**PBMC isolation**

* Obtain blood in heparin-coated vacutainer
* Allow blood to equilibrate at RT for approximately 30 minutes
* Dilute 10ml of blood into 25ml PBS+2mM EDTA
* Using serological pipette, carefully add 13ml Ficoll underneath blood
* Centrifuge 800G, 30min, brakes off, medium acceleration, room temperature
* Transfer the buffy coat (along with residual plasma) to a new 50ml tube, pooling 2 tubes together where possible
* Top up the tube to 45ml with PBS+2mM EDTA
* Centrifuge 500G, 15min, room temperature (brakes and acceleration max from now on)
* Decant supernatant and resuspend pellet in 10ml PBS+2mM EDTA
* Merge pairs of tubes
* Centrifuge 200G, 10min, room temperature
* Decant supernatant, resuspend in 1ml of PBS+2mM EDTA (or media), and count
* Proceed to either cryopreserve or culture the isolated cells