Blood and Fluid Collection and Processing SOP

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We use this protocol and it's working

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ABSTRACT

This protocol describes the basic processing of blood and urine specimens from surgical resection patients. Blood is obtained for serum, EDTA plasma, and PBMCs. This is a standard protocol that can be adapted to fit most laboratory conditions and needs.

GUIDELINES

This protocol should be performed in a Bio-Safety cabinet and is located within a qualified BSL2 laboratory. The user of this protocol should also conform to all institutional guidelines when handling biological fluids obtained from human participants.

MATERIALS

Centrifuge, 50 ml centrifuge tubes, 15 ml centrifuge tubes, Ficoll, trypan Blue, Phosphate buffered saline (PBS), storage tubes, hemacytometer, Mr. Frosty or equivalent for short term storage of PBMC, minus 80 Freezer, microcentrifuge tubes, microcentrifuge

SAFETY WARNINGS

Use universal precautions to protect from possible blood born pathogens

ETHICS STATEMENT

All blood and urine specimens must be obtained after written informed consent, and must conform to all local IRB regulations.

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1 In general we collect 3 EDTA anticoagulation tubes (purple) and one plain serum tube (red). Collection of the blood usually from a central arterial line or at the time of the IV placement. Urine is collected from Foley catheter and placed into a 50ml centrifuge tube.

2 Blood and urine are retrieved from anesthesia in the OR and returned to the lab.

3 Urine and the serum (red) tube are placed in to the centrifuge and spun at 3,000 rpm for 10 minutes

4 Carefully aspirate the serum and place 0.5ml in to labeled storage tube, record the number and amount in each tube on the Tissue Bank Specimen Registry sheet.

5 Carefully aspirate urine and place 1.8 ml in to labeled storage tubes, record the number and amount in each tube on the Tissue Bank Specimen Registry sheet.

6 Store urine and serum in the designated minus 80 freezer

7 record in the master specimen registry book, or similar record keeping tracking system.

8 Measure the amount of EDTA blood by placing in to a 50 ml centrifuge tube and then in a separate 50 ml centrifuge tube slowly layer the blood over an equal amount of Ficoll-Pacque Plus.

9 Spin the blood/Ficoll tube at 400 x G for 30 minutes, turn off the centrifuge brake.

10 Carefully aspirate the plasma (top layer) and place 1ml in to microcentrifuge tubes. Spin the plasma in the microcentrifuge tubes at 16,000 x G for 10 minutes.

11 Carefully aspirate the resulting EDTA Plasma from each tube, making sure you can see any pellet that has formed and avoid disturbing the pellet, place in to storage tubes, record the number and amount in each tube on the Tissue Bank Specimen Registry sheet. Place in to in the designated minus 80 and record in the master specimen registry book.

12 Carefully remove the Buffy coat below the plasma layer and place in to 50 ml centrifuge tubes. Fill the tube with PBS up to 50 ml and spin at 400xG for 10 minutes.

13 Pour off the supernatant, resuspend pellet in 50 ml of PBS and spin again at 400xG for 10 minutes.

14 Pour off the supernatant, resuspend the pellet in 10-30 mls of PBS depending on the size of the pellet (you will need to judge the amount for efficient counting). Remove 10 microliters and place in to a tube with 10 microliters of trypan Blue. Mix well and place on to hemacytometer for counting.

15 Calculate the number of cells by taking the counted number, multiply by 2 (dilution factor of trypan blue), then multiply by 10,000, and then finally multiply by the total volume of PBS the pellet was resuspended in (e.g. 10-30mls). This will give you the total number of cells.

16 Bring up the tube to 50 ml with PBS and spin one more time, 400xG for 5 minutes. (This step can done while you are counting).

17 Resuspend the cell pellet in the appropriate amount of freezing media. Each tube should contain 5x106 cells. Record the number and amount in each tube on the Tissue Bank Specimen Registry sheet. Place in to a Mr. Frosty, labeled with the Patient ID and the number of tubes (e.g. n= x) in the designated minus 80 and record in the master specimen registry book.