Establishing New Cultures from Frozen Stocks

Reagents:

Note: Sterilize all solutions by 0.22μm filtration and store at 4.0°C.

- Solution A: 12% w/v NaCl in ddH_20
- Solution B: 1.6% w/v NaCl in ddH_20
- Solution C: RPMI complete medium with 10% human serum

Procedure:

1. Remove cryovial containing parasitized red blood cells from cryotank and place at room temperature to defrost. (Cryovial should contain ~500-1000μL)
2. For each 500μL thawed blood, add 100μL of Sol A drop by drop and mix slowly.
3. Transfer the culture to a sterile 15mL tube and let it stand for 3 min. (Move quickly to Step 4)
4. For each original 500μL of thawed blood, add 5mL Sol B drop by drop and mix slowly by pipetting up and down.
5. Centrifuge at 2000 rpm (800xg), 5min at RT. (Don't forget to set the brake to low)
6. Remove and discard supernatent.
7. For each original 500μL of thawed blood, add 5mL Sol C.
8. Centrifuge at 2000rpm (800xg), 5min at RT.
9. Remove and discard the supernatent.
10. Resuspend the culture in 10 mL of Sol C in a small culture flask, adding 500μL of fresh washed 50% hematocrit blood to obtain a final hematocrit of ~5%.
11. Gas the culture (1% O_2, 3%CO_2, 96% N_2) and place in 37°C incubator.
12. Change the medium and smear daily to monitor the parasitemia. When the parasitemia reaches 8-10%, the stock culture should be diluted down and large cultures may then be set up using complete medium with 5% human serum and Albumax.