BIOBANQUE QUÉBÉCOISE DE LA COVID-19

PON07EN01WEB

Title	**Blood sample processing**
Codification	SOP07-WEB
Pages	5

Version history

Date jj/mm/aaaa	**Version**	**Page**	**Description of the amendement**
27-07-2020	01	5	Creation of a short version of the SOP

Historique de la mise en place de la PON

Version	Date jj/mm/aaaa	Version	Date jj/mmm/aaaa	Version	Date jj/mmm/aaaa

Approbation de la PON du laboratoire

	Signature	Date jj/mmm/aaaa

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1. Sample processing

Depending on the possibilities for the blood samples to be processed, we collect 1 PAXgene® RNA tube, 4 ACD tubes and 1 red-capped tube (serum): to perform DNA profiling, RNA profiling, to isolate plasma and PBMCs and to collect serum.

All tubes kept at RT before processing; the faster after blood draw the better, ideally <6 hours; < 12 hours fine for most assays; >12h: a number of functional assays will become less reliable. Please indicate in the comment section on retrieval the time between treatment and venipuncture.

Type of specimens collected
PAXgene (RNA extract)
Whole blood (DNA extract)
Plasma
PBMC
Serum

Solution preparation

HEPES 1M

1. 5ml aliquots and store at 4^0C.

Penicillin Streptomycin (10 000U/ml)

1. 5ml aliquots ans store 4^0C or -20^0C.

FBS decomplemented

1. Heat at 56^{0}C for 30 minutes to decompliment.
2. 50 ml aliquot and store -20^{0}C.
3. Thaw before use.

R+

1. Take a 50ml bottle of RPMI 1640.
2. Add 5mL of HEPES 1M.
3. Add 5mL of Pen/Strep 10000U.
4. Can be stored at 4^{0}C.

R10FBS

1. Take 45ml of prepared R+ medium.
2. Add 5mL of decomplemented FBS.

FBS 20%DMSO :

1. Take 40ml of decomplemented FBS.
2. Add 10mL of DMSO.
3. Store at 4°C for 1 week.
4. It will be used as a freezing medium.
5. Note the date on which the solution is prepared.

Procedure

PAXGENE RNA

1. Allow the tube to stand at room temperature overnight. Record the date and time on the worksheet (BQC19 – Biobanking sample logsheet).
2. After overnight at room temperature, transfer PAXgene® RNA tubes at -20°C for 24 hours. Record the date and time on the worksheet (BQC19 – Biobanking sample logsheet).
3. Transfer to -80°C for long term storage. Record date, time and location on the worksheet (BQC19 – Biobanking sample logsheet).

SERUM : SERUM RED TUBE

1. Centrifuge serum red cap tube at 2000g for 10min at RT BRAKE ON.
2. Recuperate serum in 15mL tube. BE CAREFUL not to disturb the base / clot in the bottom of the tube.
3. Aliquot the serum in volumes of 250µl and volumes of 500µl in the screwcap tubes. For work optimization, this step can be done during the whole blood centrifugation.
4. Complete the worksheet (BQC19 – Biobanking sample logsheet).

WHOLE BLOOD: ACD TUBES

1. Transfer blood from ACD tubes into 50mL Falcon tube (pooled) usual volume is approximately 30 mL. Record the volume collected on the worksheet (BQC19 – Biobanking sample logsheet).
2. Transfer aliquot of 500µL of whole blood into cryotubes.

PLASMA : TUBES ACD

3. Centrifuge whole blood tubes at 850 g for 10 min, at RT, BRAKE OFF.
4. Remove the plasma layer with plastic transfer pipet (10 mL) and transfer into a 15mL tube.
5. Transfer the plasma into tubes with screw caps, containing 500µL and 250µL/tube.
6. Store tubes at -80°C and note their location in the worksheet (BQC19 – Biobanking sample logsheet).

FICOLL-HYPAQUE OVERLAY METHOD FOR PBMC ISOLATION

1. After Plasma collection, top up blood to 30 mL with HBSS+ medium (30mL total). We need to dilute blood in 1:1 ratio. Mix gently and thoroughly.
2. Take 50 mL tubes with 15mL Ficoll. Plan 2 parts diluted blood for one part of Ficoll (usually 15 ml of Ficoll and 30mL of blood).
3. **Carefully and slowly** pipette blood on top of Ficoll solution in 50 mL centrifuge tubes (gently allow mixture to flow down along the side of tube).
4. Centrifuge tubes at RT for **30 minutes at 400g BRAKE OFF**. Handle carefully and make sure that tubes are balanced to not disrupt layering
5. After Ficoll separation, gently collect the PBMC layer by aspirating it using a plastic Pasteur pipette. Start from about 1 mm away, beginning by the sides of the tube. Transfer the cells to a clean 50ml tube (maximum 20ml/tube).
6. **First wash:** Top up cells suspension to 45mL with **HBSS**. Centrifuge at 400g, ~~(1500rpm)~~ for 10 min, **BRAKE ON**.
7. Decant media after centrifuging.
8. **Second wash:** Use a 1ml in pipette tips to break up pellet. Wash again adding 44mL R+. Spin at 400g, ~~(1500rpm)~~ for 10 min, **BRAKE ON**.
9. Decant media and resuspend cells in about 5 mL of R10FBS. Set centrifuge at 4°C.

ALTERNATIVE: SEPMATE METHOD FOR PBMC ISOLATION

1. After Plasma collection, top up blood to 30 mL with PBS-2%FBS (30mL total). Mix gently and thoroughly.
2. Take 2 SepMate 50 mL tubes per sample and fill it carefully by pipetting it through the central hole with 15mL Ficoll.
3. Keeping the SepMate vertical, add the 15mL diluted blood by pipetting it down the side of the tube (2 tubes per sample).
4. Centrifuge tubes at RT for 10 minutes at 1200g **brake OFF**. Handle carefully and make sure that tubes are balanced to not disrupt layering.
5. After centrifugation pour off the top layer of into a new tube by inverting tube not more longer than 2 seconds.
6. **First wash**: Top up cells suspension to 45mL with PBS-2%FBS Centrifuge at 300 g for 8 min, **brake ON**.

7. **Second wash**: Top up cells suspension to 45mL with PBS-2%FBS Centrifuge at 300 g for 8 min, **brake ON.**
8. Decant media and resuspend cells in about 5 mL of R10FBS. Set centrifuge at 4°C.

PBMC COUNT

1. *Manual cell counting* Count and record the number of viable PBMCs per mL. If done via an automatic cell counter, follow the supplier's instructions.

FREEZING PBMCS

1. Count cells to be frozen and keep in fridge until ready to spin and freeze. Write number on SOP blood processing log sheet (BQC19 – Biobanking sample logsheet).
2. Note: **we usually freeze down 10M PBMC per vial minimum for storage in nitrogen tank**. Low number of cells per tube leads to lower relative recovery in terms of cell number.
3. Spin cells (from previous step 8) in a cold centrifuge (4°C) for 10 minutes at 400g.
4. After the spin, aspirate supernatant until 100uL of residual volume is left and resuspend pellet.
5. Resuspend PBMC in cold pure decomplemented FBS at 20M/mL.
6. Add freezing solution (20% DMSO FBS) 1:1 (same volume as FBS at step 5) drop-by-drop while CONTINUOUSLY shaking the tube.
7. Transfer 1 mL to each labeled Nalgene cryovial.
8. Once cells are in freezing solution, place cells in Mr Frosty box inside the -80°C freezer.
9. Do not keep the vials containing cells and freezing solution on ice for too long before they are placed in the -80°C freezer. DMSO is toxic to cells, so their viability will suffer if they are not frozen quickly enough. Do not prepare too many tubes simultaneously if you lack experience.
10. Cells transferred to LN_2 the next day.