SOP Name	Whole blood processing
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1. **SCOPE:** This SOP applies to all staff, visitors, researchers and research staffworking in VACCELERATE and affiliated labs.

2. **PURPOSE:** To outline the appropriate policies and standards for processing whole blood samples to isolate and store serum, plasma, miRNA from plasmaand Platelet Free Plasma (PFP) samples.

3. **POLICY:** VACCELERATE works within the guidelines and regulations of the EU CT Directive 2001/20/EC, GCP Commission Directive2005/28/EC, ICH/GCP and with all other local and international applicableregulatory requirements.

4. **ROLES AND RESPONSIBILITIES**

- The investigators should stipulate the appropriate processing requirements for all prospective studies with local laboratory staff prior to the study's initiation.
- It is the responsibility of the research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

5. **DEFINITIONS**

PFP - Platelet Free Plasma

6. **RELATED DOCUMENTS**

SPREP002 Collection and handling of bio materials
SPREP003 Core lab sample receipt and storage
SPREP004 Courier packages
SPREP005 Sample handling
SPREP001 Venepuncture

7. PROCEDURES

These procedure should be carried out under a laminar flow hood/biosafety Class II cabinet.

7.1 PROCEDURE FOR SERUM PROCESSING FROM WHOLE BLOOD

7.1.1 Recommended Materials:

- Transfer pipette 3.5 ml bulb, Sarstedt LTD 86.1171.001.
- Cryovials: Corning, 2ml: 430659
- Pre-printed cryogenic sample labels: LabTag, JTT-160

7.1.2 Procedure:

1. The procedure shall ideally be carried out within one hour following blood collection. It is recommended that the blood be allowed to sit for 30 to 60 minutes to allow for coagulation.
2. Ensure correct collection tube for serum processing: Blood tubes (BD vacutainer 'gold' CODE: 367954; or Sarstedt serum-gel'white' CODE:5034511).
3. Allow blood tube to remain upright to ensure a separation layer and to prevent coagulation and clots from blocking tube following centrifugation.
4. Centrifuge the blood tube at an appropriate speed (1000 x g for BD tubes, 2000 x g for Sarstedt tubes) for 10 minutes at room temperature with the brake on (standard setting).
5. Label cryovials with pre-printed cryovial labels. Transfer serum to pre-labelled cryovials in 500 µl aliquots.
6. Store at -80°C.

7.2 PROCEDURE FOR PLASMA PROCESSING FROM WHOLE BLOOD

7.2.1 Recommended Materials:

- Transfer pipette 3.5 ml bulb, Sarstedt LTD 86.1171.001.
- Cryovials: Corning 2ml, 430659
- Pre-printed cryogenic sample labels: LabTag, JTT-160

7.2.2 Procedure:

1. The procedure shall ideally be carried out within one hour following blood collection. Where advised to spin at 4°C, it is recommended that vacutainers be kept on ice until centrifugation.
2. Ensure correct collection tube for plasma processing: Blood tubes (BD vacutainer 'light green' CODE: 367375; or Sarstedt plasma-gel 'orange': CODE: 367958).
3. Centrifuge the blood tube at an appropriate speed (1300 x g for BD tubes, 3000 x g for Sarstedt tubes) for 10 minutes at room temperature with the brake on (standard setting).
4. Label cryovials with pre-printed cryovial labels. Transfer plasma to pre-labelled cryovials in 500 µl aliquots.
5. Store at -80°C

7.3 PROCEDURE FOR PLASMA PROCESSING FOR miRNA EXTRACTION FROMWHOLE BLOOD

7.3.1 Recommended Materials:

- Transfer pipette 3.5 ml bulb, Sarstedt LTD 86.1171.001.
- Cryovials: Corning 2ml, 430659
- Pre-labelled cryogenic sample labels: LabTag, JTT-160
- Pl000 pipette and tips (DNase and RNase free)

7.3.2 Procedure:

1. The procedure shall ideally be carried out within one hour following blood collection.
2. Ensure the correct collection tubes for miRNA extraction: Blood tubes (BD vacutainer 'purple' CODE: 368857; or Sarstedt K-EDTA 'red/pink': CODE: 5032611).
3. Centrifuge the EDTA tube at an appropriate speed (1300 x g for BD tubes, 2500 x g for Sarstedt tubes) for 10 minutes at room temperature with the brake on (standard setting).
4. Label cryovials with pre-printed cryovial labels. Transfer plasma to pre-labelled cryovials in 500 µl aliquots.
5. Store at -80°C.

7.4 PROCEDURE FOR PLATELETS FREE PLASMA PROCESSING FROM WHOLE BLOOD

7.4.1 Recommended Materials:

- Transfer pipette 3.5 ml bulb, Sarstedt LTD 86.1171.001
- 1.5 ml Eppendorf tubes
- Cryovials: Corning 2ml, 430659

7.4.2 Procedure:

1. The procedure shall ideally be carried out within an hour from blood collection.
2. Ensure correct collection tubes for platelet free plasma processing: EDTA blood tubes (BD vacutainer 'purple' CODE: 368857; or Sarstedt K-EDTA'red/pink': CODE: 5032611)
3. Centrifuge the EDTA tube at an appropriate speed (1300 x g for BD tubes, 2500 x g for Sarstedt tubes) for 10 minutes at room temperature with the brake on (standard setting).
4. Carefully transfer plasma layer in to a 1.5 ml Eppendorf tube, being careful to not transfer any buffy layer or red cells.
5. Centrifuge the isolated plasma at 10,000 x g for 10 minutes at 4°C.
6. Carefully transfer PFP to pre-labelled cryovials in 500 µl aliquots, avoiding disrupt the cell pellet that has formed on the wall of the tube.
7. Store PFP at -80°C.

7.5 PROCEDURE FOR CITRATED PLASMA AND BUFFY COAT FROM WHOLE BLOOD

7.5.1 RECOMMENDED MATERIALS

- Transfer pipette 3.5 ml bulb, Sarstedt LTD 86.1171.001
- 1.5 ml Eppendorf tubes
- Cryovials: Corning 2ml, 430659

7.5.2 PROCEDURE

1. The procedure shall ideally be carried out within an hour from blood collection.
2. Ensure correct collection tubes for citrated plasma and buffy coat processing: (BD Vacutainer 'Light Blue' CODE: 363095; or Sartedt 'Light Green' CODE: 05.1165.001)
3. Centrifuge the blood tube at an appropriate speed (1000 x g for BD tubes, 2000 x g for Sarstedt tubes) for 10 minutes at room temperature with the brake on (standard setting).
4. Label cryovials with pre-printed cryovial labels. Transfer citrated plasma and then buffy coat (white layer in between the plasma and the whole blood) to separate pre-labelled cryovials in 500 µl aliquots.
5. Store at -80°C.

8. REVIEW AND REVISION

This SOP should be reviewed at least every two years and revised accordingly.

9. DOCUMENT HISTORY

Version Number	Effective Date:	Summary of changes from previous version:	Edited by: (name and role)
01	01/06/2021		