Standard Operating Procedures Clinical Protocol Ic Blood Sample Collection - SERUM

** NOTE: The following procedure is to be performed wearing laboratory coat, gloves, eye protection, and mask.

PRINCIPLE

Arterial or mixed venous blood will be collected from patients at the indicated time points following randomization.

The collection of blood should be obtained from an existing arterial or venous line, or by venipuncture (with a 20 gauge needle or bigger), and should be performed by someone experienced in the technique and familiar with infectious precautions.

The use of pneumatic tube system is not recommended since it can activate white blood cells and promote unwanted release of mediators.

Processing of the blood should be done as soon as possible after collection in order to prevent the unwanted released of mediators caused by dying cells.

Specimen Collection & Handling

1.Label all 1 BD serum tube (no. 367812), 1x15mL conical tube and 6 cryogenic freezing tubes per patient.

2.Fill the tube with whole blood in the identified collection tube. Inverted gently several times to mix the blood with the contained pro-coagulant.

3.Allow the blood to clot by standing tube vertically at room temperature (22 oC) for 60 minutes.

4.Place the tube in wet ice for no longer than 2 hours before centrifuging.

5.Centrifuge at 1200 x g (calculate the speed in rpm taking into account the size of your rotor) for 10 minutes at 4 C with brake off, in a horizontal, swinging bucket centrifuge.

6.Carefully collect the serum using a disposable plastic pipette or sterile glass pipette from the vacutainer and transfer it into a new labeled 15mL conical tube making sure not to withdraw any of the white interfacial layer.

7.Discard the pellet.

8.Aliquot serum into 2mL cryogenic freezing tubes by placing 250µL of serum per tube. Store at ≤ –70° C until processing.

Special Note 1.1: only collect the serum fraction to within 0.2mL of the interface layer. Note that after centrifugation you should have approximately half the volume of blood as supernatant per tube.

Special Note 1.2: Processing and handling of Blood for analysis – please remember endotoxin is ubiquitous and can change expression of all mediators being assayed. Endotoxin free precautions should be taken for handling of all procedures (sterile precautions and using endotoxin free solutions should suffice).
