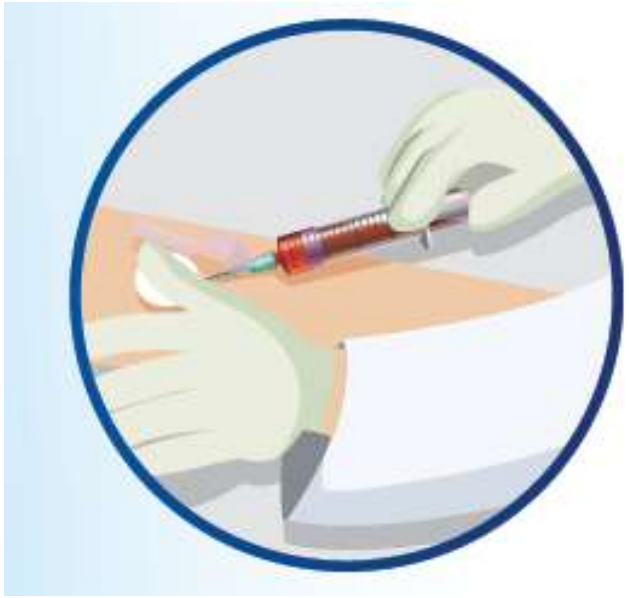
Blood Collection, Plasma and Serum Processing Recommendations

Items Required

- BD Vacutainer® needle 21G (Cat. no.: 360213) or 22 G (Cat. no.: 360211)
- BD Vacutainer® one-use Holders (Cat. no.: 364815)
- BD Vacutainer® Serum tube 3.5 mL (Cat. no.: 366703)
- BD Vacutainer® K2EDTA tube 6 mL (Cat. no.: 367863)
- BD Vacutainer® Serum tube 6 mL (Cat. no.: 367815)
- Greiner Cryogenic (Red Cap) vial 2.0 mL (Cat. no.: 126279)
- Greiner Cryogenic (Blue Cap) vial 2.0 mL (Cat. no.: 126279)
- Greiner 15 mL Centrifuge tubes (Cat. no.: 188271)

Blood Collection Procedure

- Using aseptic technique, obtain venous blood by using BD Vacutainer® needle 21G or 22G
- Note: Do not apply the tourniquet for longer than 1 min as this may lead to haemolysis. Needles finer than 22G are not recommended as it may lead to haemolysis as well.
- Draw whole blood into BD Vacutainer® Serum tube 3.5 mL (Cat. no.: 366703). This 1st Serum tube is to be discarded and only need to be filled for around 1 ml of blood.



Note that this 1st tube is meant to be discarded as it may be exposed to hemolysis

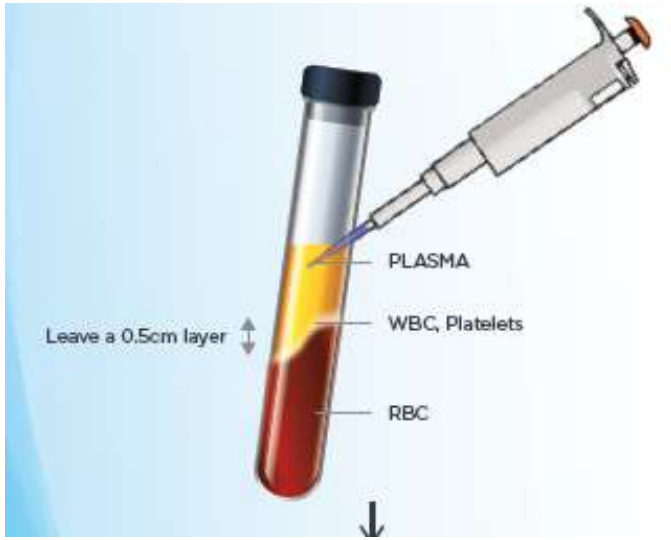
Blood Collection Procedure

- Insert the BD Vacutainer® Serum tube 6 mL (Cat. no.: 367815). Be sure to draw blood till the fill.
- Subsequently, insert the BD Vacutainer® K2EDTA tube 6 mL (Cat. no.: 367863). Be sure to draw blood till the fill line to ensure the correct blood-to-anticoagulant ratio
- Serum tubes should be drawn before K2EDTA tubes to avoid contamination of the anti-coagulants.
- Gently invert the tubes for 8 to 10 times. Do not shake the tubes.
- Record the time of blood draw – Highly recommended

- Allow serum tube to clot for minimum 30 minutes but not more than 60 minutes before proceeding to serum processing procedure.
- Note: Clotting time less than 30 minutes may lead to haemolysis while more than 60 minutes may cause a high backgrounding miRNA expression
- Process the K2EoTA tube within 60 minutes according to Plasma Processing Procedure

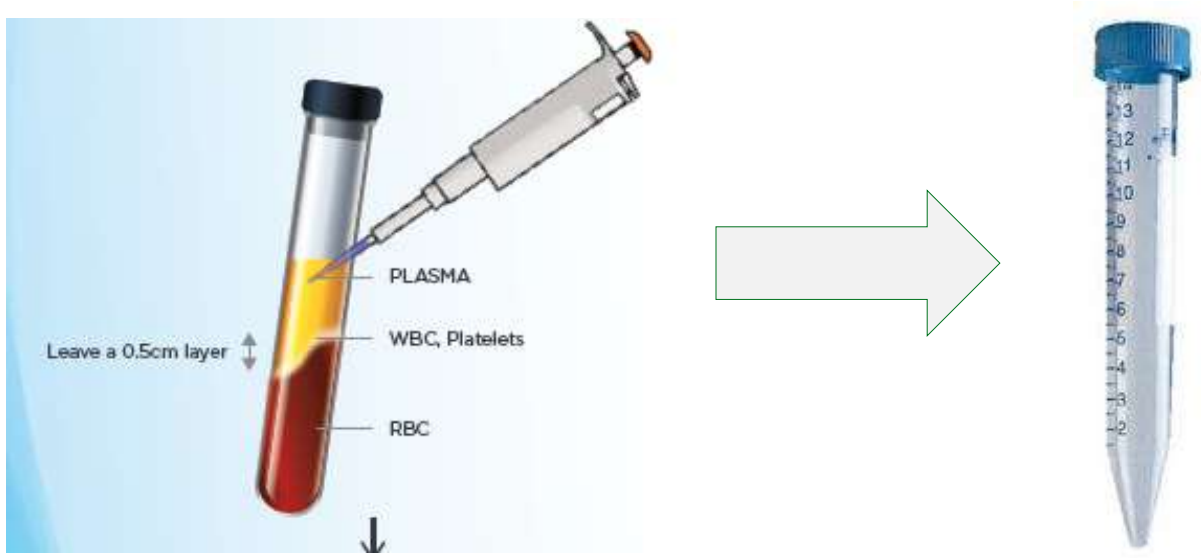
Plasma Processing Procedure

- Centrifuge K2EDTA tube within 60 minutes after blood draw at 1500 RCF at room temperature for 15 mins. Record the time after centrifugation
- (See figure below) This will give three layers: (from top to bottom) plasma, leucocytes (buffy coat), erythrocytes (RBC).



Plasma Processing Procedure

- Immediately after step (ii), carefully aspirate 2.0 mL of the supernatant by using a 1 mL pipette to transfer 2 times (1.0 mL x 2 aspiration) and dispense into Greiner 15 mL Centrifuge tubes (Cat. no.: 188271)
- Make sure always aspirate from the TOP of the supernatant and leave at least 0.5 cm layer above the blood cells. Reduce aspiration volume if the volume of the supernatant is insufficient.



Plasma Processing Procedure - Platelet-Poor Plasma (PPP)

- Re-centrifuge the collected plasma in 15 mL centrifuge tubes between 2500 -3000 RCF at room temperature for 15 mins. Do not use brake to stop centrifuge.
- Transfer 1 mL of the supernatant into the Greiner Cryogenic (Blue Cap) vial 2.0 mL (Cat. no.: 126279). Make sure aspirate from the TOP of the supernatant and do not disturb any cells at the bottom of the 15mL tube. Leave at least 0.5 cm layer from the bottom of the 15mL tube.

Serum Processing Procedure

- After allowing the serum tube to clot for 30 mins, centrifuge serum tube for 15 min at 1500 RCF under room temperature. Record the time after centrifugation
- (See figure below) Blood will be separated into 2 layers (serum and clot). Immediately and carefully aspirate 1.0 mL of the supernatant by using a 1 mL pipette and aliquot into Greiner Cryogenic (Red Cap) vial 2.0 mL (Cat. no.: 126279)
- Make sure aspirate from the TOP of the supernatant and leave at least 0.5 cm layer above the clot.

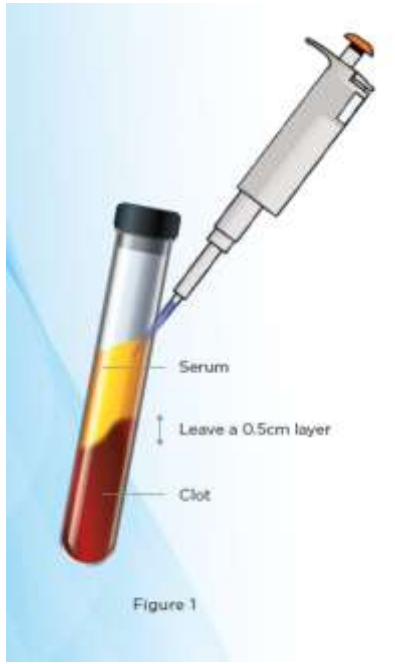
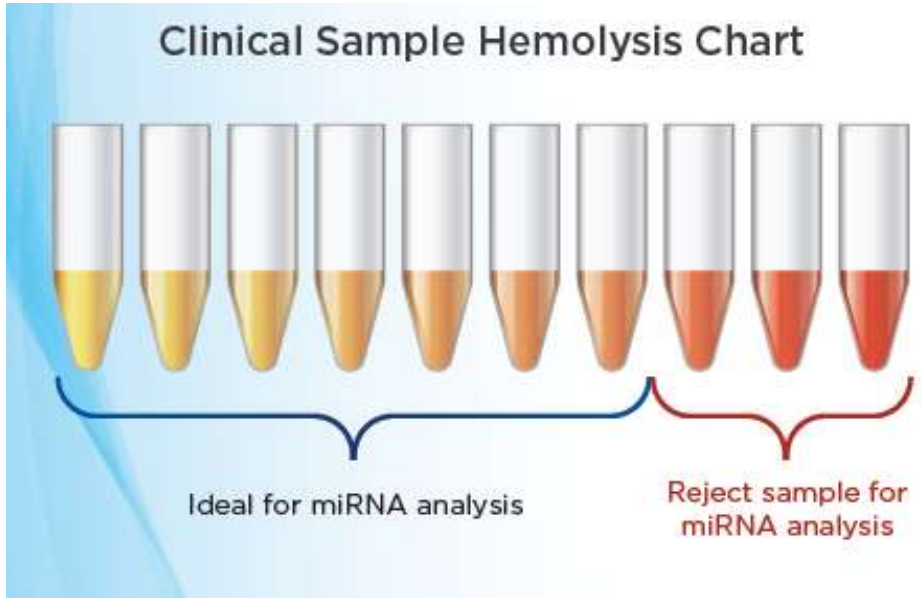


Figure 1

Plasma Processing Procedure

- All samples should be assessed for haemolysis by laboratory personnel with normal colour vision. Refer to the haemolysis chart.
- Store cryovials at -80 °C (or -20 °C). Ensure that the cryovials are adequately labelled with the relevant information



Circulating cell free miRNA are susceptible to contamination by blood cell miRNAs in the event of haemolysis.

Colometric assessment can provide an indication of the extend of haemolysis.

Re-collection of blood is recommended if the sample is haemolysed

Storage and shipment of Samples

- Store the samples at -80 °C freezer (-20 °C freezer if -80 °C freezer is not available).
- DO NOT thaw samples during shipment, samples are to be shipped in frozen condition
- Repeated freeze-thaw must be avoided.
- Place the samples into the Biohazard ziplock bag and seal it. Place the bag back into the box.
- Ship the box out under dry ice condition
- 10kg of dry ice is good for 72-96 hours of shipping period in 20kg box