Strain Designation: WB
Deposited Name: Giardia lamblia (Lambl) Stiles
Depositor: FD Gillin
Isolation: Duodenal aspirate, 30-year-old human male, Bethesda, MD, 1979

Growth Conditions
Temperature: 35°C
Atmosphere: Anaerobic
Culture System: Axenic

Medium
ATCC® Medium 2695: Keister's Modified TYI-S-33
ATCC® Medium 2155: LYI Giardia Medium (filtered)

Instructions for Complete Medium
Medium: ATCC Medium 2695† (Quality controlled freeze-dried lots of this medium are commercially available as cat. no. PRA-2695)
†previously ATCC Medium 1404
Alternate Medium: ATCC Medium 2155 (Quality controlled freeze-dried lots of this medium are commercially available as cat. no. PRA-2155; some strains may not grow equally well in alternative media)

Protocols
Storage and Culture Initiation
Frozen ampules packed in dry ice should either be thawed immediately or stored in liquid nitrogen. If liquid nitrogen storage facilities are not available, frozen ampules may be stored at or below -70°C for approximately one week. Do not under any circumstance store frozen ampules at refrigerator freezer temperatures (generally -20°C). Storage of frozen material at this temperature will result in the death of the culture.

1. To thaw a frozen ampule, place it in a 35°C water bath, until thawed (2-3 min). Immers the ampule just sufficient to cover the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically transfer contents to a screw-capped test tube containing 13 mL ATCC Medium 2695. Incubate the tube on a 15° horizontal slant at 35°C.

Culture Maintenance
1. When the culture has reached or is near peak density, place the tubes on ice for 10 minutes.
2. Gently invert the culture tube 10 times and aseptically transfer a 0.1-0.4 mL aliquot to a screw-capped test tube containing 13 mL ATCC Medium 2695.
3. Incubate the culture on a 15° horizontal slant at 35°C.
4. Transfer the culture every 3-4 days as described in steps 1-2. The transfer interval will depend on the size of the inoculum and the quality of the medium. This should be empirically determined by examining the culture on a daily basis until the growth cycle has stabilized. Do not allow the culture to overgrow. The culture crashes soon after reaching peak density.

Cryopreservation

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 x g for 5 minutes.
2. Adjust the concentration of cells to 2 x 10^7/mL in fresh medium.
3. Before centrifuging prepare a 24% (v/v) solution of sterile 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 filter sterilize through a 0.2 μm filter;
   b. Add 2.4 mL of DMSO to an ice cold 20 x 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
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% sucrose (w/v) and 10^7 cells/mL. The time from the mixing of the cell preparation and DMSO stock solution to the start of the freezing process should be no less than 15 min and no longer than 30 min.

5. Dispense in 0.5 mL aliquots into 1.0 - 2.0 mL sterile plastic screw-capped cryules (special plastic vials for cryopreservation).

6. Place the vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through the heat of fusion. At -40°C plunge into liquid nitrogen. Alternatively, place the vials in a Nalgene 1°C freezing apparatus. Place the apparatus at -80°C for 1.5 to 2 hours and then plunge ampules into liquid nitrogen. (The cooling rate in this apparatus is approximately -1°C/min.)

7. The frozen preparations should be stored in either the vapor or liquid phase of a nitrogen refrigerator. Frozen preparations stored below -130°C are stable indefinitely. Those stored at temperatures above -130°C are progressively less stable as the storage temperature is elevated.

8. To establish a culture from the frozen state place an ampule in a water bath set at 35°C. Immerse the vial just to a level just above the surface of the frozen material. Do not agitate the vial.

9. Immediately after thawing, do not leave in the water bath, aseptically remove the contents of the ampule and inoculate a 16 x 125 mm mm plastic-capped test tube containing 13 mL ATCC Medium 2695.

10. Incubate the culture on a 15° horizontal slant at 35°C.