## Sorbitol Synchronization

1. Transfer flask contents to a 50 mL tube and spin on Protocol 1 on the centrifuge.
2. Aspirate (remove) supernatant (REMEMBER TO OPEN SIDE VALVES & USE DIRTY TUBE).
3. Add 5 mL sorbitol via pipette; resuspend the pellet.
4. Incubate (dry) for 5 min at 37 C.
5. Add 30 mL red ICM and spin again on Protocol 1.
6. Aspirate the supernatant again.
7. Resuspend in enough MCM to make solution of about 5% hematocrit (about 20 mL works for 1 mL of RBC pellet).

## Flow cytometry (for parasitemia calculation)

1. Prep tube. 1 tube of 500 uL total requires:
   1. 100 uL culture
   2. 400 uL clear ICM
   3. 0.5 HO & TO dyes each
2. Incubate at 37 C for 25 minutes.
3. At the computer, login (Gaelle, flow), click Acquisition, use template HOTO\_Tubes\_2022-09-01.
4. Add enough PBS so that about 1/3 of the tube is filled.
5. Attach tube/stick it into the probe, click Record.
6. Record parasitemia percentage.

## Prepping well plate

1. In two separate tubes, prepare well plate culture solutions with 1% parasitemia and 1%/0.5% hematocrit. First calculate the amount of uRBCs needed to be added to the pellet (RESPIN if RBCs are suspended) bring down the parasitemia, then calculate the amount of MCM needed for each hematocrit condition (just use the spreadsheet honestly).
   1. NOTE: after calculating the amount of uRBCs needed, double the actual volume drawn for the solutions because we’re using 50% solution of blood.
2. Add 180 uL of the 1% or 0.5% culture solution into each well.
3. Add 20 uL of filtered PBS or SGE into the appropriate wells. MIX afterwards.
   1. If SGE is still frozen, then use warm water bath to quickly thaw.
4. Add 200 uL of distilled water into each of the edge wells.
5. Place well plate into the gas chamber and gas.
   1. Open both the gas chamber tubes.
   2. Take the tube attached to the smaller gas chamber to the chamber’s intake tube (with the filter).
   3. Turn on the gas by turning the red valve (should be in parallel to the output tube).
   4. Let the gas run for 10 seconds, then close the output (filterless) tube.
   5. After another 5-10 seconds, close the chamber’s intake tube. Shut off gas output.
6. Place gas chamber with well plates in 37 C incubator and incubate for 48 hours.

## Flow cytometry (for analysis)

1. Spin plate.
2. In the meantime, mix 1 mL of ICM (clear, for flow cytometry) & 1.25 uL of HO dye (KEEP OUT OF LIGHT). 20 uL of the ICM-dye solution goes into each needed well of a 96 round-bottom plate.
3. Take the spun culture well plate and remove 150 uL of the supernatant, then resuspend the pellet with the remaining supernatant (using the same tips).
4. Take 5 uL of each culture and add to the corresponding well plates.
5. Incubate for 10 min (NO JIGGLER).
6. Add 125 uL of PBS (yellow, 1x)
7. Run flow cytometry (template SGE-Cam-Tdt)