

# Thawing New Malaria Cultures

(04/11/02)

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

## **DAY 1:**

- 1) Get the frozen sample from the liquid N<sub>2</sub> tank and put them in liquid N<sub>2</sub>.
- 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 cm<sup>2</sup> 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 cm<sup>2</sup>) (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 cm<sup>2</sup>) (50 ml media).