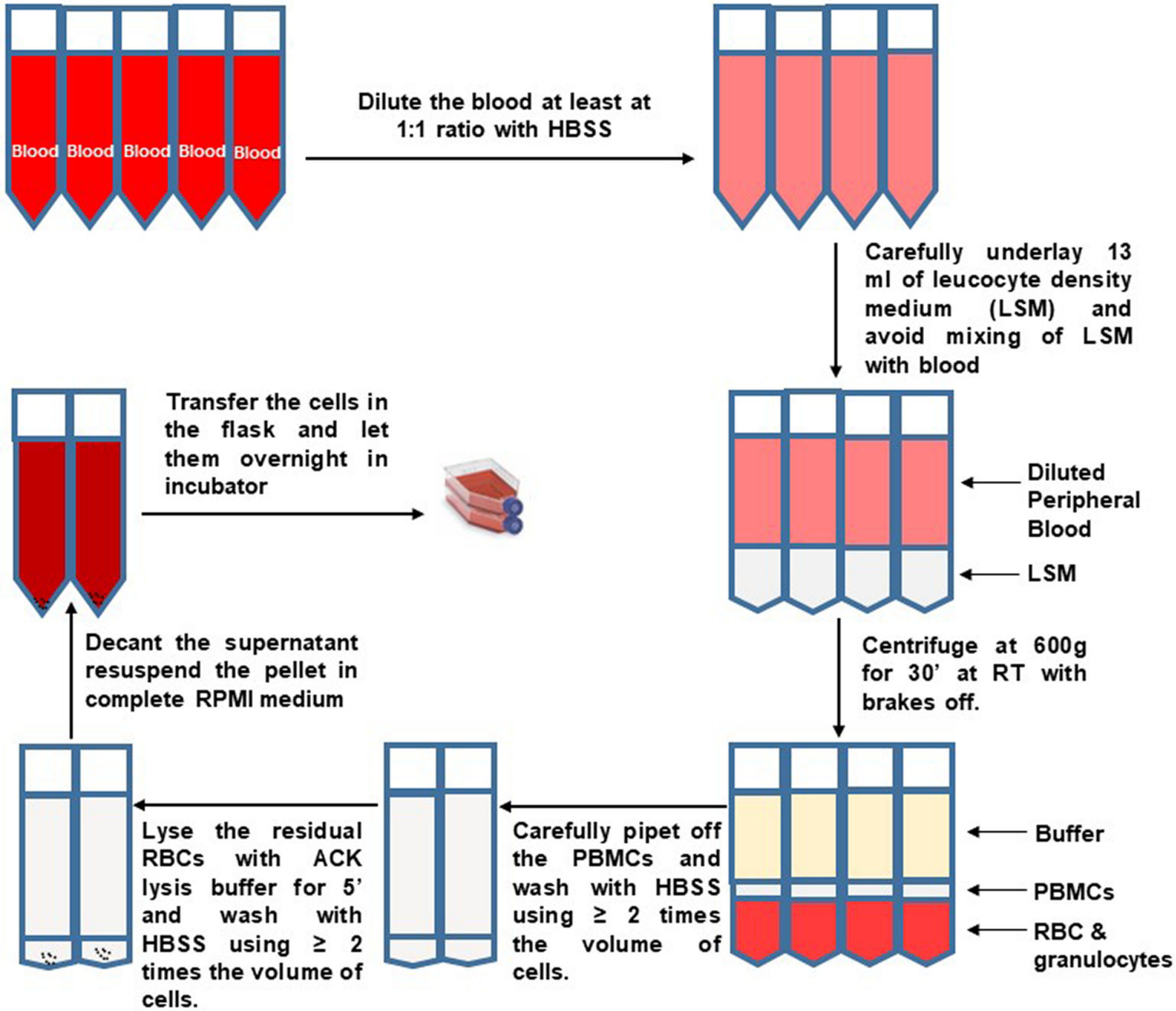
Blood
Blood
Blood
Blood
Blood
Dilute the blood at least at 1:1 ratio with HBSS
Carefully underlay 13 ml of leucocyte density medium (LSM) and avoid mixing of LSM with blood
Diluted Peripheral Blood
LSM
Centrifuge at 600g for 30' at RT with brakes off.
Buffer
PBMCs
RBC & granulocytes
Carefully pipet off the PBMCs and wash with HBSS using ≥ 2 times the volume of cells.
Lyse the residual RBCs with ACK lysis buffer for 5' and wash with HBSS using ≥ 2 times the volume of cells.
Decant the supernatant resuspend the pellet in complete RPMI medium
Transfer the cells in the flask and let them overnight in incubator