

**APPENDIX: CRYOPRESERVATION OF GIARDIA LAMBLIA**

Note: *Giardia lamblia* is also referred to as *Giardia intestinalis* and *Giardia duodenalis* (Adam, R. D. "Biology of *Giardia lamblia*." Clin. Microbiol. Rev. 14 (2001): 447-475. PubMed: 11432808.).

1. Harvest cells from a culture that is at or near peak density. To detach cells from the wall of the culture tubes place on ice for 10 minutes. Invert tubes several times until the majority of the cells are in suspension. Centrifuge tubes at  $800 \times g$  for 5 minutes.
2. Adjust the cell concentration to  $1 \times 10^7$  to  $2 \times 10^7$  cells per milliliter with fresh medium.
3. Before centrifuging, prepare a 24% (v/v) solution of sterile dimethylsulfoxide (DMSO) in fresh medium containing 8% (w/v) sucrose. The solution is prepared as follows:
  - a) Add 1.05 g sucrose to 10 mL of fresh medium and sterile filter through a 0.2  $\mu\text{m}$  filter.
  - b) Add 2.4 mL of DMSO to an ice-cold  $20 \times 150$  mm screw-capped test tube.
  - c) Place the tube on ice and allow the DMSO to solidify (~ 5 min) and then add 7.6 mL of ice-cold medium prepared in step 3a. The final concentration will be 24% (v/v) DMSO and 8% (w/v) sucrose.
  - d) Invert several times to dissolve the DMSO.
  - e) Allow to warm to room temperature.
4. Mix the cell preparation and the cryoprotective agent, prepared in step 3, in equal portions. Thus, the final concentration will equal 12% (v/v) DMSO, 4% (w/v) sucrose and  $10^7$  cells per milliliter.

Note: The time from the mixing of the cell preparation and DMSO stock solution before the freezing process is begun should be no less than 15 minutes and no longer than 30 minutes.

5. Dispense 0.5 mL aliquots into 1 mL to 2 mL sterile plastic screw-capped vials for cryopreservation.
6. Place the vials in a controlled rate freezing unit. From room temperature, cool the vials at  $-1^\circ\text{C}$  per minute to  $-40^\circ\text{C}$ . If the freezing unit can compensate for the heat of fusion, maintain rate at  $-1^\circ\text{C}$  per minute through this phase. At  $-40^\circ\text{C}$ , plunge vials into liquid nitrogen. Alternatively, place the vials in a Nalgene  $1^\circ\text{C}$  freezing container. Place the container at  $-80^\circ\text{C}$  for 1.5 to 2 hours and then plunge vials into liquid nitrogen.
7. Store in either the vapor or liquid phase of a nitrogen refrigerator ( $-130^\circ\text{C}$  or colder).