are sick, please stay at home!

Before starting these procedures, everyone must be trained and sign a responsibility term.

Description of Risk	Risk Control Measure
Biological Hazard - picking up samples in an Hospital	● Maintain, whenever possible, a safe social distance. ● The sample must be transported in a double container. ● The sample tube and transport box must be decontaminated by the hospital personnel, before delivering it; ● Do not accept a sample box in bad shape, dirty or with leakage signs; If the person needs to enter the hospital installations: ● Use PPE: lab coat, gloves and surgical mask (optional). ● Before entering the EMB, remove the mask and one glove to open doors and push elevator buttons. ● Do not touch the mask or eyes with gloves or unclean hands. ● Wash your hands after delivery of the samples.
Biological hazards - Virus room COVID-19 Medium risk	● Work must be performed under BSL-2+ conditions. ● Use a validated Biosafety Cabinet (BSC). ● Follow good practices when working in the BSC (ex: slow movements; only the essential material inside, without covering the BSC grids). ● Use absorbent material to cover the surface of the BSC, where contaminated material is going to be used. ● Use of PPE: double lab coat, double gloves (one elbow length + one wrist length); safety goggles, disposable safety mask (FFP2/ FFP3), cap and shoe covers. ● Follow the dress and undress procedures, in the following order: ○ Dress

	■ Take off adornments and tie hair, ■ Dress Lab coat, put shoe covers and disinfect hands; ■ Dress disposable lab coat, put mask and safety goggles and cap on, and disinfect hands; ■ Put elbow length gloves, on top of the lab coat and wrist gloves over those. ○ Undress ■ Take off shoe covers and disposable lab coat; ■ Take off cap and safety goggles; ■ Take off gloves and disinfect hands; ■ Take off mask; ■ Wash hands. ● PPE and all contaminated or possibly contaminated material must be disposed of in a red waste bag, for incineration. ● All contaminated or possibly contaminated material must be disposed of at the end of the procedure; ● Clean and decontaminate work area at the end of the procedure; ● The person that is working in the BSC must always be overseen by someone, to help him/her in case of need/emergency. ● Before leaving the BSC, all tubes with inactivated samples must be decontaminated and transferred to a clean rack.
Chemical Hazard - RNA extraction Room Very Low Risk	● Work must be performed in BSL-2. ● Use a validated Biosafety Cabinet (BSC). ● Follow good practices when working with BSC (ex: slow movements; only the essential material inside, without covering the BSC grids). ● Use of PPE: lab coat, wrist-length gloves, surgical mask. ● Disposable PPE material must be disposed of in white waste bag; ● All material must be disposed at the end of the procedure; ● Clean and decontaminate work area at the end of the procedure; ● Wash your hands at the end of the procedure.
Chemical Hazard - Prepare Lysis Buffer Medium risk	● Work must be performed in a Chemical/Fume hood; ● Follow good practices when working in a Chemical/Fume hood (ex: the exhaustion must be on, sach must be opened 15 cm). ● Use of PPE: Lab coat, safety goggles, gloves (check the products safety data sheet).
Chemical Hazard - RNA extraction Room Low risk	● Work must be performed in BSL-2. ● Use a validated Biosafety Cabinet (BSC). ● Follow good practices when working with BSC (ex: slow movements; only the essential material inside, without covering the BSC grids). ● Use of PPE accordingly with the area you are going to work: inactivation room or RNA extraction room (lab coat and gloves mandatory).

Chemical/Biological Hazard - RT-PCR Room Low risk 26_03_2020	● Work must be performed in BSL-2. ● Use a validated Biosafety Cabinet (BSC). ● Follow good practices when working with BSC (ex: slow movements; only the essential material inside, without covering the BSC grids). ● Use of PPE (lab coat, gloves) ● Wash hands in the end of procedure
Generation of aerosols and droplets	● Aerosol production must be avoided. ● All practices that may produce aerosols must be performed inside the BSC (ex.: vortexing) ● Using sprays is forbidden,only BLEACH WIPES (Bleach 15%) ● In Virus Inactivation Room wear FFP2 / FFP3 masks.
Emergency Procedures	
Biological spill/leaking sample	● If the spill occurs inside the BSC: ○ Cover with bleach wipes ○ Remove the absorbent material in the BSC and put it in the waste bag inside the BSC; ○ Close the bag and discard ○ Discard contaminated wrist gloves and put news ones; ○ Decontaminate all the materials with bleach wipes and the work surface of the BSC. ○ Discard contaminated wrist gloves and put news ones; ○ Place new absorbent material in the work area of the BSC and continue to work. ● General iMM Spill Kit available in the inactivation room. ● Spill Kit available near the BSC in inactivation room: ○ It contains a box of BLEACH WIPES and a bottle of Bleach 15%. ○ Should never be used except for spills. ○ Prepare Fresh every 24h!!! Important: the person that is going to deal with the spill has to be fully equiped with the PPE mandatory for the room. Emergency clothing is available if necessary
Active biological material in contact with skin, eyes, mouth or nose	● Undress PPE; ● Clean the affected area with plenty of water and soap; ● Contact the overseeing person to follow the iMM contingency plan for COVID-19. ● The overseeing person must inform Safety and Compliance.
Chemical/inactivated biological material in contact with skin, eyes, mouth or nose	● Clean the affected area with plenty of water and soap; ● Follow the instructions in the Safety data Sheet.

07_04_2020

Disinfectants	
From WHO	● 62–71% ethanol; 0.5% hydrogen peroxide; quaternary ammonium compounds; and phenolic compounds, if used according to the manufacturer's recommendations. ● Other biocidal agents such as 0.05–0.2% benzalkonium chloride or 0.02% chlorhexidine digluconate can be less effective. ● Particular attention should be paid not only to the selection of the disinfectant but also the contact time (for example, 10 minutes), dilution (that is, concentration of the active ingredient) and expiry date after the working solution is prepared.

Attention:

If there is the need to conserve supplies, like particulate masks/respirators, due to the risk of depletion, a combination of two approaches is recommended, since the engineering protection and their use by the personnel at the highest risk is being applied:

- extended use, and
- limited reuse.

Extended use refers to the practice of wearing the same respirator for repeated procedures, with the same pathogen, the same risk level, in the same physical space, without removing the respirator. Extended use is favored over reuse because it is expected to involve less touching of the respirator and therefore less risk of contact transmission. Extended use has been recommended as an option for conserving respirators during previous respiratory pathogen outbreaks and pandemics.

Good practices:

- Do not use for more than 8 hours;
- Limit potential respirator's surface contamination;
- Practice the correct donning and doffing technique;
- Check the respirator's adherence;
- Discard respirators following use during aerosol generating procedures;
- Discard respirators contaminated with biological samples, chemical products, or with the user's own nasal secretions;
- Perform hand hygiene with soap and water or an alcohol-based hand sanitizer before and after touching or adjusting the respirator (if necessary for comfort or to maintain fit);
- Discard any respirator that is obviously damaged or becomes hard to breathe through.

Reuse refers to the practice of using the same respirator for multiple procedures, but removing it ('doffing') after each one. The respirator is stored in between procedures to be put on again ('donned') prior to the next one. Even when respirator reuse is practiced or recommended, restrictions should be in place to limit the number of times the

same respirator is reused. “Limited reuse” has been recommended and widely used as an option for conserving respirators during previous respiratory pathogen outbreaks and pandemics.

Good practices:

- Do not use for more than 8 hours;
- Limit potential respirator’s surface contamination;
- Practice the correct donning and doffing technique;
- Check the respirator’s adherence;
- Do a physical inspection before every donning (e.g., Are the straps stretched out so much that they no longer provide enough tension for the respirator to seal to the face?, Is the nosepiece or other fit enhancements broken?, etc.);
- Hang used respirators in a designated storage area or keep them in a clean, breathable container such as a paper bag between uses. To minimize potential cross-contamination, store respirators so that they do not touch each other and the person using the respirator is clearly identified. Storage containers should be disposed of or cleaned regularly.
- Avoid touching the inside of the respirator. If inadvertent contact is made with the inside of the respirator, perform hand hygiene as described above.
- Use a pair of clean (non-sterile) gloves when donning a used N95 respirator and performing a user seal check. Discard gloves after the respirator is donned and any adjustments are made to ensure the respirator is sitting comfortably on your face with a good seal.
- Discard respirators following use during aerosol generating procedures;
- Discard respirators contaminated with biological samples, chemical products, or with the user's own nasal secretions;
- Perform hand hygiene with soap and water or an alcohol-based hand sanitizer before and after touching or adjusting the respirator (if necessary for comfort or to maintain fit);
- Discard any respirator that is obviously damaged or becomes hard to breathe through.
- Follow the manufacturer’s user instructions, including conducting a user seal check.
- Follow the manufacturer’s maximum number of donnings (or up to five if the manufacturer does not provide a recommendation) and recommended inspection procedures.
- Pack or store respirators between uses so that they do not become damaged or deformed.
- Respirators must only be used by a single wearer.

Risks of extended use and limited reuse:

The most significant risk is of contact transmission from touching the surface of the contaminated respirator. Contact transmission occurs through direct contact with others as well as through indirect contact by touching and contaminating surfaces that are then touched by other people.

Respiratory pathogens on the respirator’s surface can potentially be transferred by touch to the wearer’s hands and thus risk causing infection through subsequent touching of the mucous membranes of the face (i.e., self-inoculation).

While contact transmission caused by touching a contaminated respirator has been identified as the primary hazard of extended use and reuse of respirators, other concerns have been assessed, such as a reduction in the

respirator's ability to protect the wearer caused by rough handling or excessive reuse. Extended use can cause additional discomfort to wearers from wearing the respirator longer than usual.

Adapted from CDC, *Recommended Guidance for Extended Use and Limited Reuse of N95 Filtering Facepiece Respirators in Healthcare Settings, 28 March 2018.*https://www.cdc.gov/niosh/topics/hcwcontrols/recommendedguidanceextuse.html

G. Attached documents

I. Organizational structure

Organizational Structure

iMM Direction - Took the initiative and designated staff to implement it

Vanessa Luís - Designated by iMM Direction to launch this initiative
(Overall mission)

Miguel Prudêncio - Group leader with vast experience in P3
(Virus inactivation detailed procedure and security)

Judite Costa - Project manager with experience in logistics/operations
(Logistics and Operations of all procedures)

All oversaw everything and agreed on measures to take.

Sara Santos - Safety Office
(IMM safety officer and Communication with FMUL)

Inês Domingues - Communication Office

Fausto Lopo de Carvalho and Finance and Operations Team (Alexandre Jesus; Inês Bilé; Madalena Reis, Sofia Santos and HR team, IT Team)

Inês Martins - Operations support

Mariana Ferreira, Marie Bordone & Nuno Agostinho - Logistics Support, communication between Vanessa, Miguel and Judite, and all iMM members; Whatsapp Groups structure all except Vanessa, Miguel and Judite.

Edgar Gomes - Advisor

All teams are "on call". There are shifts but staff is only requested to go to IMM if samples to test are confirmed. They are called with 1 hour notice and updated guidelines.

Samples transport grouped managed by Vanessa, eference person Carlos Ramos
Check-in, check-out group managed by Judite and Nuno Agostinho
Virus inactivation team, managed by Judite and Miguel, reference person Henrique Machado
RNA team, managed by Vanessa, reference person Marta Fidalgo
RT-PCR team, managed by Vanessa, reference persons Sofia Marques and Karine Serre

Note: only come to iMM if necessary. When possible work remotely.

II. List of Logistic Tasks

List of Logistic Tasks (Permanent logistics person on call)

Spreadsheets
Templates here: https://drive.google.com/drive/u/1/folders/1bBCmtxLjvLtJSQh26c5isjb5PetxJJVn

1. **Labelling of Samples (Virus Room Only)**

Store all details regarding checked-in samples (**identifier codes**, **details**, **equipment** and **staff**) in the following columns (see below schematic representation of communication between spreadsheets):

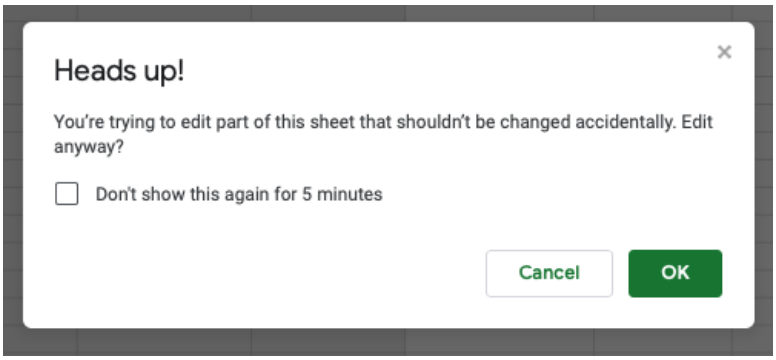
- Global identifiers: ID*, External code*, Provider*
- Sample collection: Date, Entry time, Who brought the samples, Styrofoam box number, Container box number, Priority
- Swab storage at -80: Room, Equipment code, Shelf, Swab stock box
- RNA storage at -80: Room, Equipment code, Shelf, RNA stock box
- Virus room: Check-in staff*, Date, Time, Hood*, Operator*, Oversee*, Notes*
- RNA extraction room: Check-out staff, Date, Time, Hood, Operator, Oversee, Notes
- PCR room: Date, Time, PCR ID, Operator, Oversee, Data filename, Test conclusion, Notes
- Samples check-out: Date, Exit Time, Who brought the samples

Access details: accessible online, link shared with Virus Inactivation team

NOTE: (applies to this and spreadsheets 2, 3 and 4) Columns marked with * and highlighted in gray in the snapshot below are protected against unintended editing and can only be edited in this spreadsheet (thus, in the Virus Inactivation room).

			Sample collection						Swab storage at -80			
ID	External code	Provider	Date	Entry time	Who brought the samples	Styrofoam box number	Container box number	Priority	Room	Equipment code	Shelf	Swab stock box

Also, editing of any of the other editable columns (in white) is also protected against unintended editing using a pop-up window warning that the user is editing a sensitive cell.



2. **Labelling of samples (RNA Extraction Room Only)**

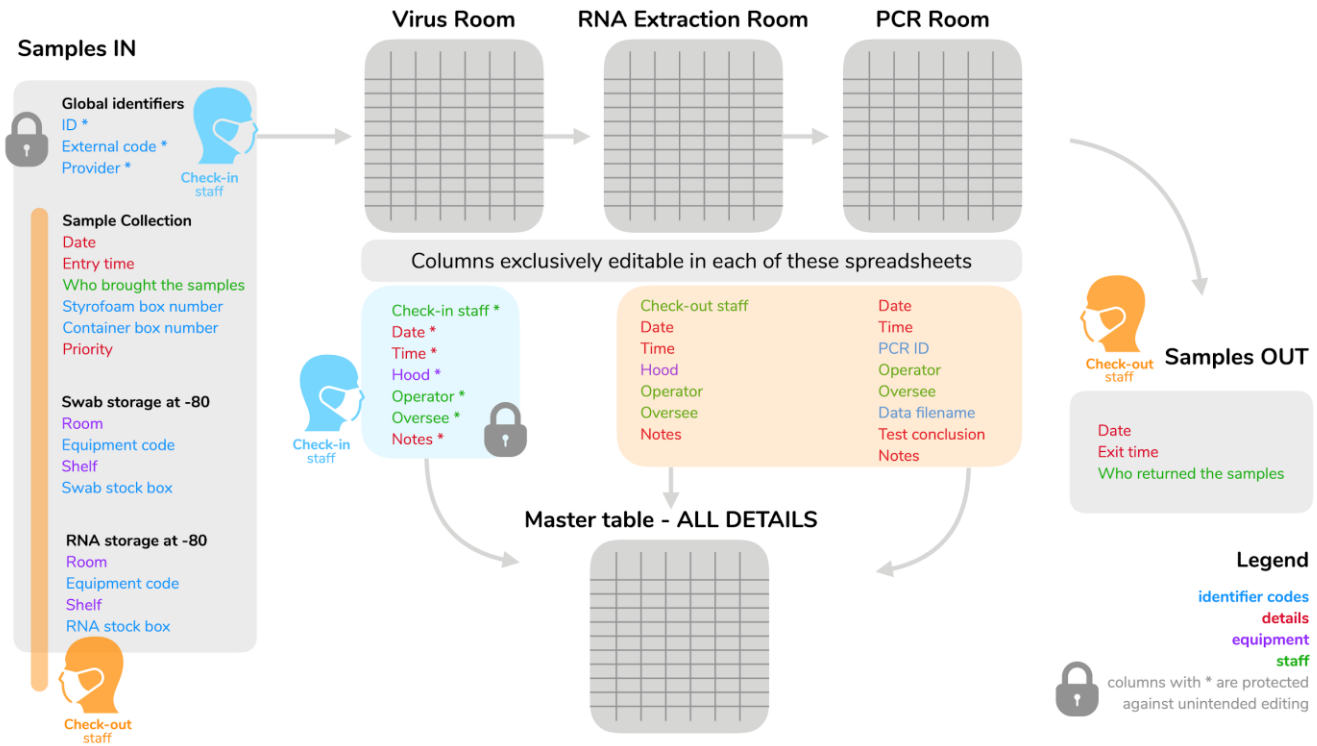
Copy of spreadsheet 1. Receives details from samples that have entered the Virus room and allows (open for editing) details regarding RNA extraction.
Access details: accessible online, link shared with RNA Extraction team

3. **Labelling of samples (RT-PCR Room Only)**

Copy of spreadsheet 1. Receives details from samples that have entered the Virus and RNA Extraction rooms and allows (open for editing) details regarding RT-PCR
Access details: accessible online, link shared with RT-PCR team

4. **Labelling of Samples - ALL DETAILS**

Fed by all details included in spreadsheets 1, 2 and 3
Access details: accessible online



Scheme explaining interaction between spreadsheets that store information about samples

5. **Tracking lab material**

- Sheet 1 - Track items borrowed from labs:
  columns Product, Quantity, Lab, Taken by, Date
- Sheet 2 - List items that are needed and are to be borrowed from labs:
  columns Product, Quantity, Lab, Taken by, Date

Access details: accessible online, link shared with all active volunteers

6. **Volunteers database**

Store all volunteers personal data (name, phone and e-mail), availability and details about lab experience (type of tasks and years of experience) and includes a column for stating willingness to read and comply with all the SOP and Risk Control Measures; allowing distribution of volunteers in teams. All personal data will be destroyed after the end of the COVID19 Diagnostic action.

WhatsApp groups management

- Manage team groups in WhatsApp and communicate all important info with volunteers

Meals

- Ensure meals are available for all volunteers: pick food from sponsors/providers and drop at iMM

Parking in HSM for volunteers

- Under social distancing measures, some volunteers that do not have regular access to iMM parking spots are taking the car. Therefore, a list of all car plates authorised to enter HSM/iMM (feeding on the volunteers database) needs to be prepared and constantly updated and given to iMM security team

III. Responsibility Term

Instituto de Medicina Molecular João Lobo Antunes

RESPONSABILITY TERM

Activity title:	Laboratory processing of COVID-19
Date:	
Laboratory/Facility:	
Name (first and last):	
ID card n. :	
Issued by:	
Valid until:	
Category (PhD, invest.):	

(PT) Declaro que fui informado(a)

e esclarecido(a) sobre:

- Procedimentos gerais e específicos de segurança e laboratoriais utilizados para o trabalho de processamento de amostras com COVID-19 no iMM;
- Riscos para a segurança e saúde no trabalho a que estou exposto, qual o seu nível e quais as medidas de prevenção e ações que devo tomar no âmbito da prevenção de riscos profissionais;
- Equipamentos de Proteção Individual que devo utilizar.
- As pessoas que correm maior risco de doença grave por COVID-19 são os idosos e pessoas com doenças crónicas (ex.: doenças cardíacas, diabetes e doenças pulmonares), sendo desaconselhada a sua participação em actividades que possam potenciar esse risco.
- O iMM considera que não devem participar neste projeto pessoas que pertençam aos grupos de risco COVID-19 (mais de 60 anos ou portador de condição médica pré-existente, como diabetes, doenças cardíacas/pulmonares, pressão alta, cancro).

(PT) Comprometo-me a:

- Cumprir escrupulosamente os procedimentos estabelecidos, de acordo com disposto acima;
- Implementar todas as medidas de prevenção e tomar todas as ações no sentido de evitar a ocorrência de acidentes e doenças profissionais;

(EN) I confirm that I was informed and understand the:

- General and specific safety and laboratory procedures implemented for the work of COVID-19's sample processing at iMM;
- Risks to health and safety at work to which I am exposed and what preventive measures and actions I should take concerning the prevention of occupational hazards;
- Which personal protective equipment (EPIs) I should wear.
- Elderly people and people with chronic conditions (eg. heart conditions, diabetes and pulmonary diseases) are advised not to participate in activities that can contribute to that risk.
- iMM considers that if you belong to the COVID-19 risk groups (over 60 years old or with a pre-existing medical condition, such as diabetes, heart/lung disease, high blood pressure, cancer) you should not participate in this project.

(EN) I hereby certify that I will:

- Scrupulously comply with the procedures established in accordance with the information above;
- Implement all measures and take all actions in order to prevent the occurrence of accidents and occupational diseases;

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Avenida Professor Egas Moniz - Edifício Egas Moniz - 1649-028 Lisboa - Portugal - P + (351) 217 999 411 – E imm@medicina.ulisboa.pt

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- Reportar ao meu superior hierárquico e ao Safety and Compliance, todas as avarias e anomalias que tenha conhecimento, bem como todos os incidentes e acidentes;
- Utilizar os Equipamentos de Proteção Individual corretamente;
- Segregar os resíduos para os locais adequados e devidamente identificados.
- Comunicar ao superior hierárquico, e ao Safety and Compliance, se tiver algum sintoma relacionado com o COVID-19.

- Report to my superior and Safety and Compliance all faults or anomalies that I have knowledge of, and all the incidents and accidents;
- Use the personal protective equipment (EPIs) as indicated;
- Segregate wastes to the appropriate and properly identified location.
- Communicate to my superior and to Safety and Compliance if any COVID-19 symptoms occur.

➢ Caso integre um dos grupos de pessoas que corre maior risco de doença grave por COVID-19, declaro expressamente que a minha participação neste projecto é feita de forma livre e esclarecida e que aceito os riscos acrescidos a que poderei estar sujeito/a,.

➢ In case the signatory is part of COVID-19 risk groups He/She expressly states that His/Her participation is this project is made in a voluntarily and informed way and that He/She accepts the increased risk that may be subject.

Signature: _____

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IV. Training Registration Forms

Training on (Activity):	**Trainer:**
Overall Debrief and Tour	Judite Costa
Check-in and Check-out	Judite Costa
Samples Transport	Vanessa Luís
RNA Extraction Room	Vanessa Luís
Virus Inactivation Room	Miguel Prudêncio
RT-PCR Room	Sofia Marques / Karine Serre

FORM

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TRAINING REGISTRATION

Activity: (one of the following) ***Overall Debrief and Tour /*** ***Check-in & Check-out / Samples Transport /*** ***RNA Extraction Room /Virus Inactivation Room*** *Filled by Trainer*			***Nº:*** *Filled by Trainer*
Date: *Filled by Trainer*		***Duration:*** *1h* *Filled by Trainer*	
Trainer: *Judite Costa / Vanessa Luís / Miguel Prudêncio / Nuno Agostinho*			
Full name	***Laboratory / Facility***	***Signature***	***Email address***

FORM.CM&RP.024 28-01-2019 Pag. 1/1

V. Evaluation of effectiveness of the training session

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EVALUATION OF THE EFFECTIVENESS OF THE TRAINING SESSION

Activity title:	Laboratory processing of COVID-19 samples
Date:	
Laboratory/Facility:	
Name (firstandlast):	
Category (phd st., invest.):	

It is important that you express your opinion, pointing out the options that best describe it.

1) Do you consider that the content of the training session and its objectives have been clearly presented?

☐ ☐ ☐

2) Do you consider that the contents of the training session were presented using an understandable and simple language?

☐ ☐ ☐

3) Do you consider that you perceived and understood the content of the training session and its objectives?

☐ ☐ ☐

Suggestions:	

Signature:	

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VI. iMM COVID-19 Procedures Flowchart

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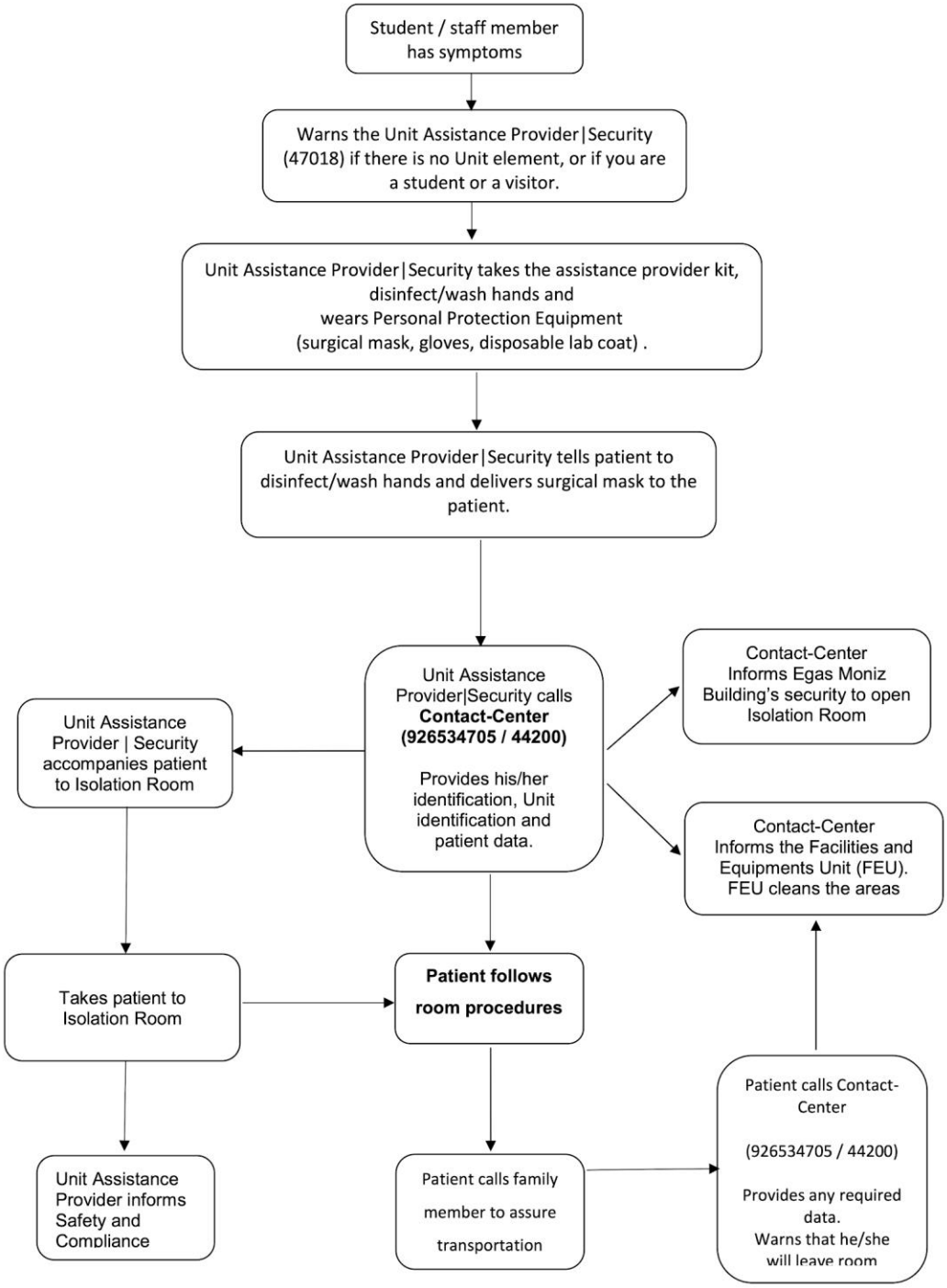
COVID-19 Procedures Flowchart



VII. DGS COVID-19 Diagnostic Guidelines

COVID-19

ORIENTAÇÃO

NÚMERO:	015/2020
DATA:	23/03/2020
ASSUNTO:	**COVID-19: Diagnóstico Laboratorial**
PALAVRAS-CHAVE:	Diagnóstico laboratorial; amostras biológicas
PARA:	Profissionais do Sistema de Saúde
CONTACTOS:	Direção de Serviços de Prevenção da Doença e Promoção da Saúde: dspdps@dgs.min-saude.pt Laboratório Nacional de Referência para o Vírus da Gripe e Outros Vírus Respiratórios: resinsa@insa.min-saude.pt

Nos termos da alínea a) do nº 2 do artigo 2º do Decreto Regulamentar nº 14/2012, de 26 de janeiro, emite-se a Norma seguinte:

1. Diagnóstico laboratorial

a. Todos os casos suspeitos de infeção pelo Novo Coronavírus (SARS-CoV-2) devem ser submetidos a diagnóstico laboratorial. O diagnóstico laboratorial será realizado, preferencialmente, em laboratório hospitalar da Rede Portuguesa de Laboratórios para o Diagnóstico do SARS-CoV-2, na rede complementar de laboratórios privados ou no Instituto Nacional de Saúde Doutor Ricardo Jorge (INSA) – laboratório de referência nacional (Anexo I);

b. As amostras biológicas (Anexo II) são enviadas ao laboratório o mais rapidamente possível, preferencialmente em ambiente refrigerado (Anexo III), logo após a colheita da amostra biológica;

c. A deteção laboratorial do SARS-CoV-2 é feita por PCR em tempo real (RT-PCR).[1,2]

2. Colheita de amostras biológicas

a. A colheita de amostras biológicas (Anexo IV) deve ser efetuada por profissionais devidamente habilitados para a realização da colheita, conservação e acondicionamento das amostras biológicas;

b. Os profissionais de saúde que realizem colheitas de amostras biológicas devem cumprir com rigor as recomendações de utilização de equipamento de proteção individual (Anexo V);

[1] WHO (2020). Laboratory Testing for 2019 novel Coronavirus in suspected human cases. WHO. Disponível em: https://www.who.int/healthtopics/coronavirus/laboratory-diagnostics-for-novel-coronavirus

[2] A possibilidade de realização do diagnóstico SARS-CoV-2 por novos métodos de diagnóstico será atualizada, nomeadamente, por testes de diagnóstico rápido que venham a ser validados e disponibilizados no mercado português.

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c. É fortemente recomendado que sejam colhidas amostras do trato respiratório inferior, sobretudo em doentes com doença mais grave;

d. Uma única amostra do trato respiratório superior pode não excluir a infeção, sendo preferível o envio de duas amostras respiratórias de locais diferentes;

e. Para o diagnóstico de COVID-19 está indicada a colheita de amostras do trato respiratório (superior e/ou inferior, de acordo com o contexto clínico), podendo estas ser completadas, para fins de controlo ou de estudo, por colheita de soro ou de outras amostras.

2.1. Amostras respiratórias

Devem ser colhidas amostras respiratórias:

a. Trato respiratório superior

   i. Exsudado da nasofaringe e exsudado da orofaringe colhido com zaragatoa em meio de transporte para vírus. As duas amostras colhidas com zaragatoas devem ser colocadas no mesmo tubo contendo meio de transporte para vírus (2-3 ml). Deve dar-se prioridade à colheita do exsudado da nasofaringe, quando não for possível a colheita dos dois exsudados;

   ii. Expetoração (se existente).

b. Trato respiratório inferior

   i. Aspirado endotraqueal ou lavado bronco-alveolar, em doentes com doença respiratória grave.

Se analisada apenas um tipo de amostra, com resultado laboratorial negativo para COVID-19, em doentes internados e se o agravamento da doença o justificar, é recomendada a colheita de uma segunda amostra para o diagnóstico laboratorial.

Em **idade pediátrica**: deve colher-se uma amostra de exsudado da nasofaringe e uma amostra de exsudado da orofaringe.

a. Instruções de colheita dos exsudados:[3]

   i. Exsudado da nasofaringe: Inserir a zaragatoa numa das narinas paralelamente ao palato até sentir uma ligeira resistência. Deixar a zaragatoa durante alguns segundos para absorção das secreções. Remover lentamente com movimento de rotação. Repetir a colheita na outra narina.

   ii. Exsudado da orofaringe: Inserir a zaragatoa na cavidade oral até à faringe posterior, evitando tocar na língua.

[3] Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19), CDC, 19 março, 2020

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2.2. Soro

a. Duas amostras de soro de fase aguda e de convalescença (2-4 semanas após fase aguda) para a realização de testes serológicos;

b. Os testes serológicos poderão suportar a investigação futura dos casos de infeção pelo SARS-CoV-2. Devem ser utilizados em complemento do diagnóstico por biologia molecular (RT-PCR).

2.3. Outras amostras

a. Em indivíduos assintomáticos com contacto próximo com doente com infeção COVID-19 confirmado, realizar a colheita de amostras do trato respiratório superior e uma amostra de soro, se houver indicação para tal;

b. Em doentes falecidos, considerar a análise de amostras respiratórias e material de autópsia incluindo material de tecido pulmonar, se a autópsia for considerada necessária[4];

c. Em doentes sobreviventes considerar a colheita e conservação de um par de soros (fase aguda e de convalescença) para o estudo imunitário retrospetivo.

3. Comunicação com o laboratório

Para assegurar uma boa comunicação com o laboratório, o laboratório deve ser informado da proveniência das amostras biológicas. As amostras devem ser corretamente identificadas e acompanhadas da notificação do SINAVE com a informação relativa ao doente, se aplicável.

4. Condições de segurança no acondicionamento e transporte de amostras biológicas

Os profissionais de saúde devem cumprir as regras de prevenção e controlo de infeção aplicáveis bem como a correta e adequada utilização de EPI, de acordo com a Orientação n.º 003/2020 da DGS e o Anexo V da presente Orientação.

De modo a acondicionar e transportar corretamente as amostras, deve proceder-se da seguinte forma:

a. Após a colheita de amostras biológicas, os tubos e recipientes devem ser bem vedados;

b. Os tubos e recipientes devem ser desinfetados exteriormente, no local da colheita, com solução de hipoclorito de sódio a 1% - 10.000ppm (1 parte de hipoclorito de sódio em 9 partes iguais de água) e de seguida, com álcool a 70%;

c. Por fim, a tampa do tubo deve ser selada com película parafilme;

[4] Consultar Norma nº 002/2020 de 16/03/2020 atualizada a 19/03/2020, disponível em https://www.dgs.pt/directrizes-da-dgs/normas-e-circulares-normativas/norma-n-0022020-de-16032020.aspx

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d. Acondicionar as amostras biológicas, seguindo as normas de embalagem de substâncias infeciosas recomendadas pela Organização Mundial de Saúde[5] para amostras classificadas de categoria B (UN 3373);

e. Utilizar o sistema de embalagem tripla com as seguintes características:

   i. **Contentor primário** é o que contém a amostra; deve estar devidamente identificado e tem de ser estanque a líquidos e a sólidos; tem de ser embalado em material absorvente suficiente para absorver todo o conteúdo em caso de quebra ou derrame;

   ii. **Contentor secundário** é o que leva os contentores primários (tubos de amostras) e é resistente, à prova de água e estanque a líquidos e a sólidos; pode conter vários tubos de amostras desde que estes sejam protegidos com material absorvente e amortecedor, individualmente e separados, a fim de evitar o contacto;

   iii. **Contentor exterior** é a embalagem de transporte externa com material de acolchoamento adequado, onde se colocam os contentores secundários. A menor dimensão externa global utilizada deve ser de 10 x 10 cm.

f. O transporte das amostras deve ser realizado, preferencialmente, por uma empresa certificada e autorizada para o efeito (Categoria B, UN 3373) ou em alternativa, em transporte próprio da unidade de saúde, cumprindo as indicações de acondicionamento anteriormente referidas;

g. Este procedimento é da responsabilidade da unidade de saúde que realiza a colheita das amostras biológicas e deve ser acompanhado de impressão da notificação efetuada no SINAVE.

5. Testes laboratoriais

O tratamento das amostras biológicas e a inativação do RNA viral deve ser realizada em laboratório com câmara de biossegurança de nível 2 (BSL-2).

5.1. PCR em tempo-real para o SARS-CoV-2

O diagnóstico específico do novo coronavírus é realizado pela metodologia de amplificação dos ácidos nucleicos, pela reação de polimerase em cadeia (PCR).

O teste PCR para a deteção do novo coronavírus deverá incluir pelo menos um dos seguintes testes:

[5]WHO (2020). Guidance on regulations for the transport of infectious substances 2019–2020. WHO. Disponível em: https://apps.who.int/iris/bitstream/handle/10665/325884/WHO-WHE-CPI-2019.20-eng.pdf
IATA (2011). PACKING INSTRUCTION 650. IATA. Disponível em: https://www.iata.org/contentassets/b08040a138dc4442a4f066e6fb99fe2a/dgr-61-en-pi650.pdf

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a. Teste de *screening*, que permita a deteção do sub-género Sarbecovirus (SARS-CoV, SARS-CoV-2 e outros coronavírus de origem animal);

b. Teste confirmatório, específico para o novo coronavírus SARS-CoV-2.

Um caso confirmado laboratorialmente apresentará as duas reações de PCR positivas. As amostras duvidosas ou que necessitem de análise complementar devem ser enviadas ao Laboratório Nacional de Referência para o Vírus da Gripe e outros Vírus Respiratórios do Departamento de Doenças Infeciosas, do INSA.

Em áreas onde existe transmissão comunitária ativa de COVID-19, a deteção dos casos por um único teste discriminatório, pela metodologia de RT-PCR para um alvo, será considerada suficiente[6]. Os testes confirmatórios devem ser realizados apenas para resultados inconclusivos ou com um valor de ciclo de amplificação do RT-PCR superior a 35. Recomenda-se nestes casos a repetição do teste e/ou da colheita da amostra biológica.

Os laboratórios da Rede Portuguesa de Laboratórios para o Diagnóstico do novo coronavírus devem enviar ao INSA todas as amostras positivas para SARS-CoV-2 e as 5 primeiras amostras negativas para avaliação da comparabilidade de resultados.

5.2. Sequenciação do genoma viral [6,7]

A metodologia de sequenciação de nova geração (NGS) está disponível no INSA, para a realização do estudo do genoma do SARS-CoV-2.

Para além da confirmação de casos de infeção pelo SARS-CoV-2, a integração da análise genómica do novo coronavírus com a informação epidemiológica e clínica dos casos de COVID-19 é crítica para o estudo e controlo da transmissão do vírus na comunidade, monitorização da doença e de marcadores genómicos associados à gravidade da doença.

O conhecimento do genoma viral permite ainda avaliar o desempenho dos testes de diagnóstico laboratorial.

É fortemente recomendada a partilha das sequências virais em bases de dados, como o GISAID, que garantem os direitos de autor e de submissão.

Devem ser enviadas ao Laboratório Nacional de Referência para o Vírus da Gripe e outros Vírus Respiratórios do Departamento de Doenças Infeciosas, do INSA, para sequenciação do genoma viral todas as amostras positivas de casos de COVID-19.

[6] ECDC. Novel coronavirus disease 2019 (COVID-19) pandemic: increased transmission in the EU/EEA and the UK – sixth update, ECDC, 12 março, 2020. Disponível em: https://www.ecdc.europa.eu/sites/default/files/documents/RRA-sixth-update-Outbreak-of-novel-coronavirus-disease-2019-COVID-19.pdf

[7] Plano Nacional de Preparação e Resposta à Doença por novo coronavírus (COVID-19), DGS, 2020. Disponível em: https://www.dgs.pt/documentos-e-publicacoes/plano-nacional-de-preparacao-e-resposta-para-a-doenca-por-novo-coronavirus-covid-19-pdf.aspx

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A caraterização virológica deverá incluir sequências de vírus que representem diferentes momentos da epidemia, grupos etários e áreas geográficas[8].

6. Monitorização laboratorial de casos confirmados[9]

Recomenda-se o envio ao INSA de colheitas sequenciais de amostras respiratórias e de outras origens para o estudo da duração da excreção do vírus.

O RNA viral pode permanecer detetável em fluídos corporais do doente por longo período de tempo, não significando que o vírus se encontre no seu estado infecioso5.

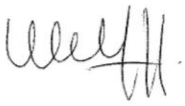
Os critérios para libertação do isolamento consensualizados pelo ECDC5 implicam:

a. Evidência de amostras do trato respiratório superior negativas para SARS-CoV-2;
b. Resolução da sintomatologia.

O critério para a declaração da completa eliminação do vírus e resolução da doença COVID-19 implica:

a. Pelo menos 2 amostras do trato respiratório superior negativas para SARS-CoV-2, colhidas com um intervalo de 24h:
    i. Em doentes que foram sintomáticos e após a resolução dos sintomas (após 3 ou mais dias sem febre e sem outra sintomatologia) - colher duas amostras (com intervalo de 24 horas), pelo menos 7 dias após o início dos sintomas iniciais;
    ii. Em doentes assintomáticos com infeção por SARS-CoV-2 (teste inicial positivo para SARS-CoV-2), colher duas amostras (com intervalo de 24 horas) no mínimo 14 dias após o resultado laboratorial positivo inicial.

Devido à crescente evidência de excreção do vírus através das fezes de doentes em fase de convalescença, particularmente nas crianças, recomenda-se um reforço da higiene pessoal após libertação do isolamento.



Graça Freitas
Diretora-Geral da Saúde

[8] Plano Nacional de Preparação e Resposta à Doença por novo coronavírus (COVID-19), DGS, 2020. Disponível em: https://www.dgs.pt/documentos-e-publicacoes/plano-nacional-de-preparacao-e-resposta-para-a-doenca-por-novo-coronavirus-covid-19-pdf.aspx

[9] ECDC. Novel coronavirus (SARS-CoV-2) Discharge criteria for confirmed COVID-19 cases – When is it safe to discharge COVID-19 cases from the hospital or end home isolation? ECDC Technical Report, 10 março, 2020

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DGS. Norma nº 002/2020 de 16/03/2020 atualizada a 19/03/2020, disponível em https://www.dgs.pt/directrizes-da-dgs/normas-e-circulares-normativas/norma-n-0022020-de-16032020.aspx

DGS. Plano Nacional de Preparação e Resposta à Doença por novo coronavírus (COVID-19), DGS, 2020. Disponível em: https://www.dgs.pt/documentos-e-publicacoes/plano-nacional-de-preparacao-e-resposta-para-a-doenca-por-novo-coronavirus-covid-19-pdf.aspx

ECDC. Novel coronavirus disease 2019 (COVID-19) pandemic: increased transmission in the EU/EEA and the UK – sixth update 12 March 2020. Disponível em: https://www.ecdc.europa.eu/sites/default/files/documents/RRA-sixth-update-Outbreak-of-novel-coronavirus-disease-2019-COVID-19.pdf

ECDC. Novel coronavirus (SARS-CoV-2) Discharge criteria for confirmed COVID-19 cases – When is it safe to discharge COVID-19 cases from the hospital or end home isolation? ECDC Technical Report, 10 março, 2020

IATA (2011). PACKING INSTRUCTION 650. IATA. Disponível em: http://www.iata.org/whatwedo/cargo/dgr/Documents/DGR52_PI650_EN.pdf

WHO (2020). Laboratory Testing for 2019 novel Coronavirus in suspected human cases. WHO. Disponível em: https://www.who.int/healthtopics/coronavirus/laboratory-diagnostics-for-novel-coronavirus

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COVID-19

Anexo I
Laboratórios com capacidade para diagnóstico laboratorial (sujeito a atualização contínua)

Laboratórios
Laboratório Nacional de Referência
Instituto Nacional de Saúde Dr. Ricardo Jorge, I. P. - Lisboa
ARS Norte
Centro Hospitalar Universitário de S. João, E.P.E. - Porto
Centro Hospitalar Universitário do Porto, E.P.E. - Porto
Centro Hospitalar de Vila Nova de Gaia/Espinho, E.P.E. - Gaia
Hospital da Senhora da Oliveira Guimarães E.P.E. - Guimarães
Unidade Local de Saúde de Matosinhos, E.P.E. - Hospital Pedro Hispano - Matosinhos
Hospital de Braga, E.P.E. - Braga
ARS Centro
Centro Hospitalar Universitário de Coimbra, E.P.E. - Coimbra
Centro Hospitalar Cova da Beira, E.P.E. - Covilhã
Unidade Local de Saúde da Guarda, E.P.E., - Guarda
ARS Lisboa e Vale do Tejo
Centro Hospitalar e Universitário de Lisboa Central, E.P.E. - Lisboa
Centro Hospitalar Universitário de Lisboa Norte, E.P.E. - Lisboa
Centro Hospitalar Lisboa Ocidental, E.P.E. - Lisboa
ARS Alentejo
Hospital Espírito Santo de Évora, E.P.E. - Évora
ARS Algarve
Laboratório Regional de Saúde Pública Dra. Laura Aires - Faro
Região Autónoma dos Açores
Hospital de Santo Espírito da Ilha Terceira, E.P.E.R. - Ilha Terceira
Hospital do Divino Espírito Santo de Ponta Delgada, E.P.E. - Ilha de S. Miguel
Região Autónoma da Madeira
Serviço de Saúde da RAM, E.P.E. – Hospital Dr. Nélio Mendonça - Ilha da Madeira
Rede complementar de laboratórios privados

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Anexo II
Colheita de amostras biológicas

	Diagnóstico laboratorial	Tipo de amostra	Amostras biológicas	Momento da colheita	Observações
Doente	RT-PCR	Trato respiratório superior	Exsudado da nasofaringe Exsudado da orofaringe Expetoração	Fase inicial da doença Colheita no momento de alta hospitalar	Zaragatoas da naso e orofaringe devem ser colocadas no mesmo tubo com meio de transporte viral
		Trato respiratório inferior	Lavado bronco-alveolar Aspirado endotraqueal		----------
	Serologia	Soro	Um par de soros	Soro de fase inicial e soro de fase de convalescença	Amostra de importante valor para futuro diagnóstico laboratorial e avaliação da imunidade
Contactos (surtos em unidades de saúde ou sintomáticos em outras instituições ou assintomáticos com estreito contacto com casos confirmados de COVID-19)	RT-PCR	Trato respiratório superior	Exsudado da nasofaringe Exsudado da orofaringe	Período de incubação do último contacto	Zaragatoas da naso e orofaringe devem ser colocadas no mesmo tubo com meio de transporte viral
	Serologia	Soro	Um par de soros	Soro de fase inicial (colhido o mais cedo possível durante o período de incubação) e soro de convalescença (2-4 semanas após o contacto)	

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Anexo III
Transporte e acondicionamento de amostras biológicas

Tipo de amostra	Dispositivo de colheita	Transporte	Acondicionamento	Observações
Exsudado da nasofaringe e orofaringe	Zaragatoa de dracon ou floculada	2-8ºC	≤5 dias: 2-8ºC ≥5 dias: -70ºC	Zaragatoas da naso e orofaringe devem ser colocadas no mesmo tubo com meio de transporte viral
Expetoração	Contentor estéril	2-8ºC	≤48 horas: 2-8ºC ≥48 horas: -70ºC	
Lavado bronco-alveolar	Contentor estéril	2-8ºC	≤48 horas: 2-8ºC ≥48 horas: -70ºC	Amostra de importante valor para o diagnóstico laboratorial
Aspirado endotraqueal, nasofaríngeo, ou lavado nasal	Contentor estéril	2-8ºC	≤48 horas: 2-8ºC ≥48 horas: -70ºC	
Soro	Tubo seco (3-5 ml)	2-8ºC	≤5 dias: 2-8ºC ≥5 dias: -70ºC	Pares de soros: agudo-1ª semana de doença; convalescença-2-3 semanas depois)
Tecidos de biópsia	Contentor estéril com soro fisiológico ou meio de transporte para vírus	2-8ºC	≤5 dias: 2-8ºC ≥5 dias: -70ºC	
Fezes	Contentor estéril	2-8ºC	≤5 dias: 2-8ºC ≥5 dias: -70ºC	
Urina	Contentor estéril	2-8ºC	≤5 dias: 2-8ºC ≥5 dias: -70ºC	

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COVID-19

Anexo IV

Diagnóstico laboratorial do novo coronavírus em casos suspeitos sob investigação

Procedimento para a realização de colheita de amostras biológicas e encaminhamento ao laboratório

I. Local: Unidade de saúde realiza a colheita de amostras biológicas para o diagnóstico laboratorial do Novo Coronavírus em casos suspeitos sob investigação.

II. Equipamento de proteção individual (EPI): bata impermeável ou fato de proteção integral, touca, respirador FFP2, proteção ocular, luvas, proteção de calçado.

III. Amostras Respiratórias: Devem ser enviadas 2 amostras biológicas respiratórias de diferente natureza para o diagnóstico do Novo Coronavírus (COVID-19). Juntar formulário **de notificação SINAVE-clínico**.

2 das seguintes Amostras Respiratórias:
- Exsudado da nasofaringe colhido com zaragatoa;
- Exsudado da orofaringe colhido com zaragatoa;
- Expetoração;
- Aspirado endotraqueal.

} mesmo tubo contendo meio de transporte viral

Quando não for possível a colheita dos dois exsudados, deve dar-se prioridade à colheita do exsudado da nasofaringe

IV. Instruções de colheita dos exsudados:

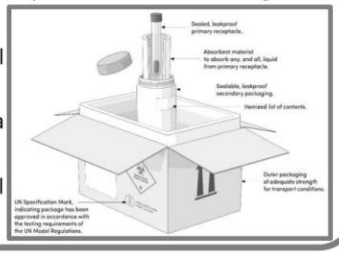
- **Exsudado da nasofaringe:** Inserir a zaragatoa numa das narinas paralelamente ao palato até sentir uma ligeira resistência. Deixar a zaragatoa durante alguns segundos para absorção das secreções. Remover lentamente com movimento de rotação. Repetir a colheita na outra narina.

- **Exsudado da orofaringe:** Inserir a zaragatoa na cavidade oral até à faringe posterior, evitando tocar na língua.

V. Acondicionamento de produtos biológicos:

Desinfetar exteriormente os tubos e recipientes no local da colheita. Selar com parafilme as tampas dos tubos. Utilizar sistema de embalagem tripla (categoria B, UN 3373). Transporte em ambiente refrigerado.

- **Contentor primário** (contém a amostra, envolver em material absorvente e colocar em saco plástico);
- **Contentor secundário** (resistente, à prova de água, estanque a líquidos e sólidos);
- **Contentor exterior** (embalagem de transporte externa, material acolchoado, dimensão mínima 10x10 cm).



VI. Transporte:

Preferencialmente por empresa autorizada para o transporte de substâncias de categoria B (UN 3373), ou em transporte próprio da unidade de saúde.

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Anexo V
Equipamento de Proteção Individual (EPI) de acordo com o nível de cuidados a prestar

EPI para a colheita de amostras biológicas	
Equipamento	**Características**
Fato de bloco operatório, se aplicável	- Composto por calças e túnica reutilizável (opcional)
Bata de laboratório, se aplicável	- Reutilizável
Respirador FFP2*	- Uso único
Óculos de proteção ou viseira de proteção facial total	- Reutilizável, após descontaminação
Bata impermeável	- Uso único
Luvas	- Uso único - 2 pares (trocar as luvas sempre que necessário) - 2 tipos de luvas: Primeiro par de nitrilo (300 mm) e segundo par de nitrilo ou latex, que cubra com uma boa margem o punho da bata)

*Para uma otimização dos respiradores:

- O profissional que faz as colheitas, pode permanecer com o mesmo respirador desde que seja escalado apenas para esta função, ou seja, num turno faz apenas colheitas de amostras respiratórias;
- O respirador tem uma durabilidade que pode ir de 4-6 horas;
- Caso o respirador fique húmido, deve ser substituído.

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VIII. iMM Contingency Plan

iMM Contingency Plan (Procedure in case of Suspicious Cases)

COVID-19 - Suspicious Cases

Instituto de Medicina Molecular | João Lobo Antunes

Who is a Suspicious Case?

Clinical criteria	Epidemiological criteria
Acute respiratory infection (fever or cough or difficulty breathing) requiring hospitalization or not	Travel history to areas with active community transmission in the 14 days before onset of symptoms. **OR** Contact with a confirmed or probable case of SARS-CoV-2 / COVID-19 infection, 14 days before the onset of symptoms. **OR** Health professional or person who has been to a health institution where patients with COVID-19 are treated.

What to do if you are a Suspicious Case?

- Inform the area assistance provider or the Building's Security (ext: 47018) the area assistance is not present;
- The area assistance provider /security will give you a mask (disinfect / wash your hands before putting the mask);
- Follow the area assistance provider /security instructions and go to the "isolation" room;
- Follow the procedures that are fixed in the room and contact **SNS 24 (808 24 24 24)**;
- Before leaving the isolation room, call the **FMUL Contact-Center (926 534 705 / 44200)**, provide the requested data and notify that you are going to leave the room.

Check the FMUL's flowchart in the attachments.

COVID-19 - Suspicious Cases

Instituto de Medicina Molecular | João Lobo Antunes

What to do if you are an Assistance Provider (AP)?

- The AP disinfect/ wash hands and wears the PPE provided;
- Deliver disinfectant tissues and a surgical mask to the "Suspicious Case";
- The AP calls the **FMUL Contact-Center (926 534 705 / 44200)** and gives the following information:
  - Name;
  - Name of the Suspicious Case (SC);
  - Institution and Lab/Facility of the SC;
- Accompany the SC to the isolation room;
- Deliver the individual kit to the SC;
- Contact Safety and Compliance and inform what happened.

Check the FMUL's flowchart in the attachments.

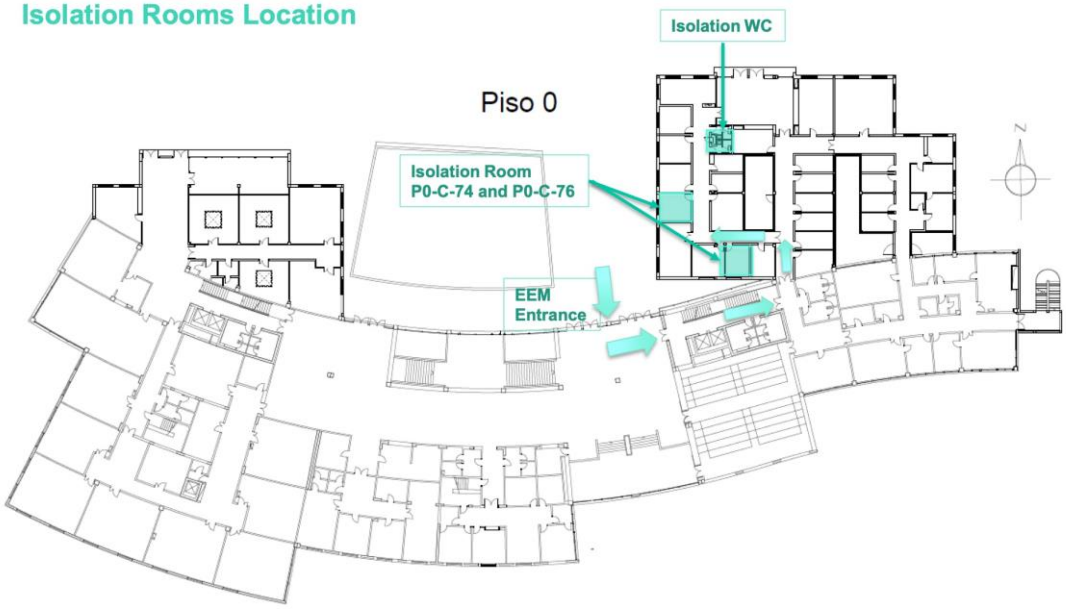
What to do after a confirmed Suspected Case?

- The FMUL's specialized cleaning team is going to clean and decontaminate the rooms where the Confirmed Case was;
- All the possible contaminated surfaces and materials (door handles, benches, desks, equipment and others), are going to be decontaminated with 70% ethanol.

COVID-19 - Isolation Room, Material and Routines

Instituto de Medicina Molecular | João Lobo Antunes

Isolation Rooms Location



COVID-19 – Contingency Plan 2020

18

IX. Overall debrief

Kit to bring from home

- ➢ Ready meals and Snacks will be provided, if you have any restrictions please bring your own food since all bars are closed.
- ➢ Also bring your own cutlery, mug and coffee cup (all of this need to be taken back by you daily).
- ➢ Hairs bands.
- ➢ Hairs pins
- ➢ Phone charger.
- ➢ Change of clothes (optional since we will have emergency change of clothes).
- ➢ Bottle to fill with water in iMM fountains.
- ➢ Preferentially bring contact lenses instead of glasses.
- ➢ Everyone must have pen in their Lab coat.

Name	Table	Task	Entry Time	Exit Time

Log in

Date:

PPE (masks and gloves) and Disinfectant Important Notes

Masks

- **Single use**: should be disposed of every time you eat, drink, smoke, etc.
- Good to **protect others** but not good to protect ourselves
- A tool for protection can become an hazard and source of contamination: **If reused** there is a huge risk of creating a "contamination hotspot" spreading everywhere
  (pocket, bag, table, workplace, home, car…)
- Only people coughing or sneezing should wear masks; everyone else should not: it's a **higher risk than to not use at all**

Máscaras

- **Utilização única**: devem ser deitadas fora cada vez que come, bebe, fuma etc.
- São úteis para proteger os outros mas não para proteger o utilizador
- Sendo usadas por protecção, podem tornar-se um risco: **se reutilizadas** há risco de criação de um ponto de contaminação que se espalha por vários locais
  (bolsos, malas, mesas, trabalho, casa, carro…)
- Só pessoas com tosse ou espirros devem usar máscaras; todos os outros não, pois **uma má utilização tem mais risco do que a não utilizacão**

Gloves

- **Should be disposed often** and not used for a long time
- **If gloves get contaminated** this can then go to face, car, doors, kids, etc.
- You can avoid using gloves and **wash your hands** frequently instead: every time there is a sink around or use hand sanitiser

Luvas

- **Devem ser trocadas frequentemente** e não usadas por muito tempo
- **Se as luvas estiverem contaminadas** podem contaminar a face, carros, portas, crianças, etc.
- Opte por não usar luvas e **lavar antes as mãos** com água e sabão sempre que possível ou usar desinfectante à base de álcool

Cleaning

- Let's not clean surfaces with disinfectants: an empty disinfectant bottle is a source of contamination
- **Use diluted (but not pure) bleach**: bleach is toxic and may cause breathing difficulties that can be confounded with symptoms of infection
  - always dilute 20% (2 parts of bleach to 8 parts of water)
  - IMPORTANT: diluted bleach loses properties need to be prepared fresh every 24hours

Limpeza

- Não limpamos superfícies com desinfectante: uma garrafa de desinfectante vazia é fonte de contaminação
- **Usar lixívia diluída**, pois a lixívia pura é tóxica e pode causar dificuldade respiratória que pode ser confundida com sintomas de infecção
  - diluição 20% (2 partes de lixívia para 8 partes de água)
  - IMPORTANTE: a lixívia diluída perde propriedades, pelo que terá de ser preparada a cada 24 horas

X. Put ON/OFF PPE, Virus Inactivation Room

How to Put Personal Protection Equipment ON

COVID-19

1	NO jewelry, NO watches and hair must be tied up	
2	Regular Lab Coat	
3	Shoe covers	
4	Desinfect Hands	
5	Disposable Lab Coat	
6	FFP2 or FFP3 mask Make sure is confortable. In some formats may be necessary to removed card bord	 *
7	Disposable Eye Goggles	
8	Head Covers	
9	Desinfect Hands	
10	Elbow Long Gloves over Lab Coat + Wriste long gloves	

Grupo de Coordenação Local do Programa de Prevenção e Controlo de Infeções e de Resistência aos Antimicrobianos
Março/2020


How REMOVE Personal Protection Equipment
COVID-19
1
Make sure you removed your ouside gloves
2
Shoe Cover
3
Disposable Lab Coat
4
Remove Gloves
5
Head Covers
6
Eye Goggles and desinfect with ethanol
7
Desinfect Hands
8
FFP2/FFP3 mask
*
9
Desinfect Hands
Grupo de Coordenação Local do Programa de Prevenção e Controlo de Infeções e de Resistência aos Antimicrobianos
Março/2020

XI. RNA TEAM CHECKLISTS

AND

RACK ORGANIZATION

RNA Room CHECKLIST

OPERATOR Checklist

FIRST SHIFT

- Make sure there are 3 Racks inside hood
- Check if there are p1000 and p200 tips
- Switch on the UV in the hood (15 min)
- Check/Prepare 70% EtOH (Prepare outside the hood)

(Prepare 50mL in Falcon: 15mL RNAse free H2O +3 EtOH)

- Check if Buffer NWR2 has EtOH added (Add EtOH outside hood)
- After UV treatment
- Trash Waste bottle(s) from previous day
- Clean hood with RNAse OUT
- Take to hood: Buffers, columns; collection tubes; Eppis; dispenser tips
- Prepare the racks according to the scheme

CLEAN YOUR HANDS WITH RNAse OUT
BEFORE START WORKING

OPERATOR Checklist

SECOND and THIRD SHIFT

- Check/Prepare 70% EtOH (Prepare outside the hood)

(Prepare 50mL in Falcon: 15mL RNAse free H2O + 35mL EtOH)

- Check if Buffer NWR2 has EtOH added (Add EtOH outside hood)
- Clean hood with RNAse OUT
- Take to hood: Buffers, columns; collection tubes; Eppis and dispenser tips
- Prepare the racks according to the scheme

CLEAN YOUR HANDS WITH RNAse OUT
BEFORE START WORKING

OVERSEER Checklist

- Prepare Waste bottle with 15% Bleach

(200mL/bottle; 2/hood)

- Wipe with RNAse OUT and place in the RNAse free area near hood
- Update the RNA Room sheet in the computer
- Wait for the call from Virus Room to pick up samples

OVERSEER Checklist

LAST SHIFT OVERSEER

- Prepare NR buffer for the Virus Inactivation Team

(2 X 50 mL falcons with buffer for 50 samples)

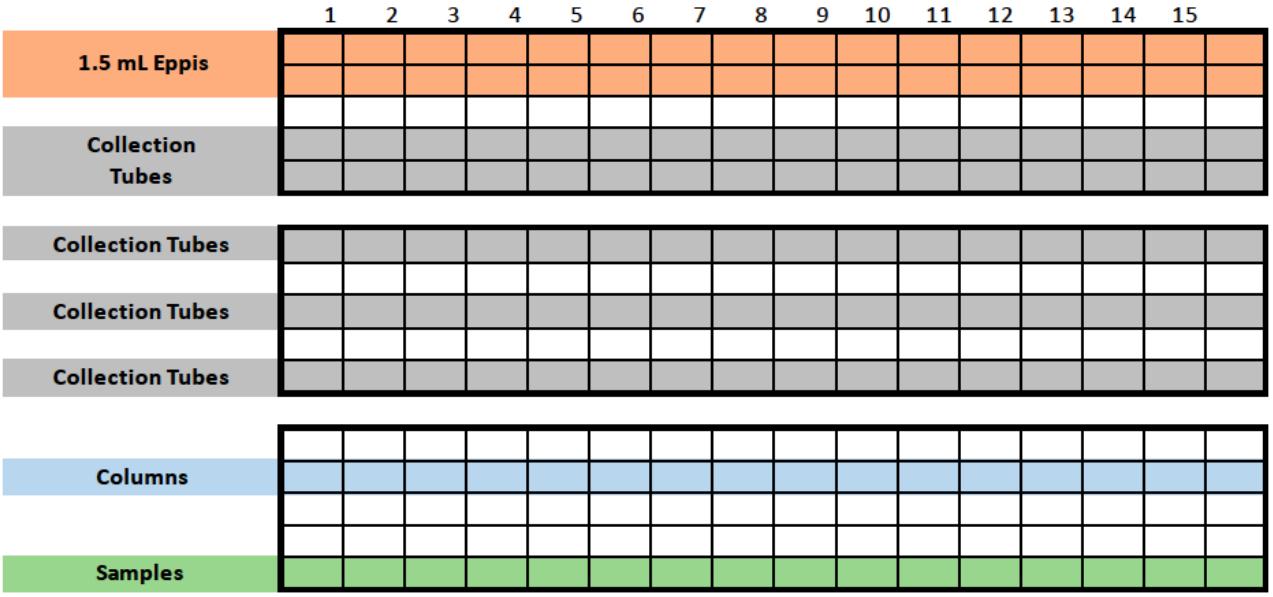
- Take Buffer to the Virus Room antechamber

RNA Room Final CHECKLIST

- Close Waste Bottle
- Turn OFF centrifuge
- Remove all buffers and solutions
- Clean hood with EtOH (EtOH in paper; DO NOT SPRAY)
- Clean hood with RNAse OUT wipes
- Turn ON UV (30 min)

Prepare the racks as follows

according to the number of samples (maximum of 16)



XII. CHECK-OUT instructions

CHECK-IN/OUT instructions

1. Open Sample label (RNA ONLY) table (shortcut in Desktop)
2. Wait for the Check-in staff to call from the Viral room (47361)
3. When the Check-in staff calls, confirm labels to print
4. Print labels for RNA room (47366):

a. Connect the label printer to the laptop via USB

b. Turn on the label printer (hold )

c. Open Sample label (RNA ONLY) table (shortcut in Desktop)

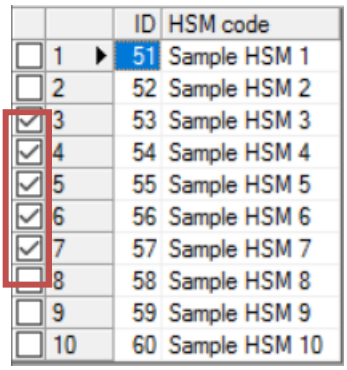
d. Select and copy (CTRL + C) the internal and external codes to print

e. Paste the values to the Sample list Excel file (shortcut in Desktop)

f. Save and close the Excel file

g. Open Covid-19 labels file (shortcut In Desktop)

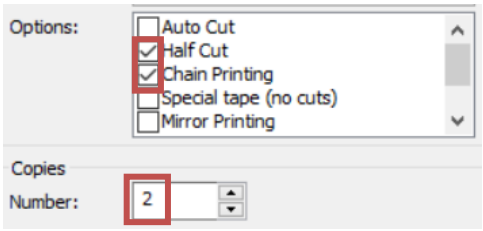
h. Select labels to print with a checkmark



		ID	HSM code
☐	1	51	Sample HSM 1
☐	2	52	Sample HSM 2
☑	3	53	Sample HSM 3
☑	4	54	Sample HSM 4
☑	5	55	Sample HSM 5
☑	6	56	Sample HSM 6
☑	7	57	Sample HSM 7
☐	8	58	Sample HSM 8
☐	9	59	Sample HSM 9
☐	10	60	Sample HSM 10

i. Click Print 

j. Check if the options "Half-cut" and "Chain Printing" are selected



k. In number of copies, insert 2

l. Click Print

m. After printing all labels, press  on the printer to cut the tape

n. If the tape starts misprinting black squares: cancel the printing, turn off the printer, replace the ink cartridge and try again

o. Turn off the label printer (hold ) when finished

data: **Official documentation on printing from Excel**

https://tinyurl.com/print-labels-from-excel

5. Leave the printed labels in the binder clip of the RNA room (47366)

6. Photograph/annotate the RNA staff scheme glued to the inside door of the RNA room (47366); if the scheme is blank/unreadable, call them to fix it

7. On the Sample label (RNA ONLY) table, write down for each sample:

a. RNA operating hood – Hood A / Hood B / Hood C

b. RNA operator name

c. RNA oversee name

8. Print labels for PCR room (XXXXX):

a. Open RT-PCR labels print (shortcut in Desktop)

b. Select the labels with a checkmark and print them following steps 4f-o

c. Call the PCR room (XXXXX) to check where to leave the printed labels

9. On the Sample label (RNA ONLY) table, fill sample transport information based on the log sheet from Transport of Samples (47376)

10. Select the newly created cells:

a. Right-click the selected data

a. Click Protect range Proteger intervalo

b. Click Set permissions Definir autorizações

c. Click Show a warning when editing this range

Mostrar um aviso ao editar neste intervalo

d. Click Done Concluído

XIII. Virus Inactivation Room CHECKLIST

Virus Inactivation Team
The Startup-Checklist

- ☐ Put on white Lab coat and wrist-length Gloves
- ☐ Switch on the UV in the biosafety cabinet
  - ☐ 15 min
- ☐ Open the Sash and start the Airflow
  - ☐ 15 min

IN THE MEANTIME:

- ☐ Check that all items you will need (see below, underlined) are in the room. If not, collect them now.
- ☐ Prepare 15% Bleach by diluting the 100% stock
  - ☐ For 500ml 15% use 75ml 100% stock plus 425mL water
- ☐ Prepare the box with Bleach wipes for the biosafety cabinet. Cut paper towel (3 layers each towel), soak in 15% bleach
- ☐ Prepare the Spill kit (large paper towels soaked in 15% bleach)
- ☐ Prepare the Liquid waste (for plastic Pasteur pipettes and tips) by filling the flask to ~⅓ with 15% Bleach
- ☐ Prepare Squirt bottle with 15% Bleach
- ☐ Prepare the Solid waste container by placing a red bag inside the beaker
- ☐ Check that all Racks intended for Falcon (Racks A, B, C) and Eppendorf (Racks 1, 2, 3) tubes are in the room
- ☐ Collect the number of 2 ml Eppendorf tubes to match the number of samples in Falcon Tubes.
- ☐ Prepare Numbered Stickers for the required amount of Falcon tubes. Cut sticker strips accordingly.
- ☐ Prepare Numbered Stickers for the required amount of 1.5 ml Eppendorf tubes. Cut sticker strips accordingly.
- ☐ Cut Parafilm strips to match the number of Falcon tubes + 5
- ☐ Check that enough Filter tips are in the box

- ☐ **PUT ON PPE**

SET-UP THE BIOSAFETY CABINET

- ☐ Place the absorbent pad inside the biosafety cabinet (do not cover the AirFlow)
- ☐ Setup the working area by placing the following items on the absorbent pad:

- ☐ Vortex
- ☐ Rack A (for incoming Falcon tubes)
- ☐ Rack 1 (for Eppendorf tubes)
- ☐ 1.5 ml Eppendorf tubes
- ☐ Filter Tips
- ☐ 1000mL Pipette
- ☐ FalconTube Rack (For the Inactivation Buffer)
- ☐ Liquid waste container (for tips)

☐ In addition Place the following in the biosafety cabinet, outside the area covered by the absorbent pad:

- ☐ Bleach wipes
- ☐ Rack B (For Falcon tubes)
- ☐ Rack 2 (for the clean tubes)
- ☐ Squirt bottle with 15% bleach
- ☐ Squirt bottle with 70% ethanol
- ☐ Dry paper towels
- ☐ Plastic Pasteur pipettes
- ☐ Solid waste container (on the opposite side of the liquid waste container)
- ☐ Rubber bands (to close solid waste bag)

☐ Check that the following are on the small Table next to the biosafety cabinet:

- ☐ Wrist-length latex or vinyl gloves of your required size
- ☐ Spill kit
- ☐ Rack C for Falcon tubes for storage
- ☐ Rack 3 for inactivated samples ready for RNA extraction

☐ Check that the following are in the Check-in area:

- ☐ Numbered stickers for Falcon tubes
- ☐ Barcode scanner

☐ Pick up the Virus Inactivation Buffer from the antechamber and place it in the rack in the biosafety cabinet

☐ Get the numbered stickers for the Eppendorf tubes and place them in the biosafety cabinet

☐ Remove paper from Parafilm strips for the Falcon tubes and place in box in biosafety cabinet

☐ Double-check everything

☐ Triple-check everything

You are ready To Pick the samples from the Antechamber!

Virus Inactivation Team - **The Startup-Checklist**

- Put on white Lab coat and wrist-length Gloves
- Switch on the UV in the biosafety cabinet
  - 15 min
- Open the Sash and start the Airflow
  - 15 min

IN THE MEANTIME:

- Check that all items you will need (see below, underlined) are in the room. If not, collect them now.
- Prepare 15% Bleach by diluting the 100% stock
  - For 500ml 15% use 75ml 100% stock plus 425mL water
- Prepare the box with Bleach wipes for the biosafety cabinet. Cut paper towel (3 layers each towel), soak in 15% bleach
- Prepare the Spill kit (large paper towels soaked in 15% bleach)
- Prepare the Liquid waste (for plastic Pasteur pipettes and tips) by filling the flask to ~⅓ with 15% Bleach
- Prepare Squirt bottle with 15% Bleach
- Prepare the Solid waste container by placing a red bag inside the beaker
- Check that all Racks intended for Falcon (Racks A, B, C) and Eppendorf (Racks 1, 2, 3) tubes are in the room
- Collect the number of 2 ml Eppendorf tubes to match the number of samples in Falcon Tubes.
- Prepare Numbered Stickers for the required amount of Falcon tubes. Cut sticker strips accordingly.
- Prepare Numbered Stickers for the required amount of 1.5 ml Eppendorf tubes. Cut sticker strips accordingly.
- Cut Parafilm strips to match the number of Falcon tubes + 5
- Check that enough Filter tips are in the box
- **PUT ON PPE**

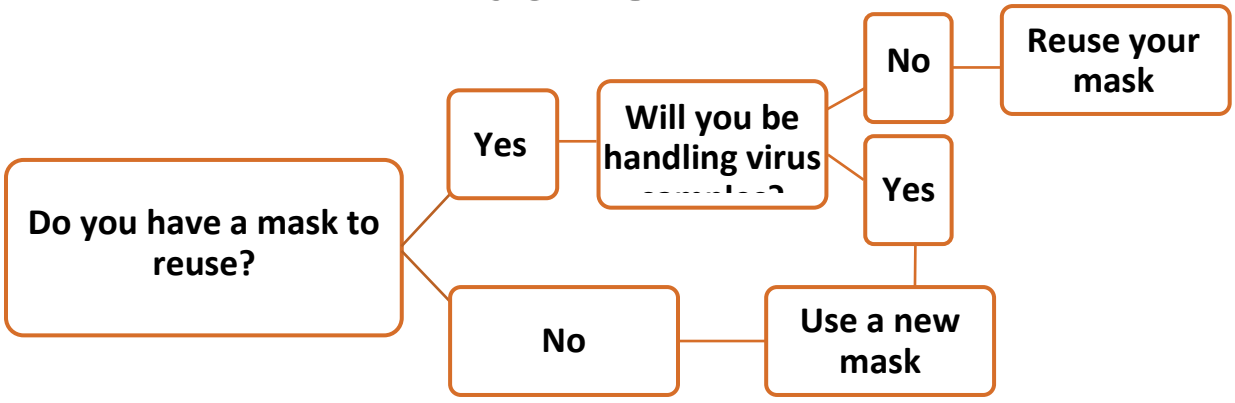
SET-UP THE BIOSAFETY CABINET

- Place the absorbent pad inside the biosafety cabinet (do not cover the AirFlow)
- Setup the working area by placing the following items on the absorbent pad:
  - Vortex
  - Rack A (for incoming Falcon tubes)
  - Rack 1 (for Eppendorf tubes)
  - 1.5 ml Eppendorf tubes
  - Filter Tips
  - 1000mL Pipette
  - FalconTube Rack (For the Inactivation Buffer)
  - Plastic Pasteur pipettes
- In addition Place the following in the biosafety cabinet, outside the area covered by the absorbent pad:
  - Bleach wipes
  - Rack B (For Falcon tubes)
  - Rack 2 (for the clean tubes)
  - Squirt bottle with 15% bleach
  - Squirt bottle with 70% ethanol
  - Dry paper towels
  - Liquid waste container (for tips)
  - Solid waste container (on the opposite side of the liquid waste container)
  - Rubber bands (to close solid waste bag)
- Check that the following are on the small Table next to the biosafety cabinet:
  - Wrist-length latex or vinyl gloves of your required size
  - Spill kit
  - Rack C for Falcon tubes for storage
  - Rack 3 for inactivated samples ready for RNA extraction
- Check that the following are in the Check-in area:
  - Numbered stickers for Falcon tubes
  - Barcode scanner
- Pick up the Virus Inactivation Buffer from the antechamber and place it in the rack in the biosafety cabinet
- Get the numbered stickers for the Eppendorf tubes and place them in the biosafety cabinet
- Remove paper from Parafilm strips for the Falcon tubes and place in box in biosafety cabinet
- Double-check everything
- Triple-check everything

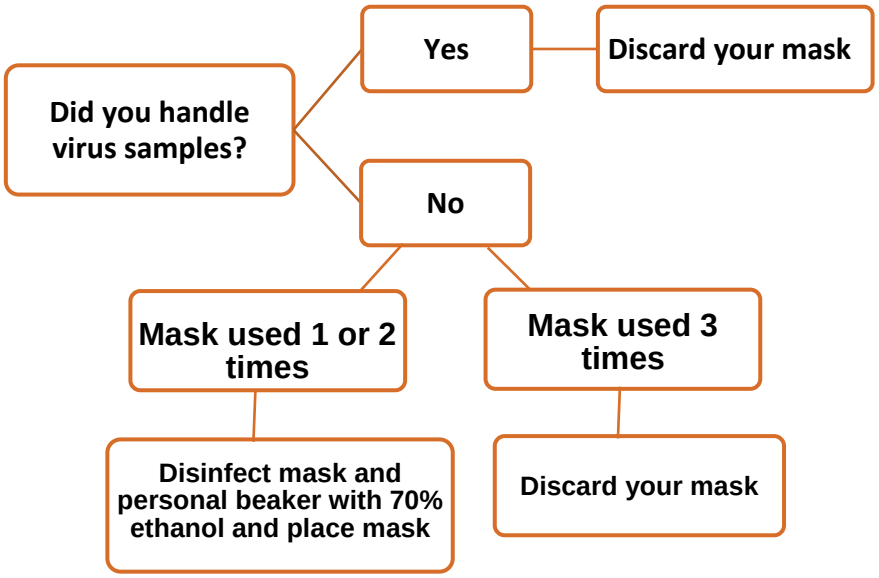
You are ready To Pick the samples from the Antechamber!

Guidelines to reuse masks in virus inactivation room

Mask On



Remove Mask



Important: Make sure you labelled your box (Name and surname) and the number of uses of the mask. Discard the mask after using it 3 times.

Example:

Name - Maria Silva

Uso - II

XIV. Virus Inactivation Short Protocol

Virus InactivationProtocol - Short Version

1. Make sure the hood is set up. Follow the Setup-Protocol!
2. Bring samples inside the hood.
3. Trash the bag, disinfect the container with bleach wipes.
4. Disinfect the tubes and move them to RACK A.
5. Overseer brings RACK A to the computer station for check-in.
6. Place the number stickers on the Eppendorf tubes in RACK 1, simultaneously, the overseer places the corresponding number on the Falcon tube .
7. Overseer brings samples back to the hood.
8. Vortex samples (10 sec each).
9. Pipette 200 µL of the samples into the corresponding Eppendorf tubes using a Pasteur pipette.
10. Add 400 µL of Virus inactivation (VI) Buffer to the Tubes.
11. Vortex the samples with VI buffer (10 sec each).
12. Wipe the Eppendorf tubes with bleach wipes and transfer them to RACK 2 (clean).
13. Wipe the original sample falcons with bleach wipes, wrap the lid with parafilm (wipe here again) and transfer them to RACK B (clean).
14. Change the gloves (trash yours inside the hood and take fresh ones outside).
15. Transfer the Eppendorf tubes to RACK 3 outside the hood.
16. Transfer the Falcon tubes to RACK C outside the hood.
17. Overseer brings Eppendorf tubes to antechamber and calls RNA room.
18. You can either clean up now…
19. ….or make sure you still have everything you need to start over with new samples. Fill up what is needed, before you get new samples inside the hood.

XV. CHECK-IN (Virus Inactivation Room) instructions

CHECK-IN (Virus Inactivation Room) instructions

1. Open Sample labels (VIRUS ONLY) table (shortcut in Desktop)
2. If not done already, write the internal codes in advance
3. Ask, write and confirm:

a. Operating hood: hood left / hood right

b. Operator name

c. Oversee name

2. For each sample:

a. If samples are NOT barcoded:

i. Wait for the operator to read the internal (e.g. 23) and external codes (e.g. 23536)

ii. Write the external code next to the respective internal code

iii. Loudly read the internal and external codes for confirmation

iv. Always ask to repeat if necessary

b. If samples are barcoded 

(e.g.):

i. Check if the barcode scanner is connected to the laptop via USB

ii. Select the cell next to the internal code of the sample

iii. Wait for the oversee to label the tube and show you the barcode; please NEVER touch the tube, it may be contaminated!
iv. Use the barcode scanner to scan the barcode
v. If the barcode is unreadable, ask the oversee to read the external code aloud, write it and loudly read it to confirm
vi. Confirm if external codes match the respective internal codes

3. Fill in the samples' provider (e.g. HSM) for all samples
4. Select the newly created cells:

a. Right-click the selected data

b. Click Protect range Proteger intervalo

c. Click Set permissions Definir autorizações

d. Click Show a warning when editing this range

Mostrar um aviso ao editar neste intervalo

e. Click Done Concluído

5. Call Check-out staff (47351) to warn the data is ready
6. When the samples containing inactivated virus are ready and outside the Viral room, call and warn the RNA room (47366) staff

a. Ensure no one opens the door inside the Viral room until the RNA room staff collects the samples to avoid external contamination

XVI. Prepare the Lysis buffer for the Virus Inactivation Room

Prepare the Lysis buffer for the Virus Inactivation Room

1. Pipette NR buffer into a 50 mL Falcon tube.
2. Add 1% of 2-Mercaptoethanol

# Samples	Volume NR	Volume 2-Mercaptoethanol
10	5 mL	50 µL
20	9 mL	90 µL
30	13 mL	130 µL
40	17 mL	170 µL
50	21 mL	210 µL

XVII. RNA Extraction Protocol – Short Version

RNA Extraction Protocol – Short Version

After receiving the samples from the Inactivation Room:

1. Centrifuge the tube at 11 000 *g* for 1min. Check for precipitates. If necessary, transfer supernatant to a new tube.
2. Add 600 µL of 70% EtOH. Mix by pipetting up and down.
3. Pipette 600 µL of the lysate onto the column.

   Centrifuge 11,000 *g* for 1'.
4. Pipette the remaining 600 µL of the lysate onto the column. Centrifuge at 11,000 g for 1'.
5. Pipette 350 µL of BufferNI. Centrifuge at 11,000 *g* for 1'.
6. Add 200 µL of BufferNWR1. Centrifuge at 11,000 *g* for 1'.
7. Add 600 µL of BufferNWR2. Centrifuge at 11,000 *g* for 1'.
8. Add 200 µL of BufferNWR2. Centrifuge at 11,000 *g* for 1'.

Do not remove the samples from the centrifuge.

9. Centrifuge again at 11,000 *g* for 1' (to dry membrane).
10. Place the column in a clean 1.5 mL eppendorf tube.
11. Add 50 µL RNase-free water directly to the column membrane (do not touch the membrane). Incubate for 1'.
12. Centrifuge at 11,000 *g* for 2' to elute the RNA.
13. Transfer 25 µL of RNA to another eppendorf tube.

    Take one RNA aliquot to the PCR freezer.

XIX. RT-PCR

XX. Other

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Practical Information

Instituto de Medicina Molecular – João Lobo Antunes
Hospital de Santa Maria
Faculdade de Medicina de Lisboa
Av. Professor Egas Moniz
1649-028 Lisboa

Lisboa
Instituto de Medicina Molecular

How to reach Instituto de Medicina Molecular in Hospital de Santa Maria?

By car:

1. If you decide drive to iMM please let us know asap, we need to request permission for you to park your car. Here is how you reach iMM parking lot:

Please use Hospital de Santa Maria main entrance on Av Prof Egas Moniz

Here you will have to take a parking card to pass the gates. **Please keep this card!!!**

Here there is a second set of gates. Please press the button to speak with security. Tell them you are an invited guest/speaker, your name, and that you were given permission to use this parking lot.

The parking lot is behind iMM. You have to walk up either side of the building to reach the front entrance of iMM.

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Avenida Professor Egas Moniz · Edifício Egas Moniz · 1649 - 028 Lisboa · Portugal · P + (351) 217 999 411 · E imm@medicina.ulisboa.pt

Lisboa
Instituto de Medicina Molecular

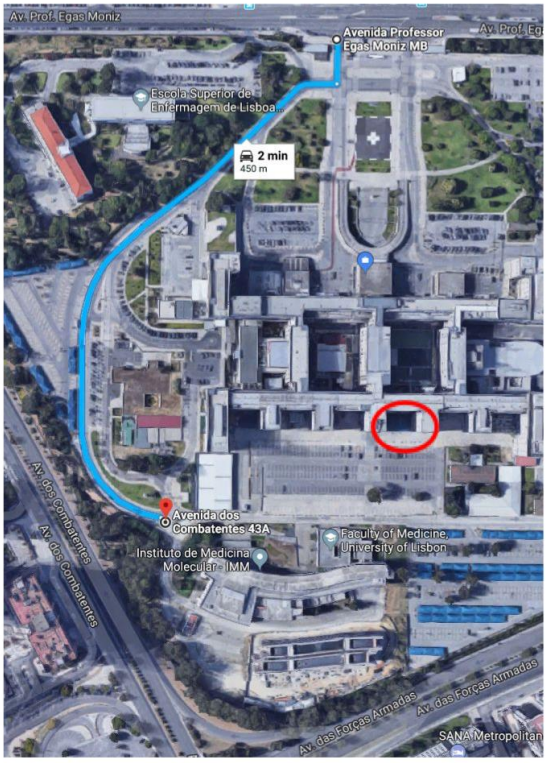
2. Once you arrive to iMM please ask security to call the person you are meeting.

They will:

- give you visitors cards.
- call the person you are visiting so they meet you by the elevators.
- Direct you to the elevators.

3. When your visit is over:
   - Go to the security of iMM to ask for a document saying you were a guest speaker at iMM.
   - Once you have this document you have to go to the parking lot agent to validate the card you collected entering the Hospital. (if you don't do this you will not be able to leave the hospital without paying for the time you spend inside the Hospital complex)

You can find the parking lot agent, in the big parking lot across iMM, here:



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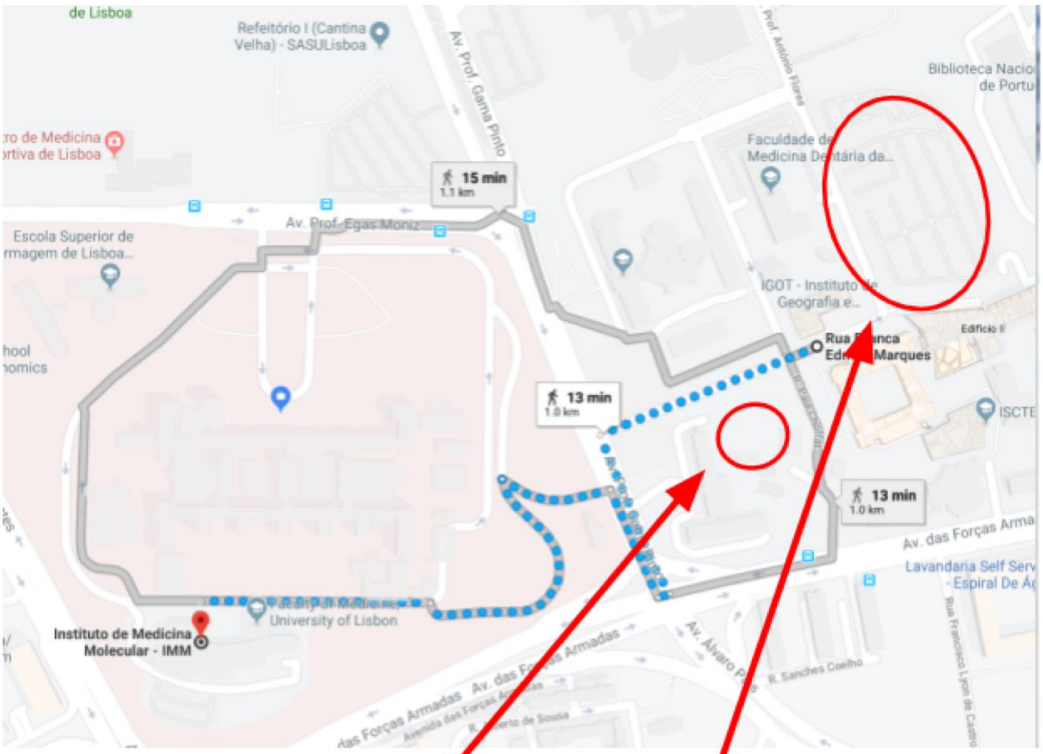
4. If for some reason you are not able to park on iMM parking lot, or if by the time you arrive the parking lot is full. There are two parking lots just across the street that are 2 or 2.50 euros for the whole day.



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How to reach Instituto de Medicina Molecular in Hospital de Santa Maria?

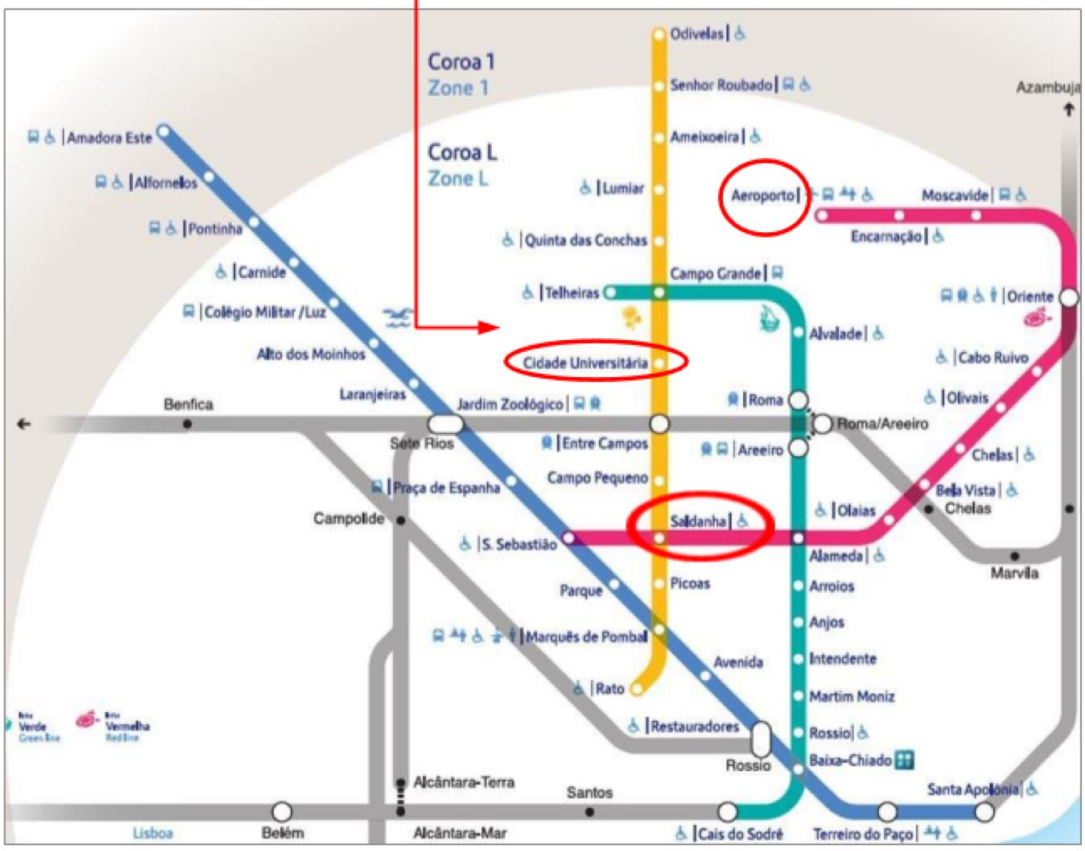
By subway

Metropolitano de Lisboa

- **From the Airport:**

1. At the airport, follow Metro station directions
2. At *Saldanha*, exit the metro train and change to **Yellow line**.
3. Take the **Yellow line** - direction *Odivelas*
4. Exit the metro station at *Cidade Universitária*
5. Walk to the Santa Maria Hospital
6. The price of the ticket is 1,45€
7. Timetable: 6:30 a.m. to 01:00 a.m.

Here is where you need to get out!



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Walking distance from "*Cidade Universitária*" Metro Station to Santa Maria Hospital (10 minutes)

By taxi:

You can take a taxi to Hospital de Santa Maria.
The average price from Lisbon airport is 15,00€.
Ask the Taxi driver to drop you in front of Egas Moniz Building/Faculdade de Medicina. (This way he will drive into the Hospital complex and drop you off in front of iMM building)
If the taxi drops you off outside the Hospital complex, please find walking directions here:

Walking distance from the main entrance of the Hospital to Instituto de Medicina Molecular (10 minutes).

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