**Thawing New Malaria Cultures**

**ALL SOLUTIONS USED IN THIS PROTOCOL SHOULD BE STERILE FILTERED!**

**DAY 1:**
1) Get the frozen sample from the liquid N₂ tank and put them in liquid N₂.

2) Thaw while holding it in your hand.

3) Transfer the cells into a 50 ml Falcon tube with a pipetman and measure the cell volume.

4) Add 12% NaCl to a concentration of 100ul per ml of cells. Do this in dropwise and slowly (IMPORTANT) into the flask, while shaking the tube gently.

5) Let the tube sit for 5 minutes at room temperature.

6) Add 10 ml 1.6% NaCl again slowly and dropwise, while gently shaking.

7) Centrifuge in a tabletop centrifuge at 1500 RPM at 20 °C for 5 minutes, low brake.

8) Aspirate off the supernatant.

9) Add 10 ml 0.9% NaCl + 2% glucose again slowly and dropwise, while gently shaking.

10) Centrifuge in a tabletop centrifuge at 1500 RPM at 20 °C for 5 minutes, low brake.

11) Aspirate off the supernatant.

12) Resuspend the pelleted cells with 10 ml culture media and place them in a 25 cm² culture flask and place in the incubator. (BE SURE TO REMEMBER TO GAS THE CULTURE!)

**DAY 2:** Change the media and check parasitaemia.
Add 400 ul 50% washed blood to the culture (Final is 2% Hematocrit).

**DAY 3:** By day 3 if the parasitaemia is 5-10% move the culture to a medium-size flask (75 cm²) (25 ml media).

**DAY 4:** Change the media and check parasitaemia.
Add 1.0 ml 50% washed blood to the culture (Final is 2% Hematocrit).

**DAY 5:** By day 5 if the parasitaemia is 5-10% move the culture to a large-size flask (150 cm²) (50 ml media).