**Isolation of Peripheral blood mononuclear cells (PBMCs)**

The purpose of the proposed experiment is to isolate PBMCs from young infants and test their number and viability upon cryopreservation (Adapted from Sigma (<https://www.sigmaaldrich.com/BD/en/technical-documents/protocol/clinical-testing-and-diagnostics-manufacturing/hematology/recommended-standard-method>) and STAR protocols (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8888982/>)).

These PBMCs will be used for single cell RNA sequencing experiments.

**April 9, 2024**

1. Prepare the six 1.5 ml sterile Eppendorf tubes containing 200 ul of PBS + 10 mM EDTA and send to BSHI microbiology lab.

**April 15, 2024**

1. Add 200 ul of venous blood to the tubes containing PBS + 10 mM EDTA and mix well.
2. Transfer the tubes immediately to CHRF single cell lab on ice packs (temp: 18-20 oC).
3. At the CHRF single cell lab, carefully layer the 400 ul blood samples on top of 300 ul of Ficoll-Paque media solution in a 1.5 ml Eppendorf tube.

\*\*Important: When layering, do not mix the Ficoll-Paque media solution and the diluted blood sample.

1. Centrifuge at 400 g for 40 min at 18 oC to 20 oC using the 15 ml falcons as adapters (in a swinging bucket rotor with slowest acceleration, deceleration)
2. Transfer the top layer till the Ficoll-Paque solution to another tube (~ 300 ul). Add 3 volumes (~900 ul) of PBS + 1mM EDTA + 2% FBS)
3. Centrifuge at 400 g for 15 mins at 18 oC to 20 oC (fixed angle, max acceleration, and deceleration)
4. Add ~ 1 ml of PBS + 1mM EDTA + 2% FBS and carefully resuspend cells.
5. Centrifuge at 400 g for 15 mins at 18 oC to 20 oC (fixed angle, max acceleration, and deceleration)
6. Resuspend cells in 200 ul of RPMI + 10% FBS.
7. Take 10 ul of cells and 10 ul of Trypan blue in a different 1.5 ml tube.
8. Add 200 ul of 20% DMSO in FBS to the tubes and put them in Mr Frosty for cryopreservation.
9. Count viable cells using hemocytometer.

**April 16, 2024**

1. Take out tubes from the Mr Frosty and thaw the cryotubes.
2. Once completely thawed, add 400 ul of RPMI + 10% FBS and mix carefully.
3. Centrifuge at 400 g for 15 mins at 18 oC to 20 oC (fixed angle, max acceleration, and deceleration)
4. Resuspend cells in 200 ul of RPMI + 10% FBS.
5. Centrifuge at 400 g for 15 mins at 18 oC to 20 oC (fixed angle, max acceleration, and deceleration)
6. Take 10 ul of cells and 10 ul of Trypan blue in a different 1.5 ml tube.
7. Count viable cells using hemocytometer and compare the number and viability obtained before freezing.