Protocols for isolation blood buffy-coat cells
(from Mr. Greg, 5th floor VAMC, ext. 6256)
From VAMC Decatur (Yoon & Boden's Lab)

1. Take 3ml of whole blood into 17ml culture media (without FBS) including 20 units of heparin in final concentration.
2. Mix gently and transfer 10ml of the above solution into two 11mm (diameter) x 175mm (height) glass tube with plastic caps.
3. Centrifuge at Room Temperature at 1000g, 10min., maximum braking.
4. Remove the supernatant until 100-200ul liquid left on the top of the cell pellet. (buffy-coat cells, the whole mixture of white blood cells will be on top of the cell pellet which are yellowish and white)
5. "Gently mixing" the cell pellet (only the buffy-coat layer) into the supernatant. This is the key step to harvest the buffy-coat cells (need practice).
6. Using 1ml serological pipette to remove all the supernatant (about 700 ul) to a 50ml centrifuge tube. Collect all the other buffy-coat supernatant into this tube.
7. Using the Unopette kit to count the buffy-coat cell numbers. (Unopette kit will lyse all the red cells contaminated in the buffy coat layer)