Standard Operating Procedures Clinical Protocol Ib Blood Sample Collection - PLASMA

** 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 larger), 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.For each patient, label two 15mL polystyrene collection tubes containing the following:

•Tube #1 contains 360µL of sterile trisodium citrate (= “CIT” tube)

•Tube #2 contains 360µL of sterile trisodium citrate + 80µl of 1M sterile benzamidine (=“CIT/BZ” tube)

** Please note that the sterile trisodium citrate can be obtained from the blue-topped Becton Dickinson vacutainer tubes (BD cat. No.366415). Each vacutainer tube should contain about 500µl of trisodium citrate, but the actual amount that can be removed is closer to 450µL.

2.Draw 2 tubes of 4mL each of blood. IMMEDIATELY transfer the content of one tube into the “CIT” tube and the content of the other tube into the “CIT/BZ” tube.

3.In laboratory, invert the tubes once to mix. Centrifuge at 1700 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.

4.Using a plastic transfer pipette, transfer each plasma into 8 cryogenic freezing tubes (ie. 16 cryogenic freezing tubes in total for each patient).

5.Write either “CIT” or “CIT/BZ” on the tops of the cryogenic freezing tubes, and also write the patient ID number and the “day” (for example, “J1, Day 1").

6.Store the plasma samples in a -80oC freezer in cardboard boxes or in Ziploc baggies (use one Ziploc bag per patient day).

Special Note 1.1: only collect the plasma fraction to within 0.2mL of the interface layer. Note that after centrifugation you should have approximately 2mL of supernatant per tube so transfer only approximately 1.5mL of plasma.

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.
