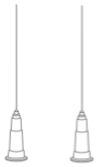
LUNGClear Blood Collection Protocol

Items needed:



BD Vacutainer® needle 21G to 22G

Cat. No.: **360213, 360211**

*Example only. Other manufacturers' 21G or 22G needles can be used.

BD Vacutainer® one-use holders

Cat. No.: **364815**

*Example only. Other manufacturers' holders can be used.

BD Vacutainer® K2EDTA or PPT Tubes

*Cat. No.: **367856, 367863, 362788**

Vacutainer Tube for discard

Cat. No.: **No Specification**

M Diagnostics Test Requisition Form (TRF)

*Check with M Diagnostics for acceptance of use before using the tube with a different catalog number.

Procedure

1. Using aseptic technique, obtain venous blood by using 21G to 22G needle.
   Note: Do not apply the tourniquet for longer than 1 min as this may lead to haemolysis.
2. Draw whole blood into a Vacutainer tube. Draw around 1ml of blood and discard the tube.
3. Draw blood into the K2EDTA/PPT tubes. (1 tube x #367863/#362788 or 2 tubes x #367856)
   Be sure to draw blood till the fill line to ensure the correct blood-clot activator ratio.
4. Invert the collected tube/s 5 times right after blood collection. Ensure that the tubes are NOT shaken vigorously, as this can lead to a hemolyzed sample.
5. Label tube/s with at least 2 identifiers. (Patient Name, Patient Identifier Number and Date of birth)
6. Record the date and the time of blood draw on the Test Requisition Form (TRF).
7. Clinic staff to sign consignment.
8. Immediate transportation is necessary to local lab for processing.

   i. Transport time cannot exceed 8 hrs. Do not freeze.
   ii. Porter / despatch to collect blood sample from clinic and provide gel packs upon collection.
   iii. Avoid direct contact of gel packs with blood tubes during transportation.

Platelet-Poor Plasma Processing Protocol

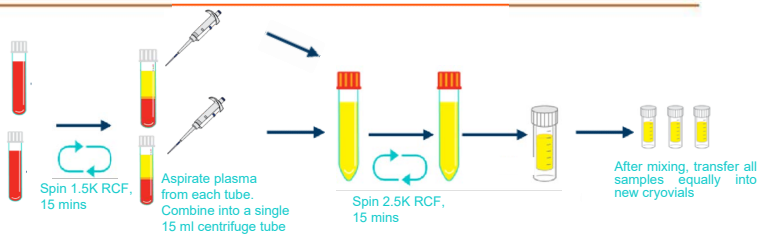
Items needed:

BD Vacutainer K2EDTA or PPT Tubes

RNase/DNase Free 15 ml centrifuge tube

RNase/DNase free 1.5ml cryovial tube

Instructions:



Procedure

1. Upon receipt of the blood tubes, immediately centrifuge at 1500 RCF at room temperature for 15 mins. Blood will be separated into 3 layers and the plasma is contained in the top layer All samples should be assessed for haemolysis by lab personnel with normal colour vision. Refer to the haemolysis chart for comparison.
   Note: Excessive centrifuge speed (over 2000 g) may cause tube breakage and exposure to blood and possible injury. If needed, RCF for a centrifuge can be calculated.
2. Immediately after step 1, carefully aspirate appropriate volume of plasma using a 1 ml pipette.(To aspirate all but remain the bottom 0.5 ml plasma. This will help to minimize disturbance to the other layers.) Transfer and dispense plasma sample into one single 15 ml centrifuge tube. Repeat for the second collection tube (if used) and combine plasma from the same subject into the same centrifuge tube.
   Note: Always aspirate from the TOP of the plasma carefully without disturbing the other layers. Reduce aspiration volume if the volume of the supernatant is insufficient.
3. Re-centrifuge the collected plasma in the 15 ml centrifuge tube at 2500 RCF at room temperature for 15 mins.
4. Carefully transfer the top plasma by using pipette and aliquot into a cryovial. Gently mix the aliquot in the cryovial with pipette to avoid miRNA gradient. Transfer at least 500ul of aliquot into a new cryovial. Repeat the process to obtain multiple cryovials of at least a total of 1 ml for testing.
   Note: Make sure to aspirate from the TOP of the plasma and do not disturb any cells at the bottom of the tube.
5. Ensure that the cryovials are adequately labelled with the relevant information, and store the cryovials at -80°C. If immediate storage at -80°C is not possible, cryovials can be stored at -20°C for up to 4 days. This storage time should include transportation time as well (transportation should always be with dry ice). Samples should not be allowed to thaw before/during/after transportation and samples must subsequently be stored at -80°C. Plasma storage condition prior delivery must be indicated on TRF for verification.

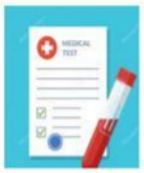
Storage and Shipment Protocol

Items needed:

2ml screw cap DNase free and RNase free cryotube

Barcode Labels

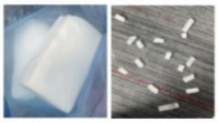
Cryobox



M Diagnostics Test Requisition Form (TRF)

Biohazard Ziplock Bag

Biological Substances Cat B Shipper Box for UN3373



Dry Ice

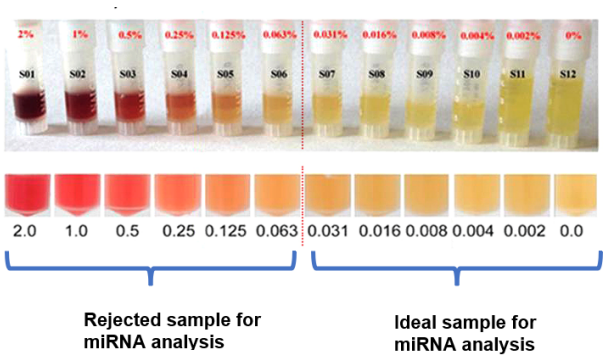
The product images shown are for illustration purposes only and may not be an exact representation of the product.

Procedure

1. DO NOT thaw samples. Frozen serum samples must be shipped using dry ice.
2. Repeated freeze-thaw must be avoided.
3. Call the M Diagnostics CS hotline 69503218 to activate the pick-up service with dry ice. This can be done one day before or actual day the samples are to be picked up. Inform the M Diagnostics CS officer to send dispatch with dry ice and the estimated volume (i.e. number of samples and package condition) to be picked up.
4. On the day of pick up, prior to the arrival of the dispatch rider/driver, the following must be checked and ensured.
   i. Ensure the TRF is completed for every sample.
   ii. Ensure that all cryotubes are labelled with labels containing at least 2 patient identifiers. Cross check that patient identifiers on cryotubes are the same as those on the TRF.
   iii. Fold and insert the TRF into the outer pocket of the Biohazard zip lock bag.
   iv. Place the cryotubes into a cryobox (maximum dimensions of L133xW133xH50mm) or sturdy outer packaging. Place the cryobox or sturdy outer packaging into a biohazard zip lock bag, remove as much air as possible from it before sealing it.
5. The dispatch driver/rider arrives with a styrofoam or cooler box for biological substances and dry ice.
6. Place the biohazard bag containing the cryobox with the TRF into the styrofoam or cooler box with dry ice.
7. Sign off any documentation required by the dispatch driver/rider where necessary.

Assessment of Hemolysis

Items needed:



Hemolysis Chart

Procedure

1. Please refer to the hemolysis chart above for preliminary assessment of hemolysis. The chart is intended to be used as a guideline and NOT a confirmatory of hemolysis in plasma.
2. Note: Hemolysis of plasma samples should be inspected by laboratory personnel with normal colour vision.

Disclaimer: Accuracy of colours and saturation may vary due to print or screen quality.

Assessment of Hemolysis

Items needed: M Diagnostics Specimen Rejection Criteria Chart

Scenarios where samples will be on hold.	Samples Rejection Criteria
Missing and/or incomplete TRF	Sample Hemolysis
Patient identifiers mismatch between TRF and Sample tube but 1 unique identifier is present.	Sample not labelled
	Spillage or leakage is observed
	Wrong blood collection tube was used
	Frozen plasma not shipped with Dry ice
	Incorrect specimen type
	Insufficient quantity of specimen

Procedure

1. Only BD Vacutainer® PPT Plasma Preparation tube or BD Vacutainer K2EDTA tube are acceptable for testing.
2. To avoid rejection, frozen plasma should be shipped to M Diagnostics by day 4 or transferred to -80°C freezer.