## Supplies needed:

- 1x 35cc syringe
- 3x 20cc syringe
- 4x 12cc syringe
- 1x 1cc syringe
- 8x 19g needle
- 1x filter needle
- 2x 19g butterflies
- 50mg Ascorbic Acid
- ACD vial
- 10-12 purple-top Vacutainer tubes
- 9 test tubes with red tops
- ~50 uCi Cr<sup>51</sup>
- Eppendorf pipetting system
- 10x Eppendorf pipettes

## Prior to patient arrival:

- 1. Assemble materials
- 2. Label red-top test tubes as follows:
  - a. 1x WB BKG
  - b. 2x WB STD
  - c. 2x PL STD
  - d. 2x POST WB
  - e. 2x POST PL
- 3. Place the filter needle in the ACD solution to vent it.
- 4. Warm ACD solution to 37 degrees Celsius

## At patient arrival:

- 1. Explain the procedure to the patient.
- 2. Get the patient's height and weight.
- 3. Draw 5mL of ACD solution into the 35mL syringe. Waste 4mL (leaving 1mL in syringe).
- 4. Place a 19g butterfly in one arm vein. Draw 30-35mL of blood into 35mL syringe.
- 5. Immediately fill one Vacutainer, label it BKG, and place it in the warmer.
- 6. Put the remainder of the blood in the ACD vial.
- 7. Put the Cr<sup>51</sup> in the ACD vial. Wait 10 minutes, periodically swirling the vial.
- 8. Draw 50mg of ascorbic acid (at 500mg/1ml concentration, that is 0.1 mL) in the 1cc syringe.
- 9. Wait 10 more minutes, periodically swirling the vial.
- 10. Draw 20mL from the ACD vial, and re-inject through the 19g butterfly.

- 11. Remove the butterfly, and instruct the pt to return in 40 minutes.
- 12. Draw all remaining blood from the ACD vial into a 20mL syringe.
- 13. Obtain 4 Vacutainers. Label one STD. Place the blood into the 4 Vacutainers.
- 14. Place the Vacutainer labeled STD into the warmer. Place the remaining three into the centerfuge.
- 15. Fill one Vacutainer with water, use to balance the centrifuge.
- 16. Ensure the centrifuge is balanced, Ensure the lid is closed and latched. Run the centrifuge at full speed for 10 minutes.
- 17. Pipette 1mL of blood (4 clicks on the Eppendorf set to #5) from the BKG Vacutainer into the BKG red-topped test tube. Cap the tube.
- 18. Pipette 1mL of blood from the (un-centrifuged) STD Vacutainer into each of the two STD red-topped test tubes. Cap the tubes.
- 19. Once the centrifuge is done, draw plasma from all three Vacutainers into the Eppendorf. Place 1mL into each of the two PL STD red-topped test tubes. Cap the tubes.
- 20. When the patient returns, draw 20mL of blood from the *opposite arm than you injected into*.
- 21. Quickly fill four Vacutainers with this blood. Label them POST.
- 22. Release the patient.
- 23. Spin down three of the POST Vacutainers as you did the STD samples.
- 24. Pipette 1mL of blood from the (un-centrifuged) POST Vacutainer into each of the two POST red-topped test tubes. Cap the tubes.
- 25. Once the centrifuge is done, draw plasma from all three Vacutainers into the Eppendorf. Place 1mL into each of the two PL POST red-topped test tubes. Cap the tubes.
- 26. Place all samples into a COBRA counting rack, with protocol #4. Leave a space between each set of samples (e.g. BKG, empty, WB STD, WB STD, empty, PL STD...).
- 27. Count the samples.
- 28. Take the *unspun* BKG, STD, and POST samples to the blood lab on the 2<sup>nd</sup> floor for a hematocrit. The STD hematocrit will always be lower, while BKG and POST should be approximately equal (with BKG slightly lower).
- 29. When the COBRA is done, input all results into the appropriate (male or female) Excel sheet. If you are not using the updated sheets with the Dubios calculations, ensure that your patient meets "ideal body weight and height" as noted in the notebook.
- 30. Ensure you dosed the patient in SYNtrac. Ensure you completed the patient in IDX. Hand in the requisition, COBRA printout, printed Excel sheet, and hematocrits from the lab to the MD.