Strain Designation: Lee (L.L.)
Deposited Name: Naegleria fowleri Carter
Depositor: DT John
Isolation: Cerebrospinal fluid of 15-year-old female, Richmond, VA, 1968

Growth Conditions
Temperature: 35°C
Culture System: Axenic

Medium
ATCC® Medium 1034: Modified PYNFH medium (Available from ATCC as ATCC cat. no. 327-X)
ATCC® Medium 710: Nelson’s Culture Medium For Naegleria
ATCC® Medium 803: M7 medium
ATCC® Medium 902: Schuster’s axenic Naegleria medium

Instructions for Complete Medium
Media: ATCC medium 1034
Alternate Media: ATCC medium 710, ATCC medium 803, ATCC medium 902

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). Immerse the ampule to a level just sufficient to cover only the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically transfer contents to a plastic screw-capped tube containing 5 mL ATCC Medium 1034. Incubate the tube on a 15° horizontal at 35°C with the cap screwed on tightly.

Culture Maintenance
1. Vigorously agitate a culture at or near peak density and aseptically transfer 0.1-0.2 mL to a fresh tube of ATCC medium 1034.
2. Incubate upright at 35°C with the caps on tightly.

Harvest and Preservation
1. Harvest cells from a culture that is at or near peak density by centrifugation at 600 x g for 5 min. Pool the cell pellets into a single tube.
2. Adjust the concentration of cells to 2.0 x 10⁶/mL. If the concentration is too low, centrifuge at 600 x g for 5 minutes and resuspend the cell pellet with a volume of supernatant to yield the desired concentration.
3. Prepare a 15% (v/v) sterile DMSO solution in ATCC medium 1034 as follows: Add the required volume of DMSO to a glass screw-capped test tube and place on ice. Allow the DMSO to solidify. Add the required volume of refrigerated ATCC medium 1034. Dissolve the DMSO by inverting several times. If the DMSO solution is not prepared on ice, an exothermic reaction will occur that may precipitate certain components of the medium.
4. Mix the cell preparation and the DMSO in equal portions. Thus, the final concentration will be 10% and 7.5% (v/v) DMSO. 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 min and no longer than 60 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 vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. If
freezing unit can compensate for the heat of fusion, maintain rate at -1 C/min through heat of fusion. At -40°C plunge the ampules into liquid nitrogen.

7. The frozen preparations are stored in either the vapor or liquid phase of a nitrogen refrigerator.

8. To establish a culture from the frozen state place an ampule in a water bath set at 35°C. Immers the vial enough to cover only 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 into 5.0 mL of fresh ATCC medium 1034.

10. Incubate the tube on a 15° horizontal at 35°C with the cap screwed on tightly.

References and other information relating to this product are available online at www.atcc.org.

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the Biosafety in Microbiological and Biomedical Laboratories from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

ATCC Warranty

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Additional information on this culture is available on the ATCC web site at www.atcc.org.

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