Instituto de Medicina Molecular | João Lobo Antunes

iMM COVID-19 Research Sample Collection

Standard Operating Procedure

Version 1.6

Authors

Helena Nunes Cabaço
(hcabaco@medicina.ulisboa.pt)
António M. Mendes
(antoniomendes@medicina.ulisboa.pt)

With the help of:
Ana Rita Pires, Patrícia Napoleão and Ana Friães

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General info & Workflow

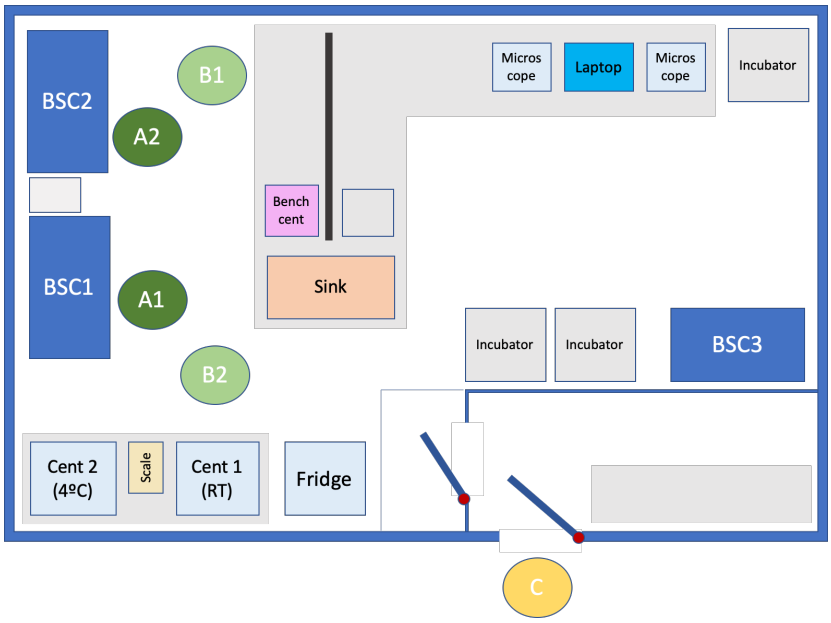
1. Working guidelines

- Appropriate personal protective equipment (PPE) should be worn by all laboratory personnel handling specimens
- All technical procedures should be performed in a way that minimizes the generation of aerosols and droplets (e.g. no vigorous vortexing or pipetting)
- Always use buckets with lids for centrifugation (only open them inside the BSC)
- Always work in double containment outside the BSC
- Change gloves every time you exit the BSC
- Disinfect all the material leaving the BSC

2. Workflow

- Work performed under BSL-2+ conditions using a **validated Biosafety Cabinet (BSC)**.
- People in the lab:
  - 2 people working in the BSC (person A1 and person A2)
  - 2 people assisting them (person B1 and person B2)
  - 1 person providing assistance outside the lab (person C)

Lab configuration



Equipment & Reagents

3. Laboratory equipment

- Centrifuges with buckets for BD Vacutainer tubes and 50ml tubes, with lids
- Microcentrifuge
- Laminar flow hood
- Vortex
- Scale
- Tube racks
- Micropipettes (20 µl, 200 µl, 1000 µl)
- Labeling material (permanent marker)
- Freezing container, isopropanol-based (e.g. Nalgene cryopreservation device “Mr Frosty”), or alcohol-free cryopreservation system (e.g. CoolCell)
- Freezer -80°C, connected to a central alarm
- Liquid nitrogen tank/ Liquid nitrogen

4. Disposable materials

- BD Vacutainer® CPT, 8ml, Sodium citrate, Ficoll (Cat. No. 362782, 60/case)
- Blood collection butterfly for CPT tubes (BD 368655)
- 15 ml and 50 ml conical polypropylene tubes, sterile
- Sterile transfer pasteur pipettes, plastic, 3.5ml
- Disposable hemocytometer (FAST READ 102®, Biosigma)
- Biobank tube labels
- 1,5 or 2 ml sterile cryotubes (Sarstedt 72.692.005 or 72.692.006; for plasma, serum and cell pellet storage at -80ºC)
- 2 ml sterile cryotubes (Greiner 122279; for PBMC cryopreservation)
- Filter tips (20 µl, 200 µl, 1000 µl)
- 2 waste bins (type IV – red)

5. Reagents

- 3% Acetic Acid with Methylene Blue (AA/MB; Stemcell Cat. No. #07060)
- DMSO (Sigma Cat. No. D2650-100ML)
- **COLD** (4ºC) PBS 1x without Ca^{2+} or Mg^{2+}, sterile, pH 7.4 (Gibco 10010056); daily: have 2x 500 ml bottles in the fridge.
- **COLD** FBS, heat inactivated, sterile (Gibco 10500064); daily: have 3 x 50 ml aliquots in the fridge (freeze the remaining aliquots and thaw when necessary)
- **Daily prepared:** 1% Virkon (Fisher Scientific Cat. No. 12328667; dissolve 1 tablet in 500 ml of tap water)
- **Daily prepared:** 15% bleach (add 75 ml bleach to 425 ml tap water)

6. PPE

- Lab coat (personal, re-usable)
- FFP2 (or equivalent; or FFP3) face mask
- Cap
- Shoe covers
- Disposable gown
- Disposable apron
- 2 pairs of gloves (1st nitrile, 2nd latex)
- Face shield (or protective goggles)

PPE donning (Annex 1)

1- Discard your surgical mask
2- Put on **face mask FFP2/3**
3- Put on **cap**
4- Put on your **regular lab coat**
5- Put on **shoe covers**
6- Put on the **disposable gown**
7- Put on **inner gloves (nitrile)** placing them over the cuffs of the overall
8- **Tape gloves** to the overall to prevent gaps
9- Put on the second pair of **gloves (latex)** over the first one
10- Put on **apron over** the disposable gown

Double-check that all PPE is correctly worn
When finished knock at the door so that the next person will enter

Enter the lab
Close the door behind you

11- In the lab: Put on face shield (or protective goggles)

Procedure Preparation

7. Lab preparation (see Annex 2 for Lab preparation per position)

- Turn the BSC UVs on for 20 minutes before opening the hood
- Prepare virkon/bleach
- Make bleach and ethanol wipes
- Prepare spill kit: box with paper towels soaked in bleach
- Place Centrifuge 2 and microcentrifuge at 4ºC
- Make sure there is cold PBS (2 bottles) and cold FBS (3 thawed aliquots)
- Open “Research samples database” excel in laptop
- Check that the “Mr Frosty” freezing containers are ready to take samples

8. Tube labeling & preparation

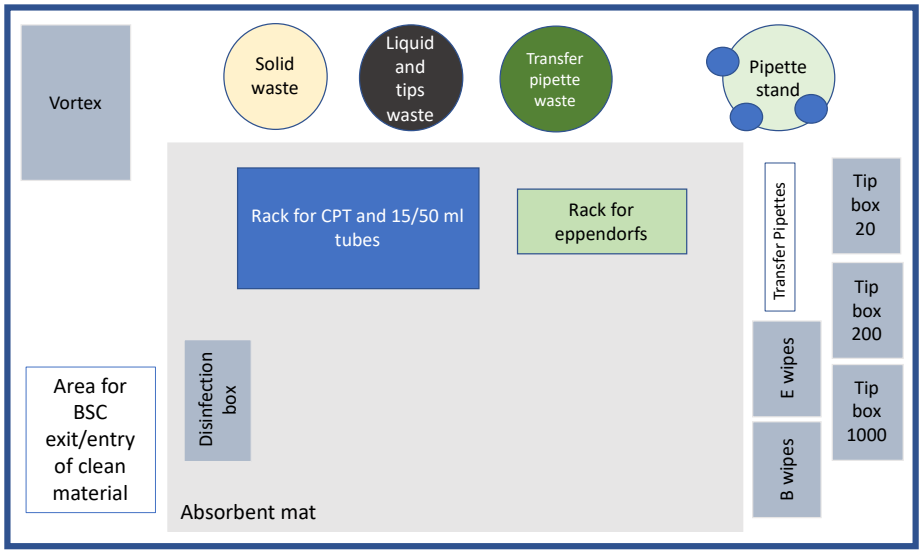
- Label cryotubes:
  - Use biobank labels
  - Place tubes in order of collection per patient (serum, plasma, cell pellets, frozen cells) in a box
  - Write the correspondence of Biobank labels and codes in the laptop

- Per patient you should have:
  - 6 tubes for serum collection
  - 6 tubes for plasma collection
  - 4 tubes for dry cell pellets
  - 6 tubes for PBMCs
  - 50 ml falcon for PBMC collection
  - 50 ml falcon with PBS for PBMC washing
  - 15 ml falcon with 10 ml PBS for PBMC resuspension before counting
  - 1 tube with 80 µl 3% Acetic Acid with Methylene Blue for cell counts

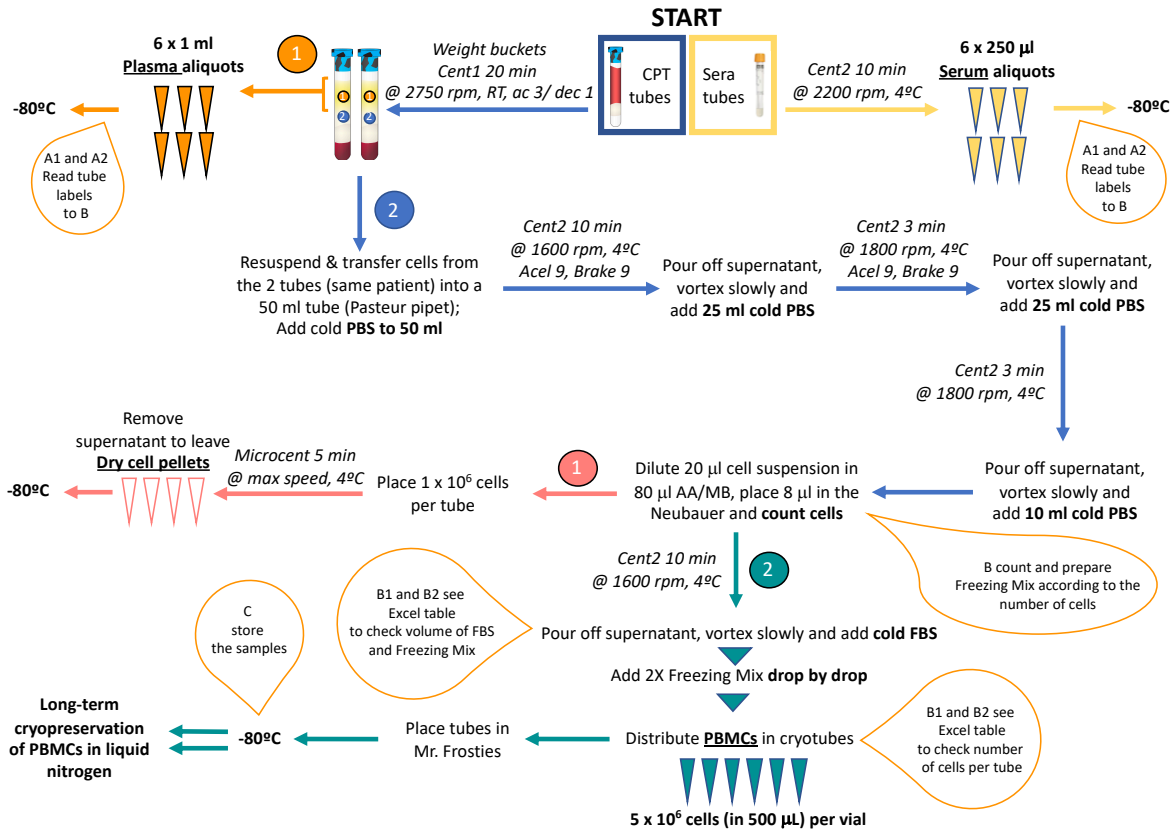
9. BSC preparation

- Place inside the BSC:
  - Absorbent mat
  - Vortex
  - Permanent pen
  - Solid waste (bucket with a bag)
  - Liquid waste (plastic bottle with ~100 ml pure bleach); also for tips
  - Transfer pipette waste with diluted bleach/virkon (tall waste bottle)
  - Pipette stand (pipettes: 20 µl, 200 µl and 1000 µl)
  - Tip boxes (20 µl, 200 µl and 1000 µl)
  - Transfer plastic pipettes
  - Racks (for CPT tubes and for eppendorfs)
  - Disinfection box (box with bleach/virkon wipes to place lids)
  - Bleach/Virkon wipes
  - Ethanol wipes

BSC layout

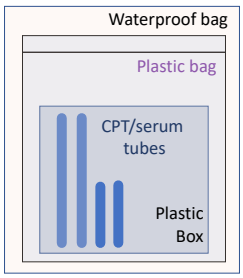


Sampling Procedure



(Annex 3)

10. Sample collection



10.1. Two CPT tubes of blood and 1 serum tube are collected per patient

10.2. Samples are brought from Hospital de Santa Maria to IMM entry in **quadruple contention**

10.3. Blood samples should be stored **upright at room temperature** and centrifuged within 4 hours of blood collection

10.4. Person C: pick up the samples and handle to person B1 in the lab

10.5. Person B: remove plastic bag with box of tubes from waterproof bag, clean plastic bag with bleach wipes and handle to person A in BSC1; go to laptop

11. CPT tube centrifugation

Note: Centrifuge 1 supports a maximum of 16 CPT tubes per centrifugation. If more than 8 patients are to be done at a time do 2 sequential centrifugations.

11.1. A1/A2: remove box from the plastic bag and wipe box with bleach wipes
11.2. Visually inspect the tubes to confirm they are ok
11.3. Carefully remove CPT tubes from the plastic box, one by one, and clean with bleach wipes; clean lid first and trash wipes, then clean sides of the tube
11.4. Dictate the SNS number to B1/B2 on the computer; B1/B2 repeats the SNS number and attributes a code to the sample (S1, S2, etc)
11.5. A1/A2: write the code on the side of the CPT tube
11.6. Remix blood sample by gently inverting the tube 8 to 10 times, place tube in the rack and proceed to next tube
11.7. Place tubes in the buckets (max 4 per bucket, all placed centrally; even opposite buckets) and close lid
11.8. Wipe buckets with bleach wipes and then ethanol wipes (make sure they are not slippery) and place in the BSC “exit” zone on the left
11.9. B1/B2: pick up one bucket and weight it, place it in Centrifuge 1
11.10. Pick up 2nd bucket and weight to confirm there is minimal difference from the 1st one (max 10 g); if not re-handle both buckets to A1 to try and redistribute the tubes or use balances
11.11. B: Centrifuge tubes at 2750 rpm (~1500 g), 20 min, 20°C, acceleration 3/break 1
11.12. During centrifugation: B1/2: handle cold PBS to A1/A2; A1/A2: label 50ml tubes with patient codes (S1, S2, …) and fill them with PBS

12. Serum collection

12.1. A1/A2: remove box from the plastic bag and wipe box with bleach wipes
12.2. Visually inspect the tubes to confirm they are ok
12.3. Carefully remove serum tubes from the plastic box, one by one, and clean with bleach wipes; clean lid first and trash wipes, then clean sides of the tube
12.4. Write the sample code of the corresponding patient in each tube
12.5. Place tubes in centrifuge buckets and close lid
12.6. Wipe buckets with bleach wipes and then ethanol wipes (make sure they are not slippery) and place in “exit” zone on the left
12.7. B1/B2: pick up one bucket and weight it, place in Centrifuge 2
12.8. Pick up 2nd bucket and weight to confirm there is minimal difference from the 1st one (max 10 g); if not re-handle both buckets to A1 to try and redistribute the tubes or use balances
12.9. B: Centrifuge tubes at 2200 rpm (~1000 g), 10 min, 4°C
12.10. Carefully remove buckets with lid and handle them to A1/A2
12.11. A1: visually inspect tubes before opening

12.12. Remove bucket lid and carefully remove each serum tube, making sure it is not damaged; clean tube with bleach wipes and place in rack
12.13. Open serum tube carefully and place lid on disinfection box, inside facing down
12.14. Collect the upper 250 µl of serum into 6 tubes (distribute the rest of the serum, if any left, through all tubes)
12.15. Close the lid of the serum tube carefully and place it in solid waste
12.16. Proceed to the next serum tube
12.17. Confirm tube labels with B1/B2
12.18. Place serum tubes in a cryopreservation box and wait until plasma is also collected

13. Plasma collection

13.1. After CPT tube centrifugation: B1/B2: Carefully remove buckets with lid from Centrifuge 1 and place them in BSC1/BSC2
13.2. A1/A2: visually inspect tubes to make sure they are not damaged
13.3. Remove bucket lid, carefully remove tubes and make sure they are not damaged
13.4. Open the CPT tubes carefully and place the lids inside down on disinfection box
13.5. Collect the upper 3x1 ml of plasma of each tube into cryopreservation tubes (total 6x1 ml aliquots; distribute the rest through all tubes until reaching just over the “8 ml” blue mark in the CPT tube)
13.6. Proceed to PBMC collection protocol with the same CPT tube
13.7. In the end of plasma collections: Confirm tube labels with B1/B2
13.8. Place plasma tubes in the same box as serum tubes, after them (organized per patient)
13.9. Wipe the box with bleach wipes and handle it to B1/B2 (through exit zone)
13.10. B1/B2 place the boxes in the fridge

14. PBMC collection

14.1. A1/A2: Resuspend cells in the remaining plasma with a sterile transfer pipette
14.2. Transfer cells from the 2 CPT tubes of the same volunteer to one 50 ml tube
14.3. Add cold PBS to each CPT tube (no need to change the pipette) and transfer cells to the 50 ml tube; repeat step until gel matrix is clean
14.4. Place pipette in the pipette waste
14.5. Close CPT tubes and place them in the solid waste
14.6. Top up 50 ml tube with cells to 50ml with cold PBS
14.7. Repeat for every CPT tube
14.8. B1/B2: handle 50 ml buckets to A1/A2 (place in BSC)
14.9. A1/A2: clean 50 ml tubes with bleach wipes, place in buckets, close lid and wipe bucket; place in exit zone
14.10. B: place buckets in Centrifuge 2; Centrifuge at 1600 rpm (~500 g), 10 min, 4°C
14.11. After centrifugation: B1/B2 handle buckets to A1/A2

14.12. A1/A2: open lid and remove tubes from buckets
14.13. Gently pour off supernatant
14.14. Resuspend pellet by slowly vortexing
14.15. Add 25 ml cold PBS (slowly pour directly from the bottle)
14.16. Clean 50 ml tubes with bleach wipes, place in buckets, close lid and wipe bucket; place in exit zone
14.17. B1: place buckets in Centrifuge 2; Centrifuge at 1800 rpm (~700 g), 3 min, 4°C
14.18. After centrifugation: B1/B2 handle buckets to A1/A2

2X

14.19. Repeat wash (steps 14.12 – 14.18)
14.20. B1/B2: give falcon tubes containing 10 ml PBS each to A1/A2
14.21. A1/A2: Gently pour off supernatant
14.22. Resuspend pellet by slowly vortexing (make sure the pellet is fully resuspended)
14.23. Add 10 ml cold PBS (slowly pour falcon tubes with 10 ml PBS) to the cells

15. PBMC counting

15.1. B1/B2: give tubes with 80 μl 3% AA/MB and counting chamber to A1/A2
15.2. A1/A2: add 20 μl of cells (agitate sample tube; 1:5 dilution) and resuspend
15.3. Place 8 μl in the disposable counting chamber and write patient code
15.4. Place chamber in a pipette tip box covered with bleach wipes and handle to B1/B2
15.5. B1/B2: count cells in the microscope; Count cells in 4 fields and write values in Research Samples Database excel sheet (see **Annex 4 – cell counting**)
15.6. Calculate the volume of FBS to add for a concentration of 20 x 10^6 cells/ml (consider ~200 μl of PBS remain in tube after centrifuging and subtract that volume)
15.7. B1/B2: Tell A1/A2 the volume to add to each patient (by code) of: cell pellets (for 1 x 10^6 cells); FBS and Freezing Mix; and the # of vials (check Research Samples Database excel sheet/**Annex 5 – PBMC distribution**)
15.8. A1/A2: write volumes in the tubes; keep 50 ml tubes in cold buckets while preparing cell pellets

16. Dry cell pellet preparation

16.1. A1/A2: place the appropriate volume of cell suspension in each of the tubes
16.2. Place tubes in a box, disinfect box and handle to B1/B2
16.3. B1/B2: place tubes in microcentrifuge
16.4. Centrifuge at max speed, 5 min, 4°C
16.5. B1/B2 places tubes in the box and handle to A1/A2
16.6. A1/A2: Remove all the supernatant to leave a dry pellet
16.7. B1/B2: give box with serum/plasma tubes to A1/A2
16.8. A1/A2: Place tubes inside the box, wipe box with bleach wipes and give it to B1/B2
16.9. B1/B2 places the box in at 4ºC until PBMCs are in the cryopreservation containers (all sample components will be placed at -80ºC later)

17. PBMC Cryopreservation

17.1. A1/A2: Close and clean buckets (with tubes with cells inside) and handle to B1/B2
17.2. B1/B2: Centrifuge at 1600 rpm (~500 g), 10 min, 4°C
17.3. During centrifugation: B1/B2 prepare Freezing Mix (20% DMSO in FBS) in BSC3 and handle FBS and Freezing Mix reagents to A1/A2
17.4. After centrifugation: B1/B2 handle buckets to A1/A2
17.5. A1/A2: open lid and remove tubes from buckets
17.6. Gently pour off supernatant
17.7. Resuspend pellet by slowly vortexing
17.8. Add volume of cold FBS to place the cells at a concentration of 20 x 10^6 cells/ml
17.9. Add to the cells, drop by drop, a volume of cold Freezing Mix equivalent to the FBS volume in the tube (final cell concentration: 10x10^6 cells/ml)
17.10. Shake gently
17.11. Transfer 500 µl of suspension (5x10^6 cells) to each cryotube (distribute remaining volume through all tubes; B1/B2: confirm labels);
17.12. Transfer the tubes into the freezing container (Mr. Frosty or CoolCell)
17.13. Wipe freezing container and handle to B1/B2
17.14. B1/B2 place the boxes and freezing containers in a bag and handle it to C
17.15. C places the freezing containers and the boxes with serum/plasma/pellets at -80ºC
17.16. Biobank staff will transfer the cryotubes into a liquid nitrogen storage container and register their position

18. BSC cleaning at the end of the protocol

18.1. A1/A2: clean material that should leave the BSC thoroughly with bleach wipes and then ethanol wipes and handle to B1/B2 through the exit zone
18.2. Close wastes, clean with bleach wipes and handle to B1/B2 to place in Class IV (red) waste container
18.3. Clean all the material staying inside the BSC (pipettes, pipette tips, trash bucket) thoroughly with bleach wipes and then ethanol wipes
18.4. Close absorbent mat towards the inside and place it in the solid waste
18.5. Clean the BSC thoroughly with bleach wipes and then ethanol wipes
18.6. B1/B2 handle 2 pieces of tape to A1/A2 and 1 bag
18.7. A1/A2: close solid waste bag with tape
18.8. Place bag with waste inside a 2nd bag
18.9. Remove gloves and place inside the 2nd bag
18.10. Put on new clean gloves and close the bag with tape
18.11. Clean 2nd bag with bleach wipes, bring it out of the BSC and place in Class IV (red) waste container
18.12. Turn BSC UVs on
18.13. Close Class IV (red) waste container and place outside with a “COVID-19” sign

19. Exiting the Lab (Annex 5)

19.1. B1/B2: save Research Samples Database file and save a backup file ending with the date (YYYYMMDD) in the Backup folder
19.2. Before exiting the experimental handling room, make sure you observed all the room procedures (A1/A2: make sure you changed gloves inside the BSC)
19.3. Remove the face shield (or protective goggles) – Disinfect it with 15% bleach and then 70% Alcohol
19.4. Remove apron
19.5. Remove shoe covers
19.6. Remove outer pair of gloves
19.7. Remove disposable gown (you may have to tear the gown since its taped)
19.8. Remove inner pair of gloves
19.9. Put a new pair of latex gloves (near you in the cabinet)
19.10. Hold your breath, remove face mask and cap and dispose of it in the appropriate bin near you
19.11. Remove gloves and put a new pair of latex gloves
19.12. Leave the lab and go to the vestibule in your regular lab coat only
19.13. Remove lab coat and hang it
19.14. Remove gloves and disinfect your hands
19.15. Put a new surgical mask on
19.16. Leave the vestibule

Emergency Procedures

If a spill occurs inside the BSC, but does not reach your overall:

- Cover with bleach wipes and leave for 10 minutes
- Remove absorbent mat in the BSC and put it in the solid waste bag
- Close the bag and discard
- Discard contaminated gloves and put new ones
- Decontaminate all the materials and work surface of BSC with bleach wipes
- Discard contaminated gloves and put news ones
- Place new absorbent mat in the BSC work area and continue to work

If a spill occurs inside the BSC and reaches your overall:

- 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 will be provided

If a minor spill occurs outside the BSC:

- Proceed to clean the spill:
  - Cover with bleach wipes and allow it to act for 20 minutes
  - Thoroughly wipe the area with bleach wipes and ethanol wipes and discard the waste into a primary solid waste bag. Close the bag, wipe it and place it in the solid container for disposal. Replace the external gloves.
  - Continue working, if necessary.

If a major spill occurs outside the BSC:

- 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 potential aerosols to settle
- Put on new PPE before entering the room
- Proceed to clean the spill (see “minor spill” guidelines)

Inform coordinators for further direction:

- Helena Nunes Cabaço
- António Mendes
- iMM Security
- Internal Emergency Numbers

Annex 1

PPE donning

1- Discard your surgical mask
2- Put on **face mask FFP2/3**
3- Put on **cap**
4- Put on your **regular lab coat**
5- Put on **shoe covers**
6- Put on the **disposable gown**
7- Put on **inner gloves (nitrile)** placing them over the cuffs of the overall
8- **Tape gloves** to the overall to prevent gaps (leave tape end bended to facilitate removal)
9- Put on the second pair of **gloves (latex)** over the first one
10- Put on **apron over** the overall

Double-check that all PPE is correctly worn
When finished knock at the door so that the next person will enter

Enter the lab
Close the door behind you

11- In the lab: Put on face shield (or protective goggles)

Annex 2

What to do before starting

B1 and B2

Turn on the computer

- ✓ Connect manually to **eduroam** with your IMM account
- ✓ Log in with your IMM account
- ✓ Open excel file "Research Samples Database"

Check the fridge:

At least 2 full PBS Bottles
3 FBS aliquots defrosted (if not take it from -20 and leave it to defrost)

Place Centrifuge 2 and microcentrifuge at 4ºC

Labeling the tubes – Use Biobank codes and labels and label tubes in the following order:

- ✓ 6 tubes for serum
- ✓ 6 tubes for plasma
- ✓ 4 tubes for dry pellets
- ✓ 6 tubes for cryopreserved PBMC

Prepare:

- ✓ BSC3
  - ✓ Turn on UVs
  - ✓ Turn off UVs after 20 minutes and open the door
  - ✓ Check for:
    - Solid waste bin
    - Pipettor and 10 ml pipettes
    - 200 µl and 1000 µl pipettes and tips
- ✓ One 50ml falcon tube for cells – Empty with label (SXX)
- ✓ One 15 ml falcon tube with 10ml of PBS (cold, then keep it in the fridge)

A1 and A2

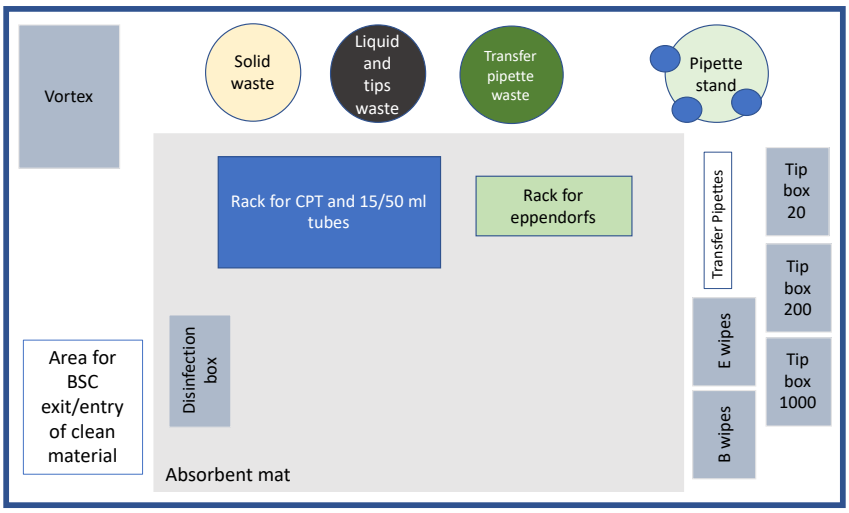
BSC 1 and 2

- ✓ Turn on UVs for 20 minutes
- ✓ Open the door

At the sink prepare:

- ✓ Bleach/virkon wipes
- ✓ Ethanol wipes

Prepare BSC 1 and 2 according to the layout (with the help of B1/B2):

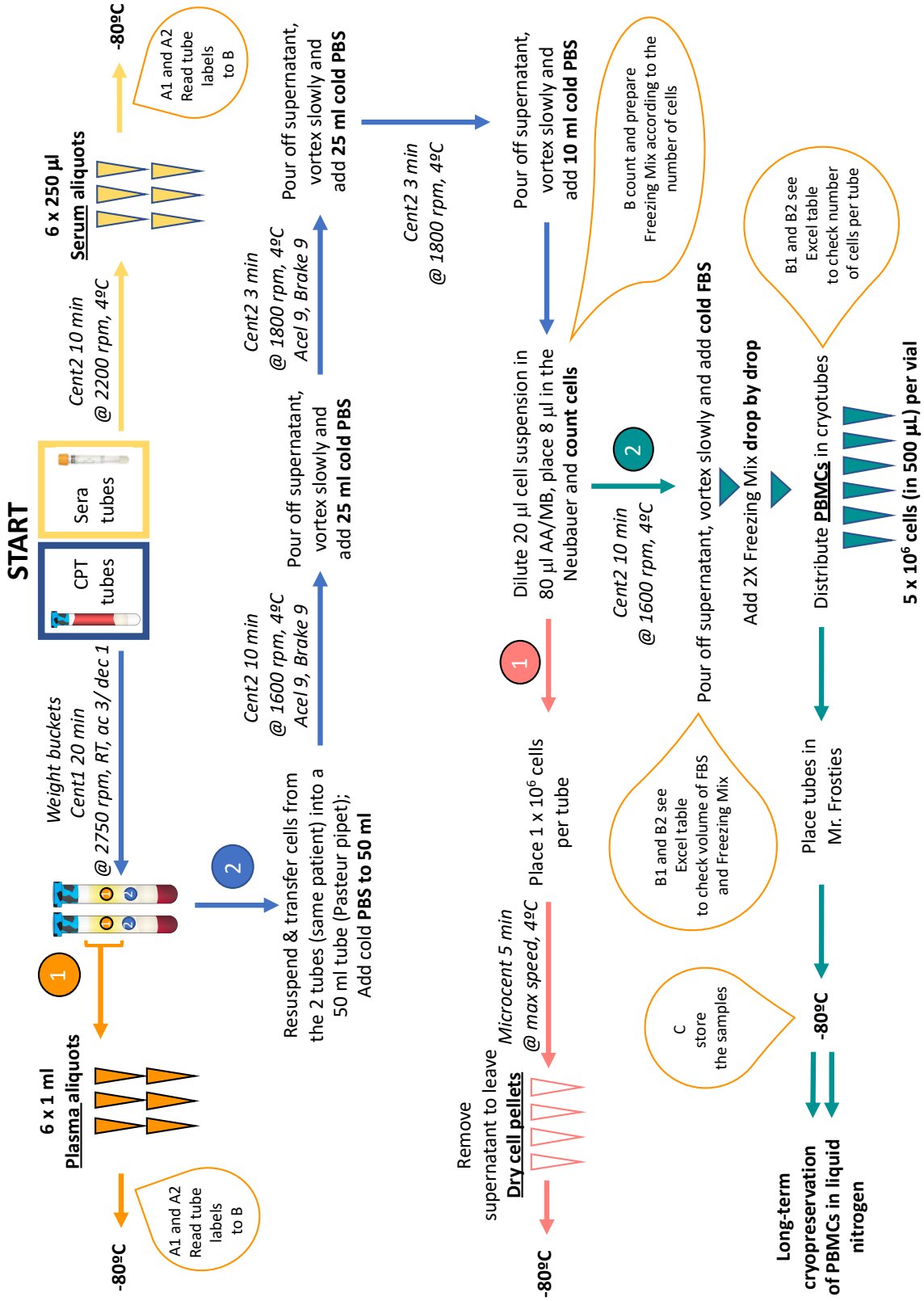


Sit down and place yourself at a working position.
Once you are working **your hands should not exit the BSC** unless you put on new gloves

C

- ✓ Be ready to receive the samples at iMM door and deliver them to your colleagues inside the lab. You will have a desk and a laptop to work on your projects while the samples are being prepared
- ✓ You will have access to the inside of the lab by phone
- ✓ Be ready to store samples at -80ºC
- ✓ At the end of the procedure make sure that stocks that need to enter for the next day are ready in the vestibule (information from inside the lab)
- ✓ Be ready to help in case of emergency

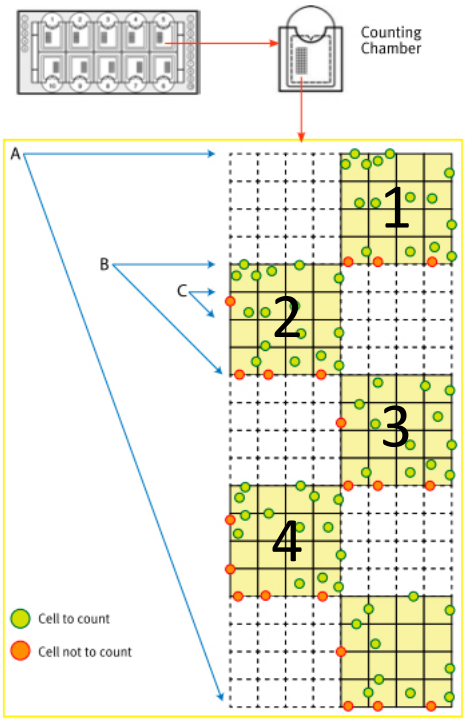
Annex 3 – Simplified Workflow



Annex 4 – Cell counting

1. Dilute cells 1:5 in 3% AA/MB (20 µl of cell + 80 µl 3% AA/MB)
2. Place 8 µl of cell dilution per FAST READ 102® counting chamber (label chamber with patient code); Each device allows analysis of 10 samples
    a. Each chamber (A) contains a GRID composed of 10 SQUARES (B) divided into 16 smaller squares (C; called SECTORS)
3. Count cells in 4 SQUARES (B; 1-4 in figure);
    a. Pay attention to the cells on the edges, you should only count those on either side, to avoid over or under counting risk
4. Calculate the cell suspension concentration using the Research Samples Database excel based on the formula (considering that each sector corresponds to 0,1 µl of diluted cell suspension):

    Cell concentration (cells/ml) = (Average of sector counts) X (dilution) X 10^4



Annex 5 – PBMC distribution

Dry cell pellets and cells to cryopreserve

Cells to cryopreserve: final suspension at 10 x 10^6 cells /mL, so 5 x 10^6 cells correspond to 500 µl (4 x 10^6 are 400 µl, …) - distribute any volume left evenly through all the tubes

* Less than 4 x 10^6 cells: add 100 µl of FBS and 300 µl of Freezing Mix and make 1 vial

** 5 to 7 x 10^6 cells: add 400 µl of FBS and 600 µl of Freezing Mix and make 2 vials

*** make calculations in excel but place less volume (less cells) per vial

NOTE: The excel sheet has been modified to make the calculations automatically.

Cells (x 10^6)	# Vials with 1x10^6 cells (cell pellets)	# Vials with 5x10^6 frozen cells (500 µl)
< 4	0	1*
5	0	2**
6	0	2**
7	0	2**
8	0	2 with ~400µl per vial ***
9	1	2 with ~400µl per vial ***
10	1	2 with ~400µl per vial ***
11	1	2
12	1	3 with ~400µl per vial ***
13	1	3 with ~400µl per vial ***
14	2	3 with ~400µl per vial ***
15	2	3 with ~400µl per vial ***
16	2	3 with ~400µl per vial ***
17	2	3
18	3	3
19	4	3
20	4	3
21	4	3
22	4	3
23	4	3
24	4	4
25	4	4
26	4	4
27	4	4
28	4	4
29	4	5
30	4	5
31	4	5
32	4	5
33	4	5
34	4	6
35	4	6
36	4	6
37	4	6
38	4	6
39	4	7
40	4	7

Annex 6 – Exiting the lab

In the lab:

1. Remove the face shield (or protective goggles) and disinfect it with 15% bleach and then 70% Alcohol
2. Remove apron > dispose in the appropriate bin near the exit
3. Remove shoe covers > dispose in the appropriate bin near the exit
4. Remove outer pair of gloves > dispose in the appropriate bin near the exit
5. Remove disposable gown (you may have to tear the gown since its taped) > dispose in the appropriate bin near the exit
6. Remove inner pair of gloves > dispose in the appropriate bin near the exit
7. Put a new pair of latex gloves (near you in the cabinet)
8. Hold your breath, remove face mask and cap > dispose in the appropriate bin near the exit
9. Remove gloves and put on a new pair of latex gloves
10. Leave the lab to the vestibule in your regular lab coat only

In the vestibule:

11. Remove lab coat and hang it
12. Remove gloves and disinfect your hands
13. Put a new surgical mask on
14. Leave the vestibule