*Thawing and preparing CBMCs*

1. Warm cRPMI in bead bath. Keep on bead bath when not in use
   1. 10% heat-inactivated HSAB, 1 % pen-strep, 1% sodium pyruvate in RPMI
2. Label \_\_\_\_ sterile 15 ml centrifuge tubes with sample IDs (polypropylene)
3. Locate samples (TS and adult; 3 different samples)
   1. TS579: 4 vials with 50 million, 4 vials with 14 million – use a vial with 50 million cells
   2. TS525: 3 vials with 50 mil, 2 vials with 25 mil – use a vial with 50 million cells
   3. TS516: several vials with 15 million each – use 2 vials
   4. TS509: vials have 20 million cells or 40 million cells – use 2 vials of 20 million cells or 1 vial of 40 million cells
   5. Adult – COVID study
4. Thaw \_\_\_ vials each of samples \_\_\_\_\_\_\_\_, \_\_\_\_\_\_\_\_, and \_\_\_\_\_\_\_\_. Remove samples from LN into dry ice and transport straight to 37oC bead bath. Stagger samples so only processing 3 at once. Incubate between 1-2 minutes with frequent checking. Avoid excess time by transferring immediately after complete thaw to labeled, sterile tubes. Combine sample aliquots.
5. Dropwise add 5 ml then complete to a full 10 ml for each sample with warmed media
6. Centrifuge cells at 300 x *g* for 10 min
7. Discard the volume by swiftly inverting into biohazardous waste
8. Resuspend the samples in 5 ml of warm media.
9. Take a sample, dilute, and calculate the live cell concentration with Trypan
   1. Stow a sample of 100,000 cells for flow
10. Repeat centrifuge step for 5 minutes
11. Resuspend cells to 50 x 106 cells/ml in a maximum of 2 ml (minimum 0.5 ml). Make multiple aliquots of cells to maximize final yield instead of discarding excess. *Keep samples separate at this point*

# Extracting monocytes (negative selection)

1. Label \_\_\_\_ 5 ml (12 x 75 mm) polypropylene round-bottom tubes
2. Add sample to new tube
3. Add 50 ul/ml Isolation Cocktail to samples.
4. Mix and incubate at room temperature for 5 minutes
5. Vortex Vortex Magnetic Particles (30 seconds). Particles should appear evenly dispersed.
6. Add 50 ul/ml Magnetic Particles to sample.
7. Mix and incubate at 4oC for 10 minutes
8. Top up to 2.5ml of recommended medium. Mix by gently pipetting up and down 2-3 times.
9. Place in magnet for 2.5 minutes at room temperature
10. Pick up the magnet, and in one continuous motion invert the magnet and tube, pouring the enriched cell suspension\*\* into a new tube. Monocytes are now in the new tube
11. Check viability and cell count
12. Transfer to sterile 15 ml centrifuge tube
13. Centrifuge at 300 x *g* for 5 minutes
14. Resuspend in 1 ml cRPMI (RPMI with 10 % human AB serum (Gibco #11875-093), 1 % sodium pyruvate, 1 % penicillin/streptomycin)

\*\* Leave the magnet and tube inverted for 2 - 3 seconds, then return upright. Do not shake or blot off any drops that may remain hanging from the mouth of the tube.

# Extracting monocytes (positive CD14+ selection)

1. Label \_\_\_\_ 5 ml (12 x 75 mm) polypropylene round-bottom tubes
2. Add sample to new tube
3. Add 50 ul/ml Isolation Cocktail to samples.
4. Mix and incubate at room temperature for 5 minutes
5. Vortex Vortex Magnetic Particles (30 seconds). Particles should appear evenly dispersed.
6. Add 50 ul/ml Magnetic Particles to sample.
7. Mix and incubate at 4oC for 10 minutes
8. Top up to 2.5ml of recommended medium. Mix by gently pipetting up and down 2-3 times.
9. Place in magnet for 2.5 minutes at room temperature
10. Pick up the magnet, and in one continuous motion invert the magnet and tube, pouring the enriched cell suspension\*\* into a new tube.

# Check purity of monocytes (leave cells at 37oC while checking purity)

1. Remove a volume for 100,000 – 200,000 cells
2. Stain (1/100 CD3[FITC], 1/100 CD19[FITC],1/100 CD14[BV421], eF780 [V440])
3. Wash x 2 in PBS
4. Run on FACS Symphony
5. Note the purity (% of monocytes in single cells/live cells)

# Aliquot cells suspension

1. Aliquot 1.25-1.5 x 106 monocytes for Rujun (RT-PCR) for each sample