

Acellular PRP & BMAC Method

PRP

Draw blood via light tourniquet and no hand squeezing via a 17-19g butterfly catheter first into a 3cc syringe (discard or test for platelet count) and then into a 60cc syringe containing 8cc ACD (overfill to 65cc).

Place 15-20cc into 50ml sterile polypropylene tubes and then secure the screw tops, preferably done under a laminar flow hood.

Spin for 15 minutes at 190 RCF (1050 RPM in Drucker 755VES) with **no** braking at the finish.

Pipette off the plasma to but not including the buffy coat (or including the buffy coat if you want WBC's) and transfer into fresh 50ml tubes (under the hood). Spin 15 minutes at 1100 RCF (2500RPM) low braking (2/5).

Remove most of the plasma (amount depending on the concentration you want) and then use the remaining plasma to gently re-suspend the platelet pellet. Repeat the mixing 3-4 times. Max 5cc PRP/60cc blood to insure $>1 \times 10^6$ Pl/uL (that assumes a native concentration of 200k/uL)

Spin 30 seconds at 2500 RPM. Pipette off the PRP, leaving any RBC's at the bottom.

BMAC

Fill 2 30cc syringes with 5cc heparin 5000U each

Have 3cc heparin in a 5cc syringe ready to inject into 1/2cc into each marrow aspiration site.

For each (of 6, 3 each side) bone marrow aspiration sites once you have loss of resistance (very superficial placement with manual method or Vidacare On Control drill) place 1/2cc of heparin into each site before aspirating 10cc marrow from each site into the 30cc syringes. Remember to mix well after each 10cc draw

Transfer the 60cc to 2-4 50ml tubes and centrifuge at 1100 RCF (2500RPM) for 15 minutes

Pipette off the buffy coat, including the somewhat thick layer. Yield should be ~2-4cc total.

Materials

IMI 16g blunt aspiration cannula part #32-34 phone 954-917-9570 \$213.86 / 100

B&D (or similar) 50ml sterile screw top (blue) polypropylene tubes (EBay 500/\$85 Lion International) **or**

Tube Centrifuge Falcon Polypropylene 50mL 30x115mm Blue 20BX/Ca (7009941) Becton-Dickinson Labware (352070) from www.HenrySchein.com 600/\$263!!

Drucker 755VES centrifuge with 6x50ml carriers \$2500-\$3120, cheapest by calling Henry Schein. Do not buy old Biomet 755VES since no variable braking. Beware that many on EBay that say 755VES are not, since it must have variable braking.

Laminar flow personal clean room hood MY-PCR32 from Mystaire.com \$2975. Phone 919-229-8511

On Care Bone Marrow Drill from Vidacare, used with their kits at ~\$130-150/each. Faster, painless, and easier than manual method of:

Bone Marrow biopsy/aspiration Needle 11gx4" J-Type Biopsy/ Aspiration Ultra Sharp Trocar 10/Ca (7991763)

Busse Hospital Disposable (630) \$156.05 www.HenrySchein.com

Test tube rocker for PRP/BMAC to keep it mixed after processing if it needs to sit for a while

Mixer Rocker Rock-It JR w/ Autoclavable Tube Pad f/ 8 Tubes Ea (1590730) Unico (L-TTR100) from www.HenrySchein.com \$217.29

ACD 50ml We get it from Sigma Aldrich C3821-50ml but you have to fill out a form and tell them it is for lab purposes, not patients!

Heparin Sodium Porcine Inj MDV 5000u/mL 30mL from Schein