Product Information Sheet for NR-46501

Naegleria fowleri, Strain CDC:V615

Catalog No. NR-46501

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Contributor:

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Manufacturer:

BEI Resources

Product Description:

Protozoa Classification: *Vahlkampfiidae*, *Naegleria*
Species: *Naegleria fowleri*
Strain: CDC:V615
Original Source: *Naegleria fowleri (N. fowleri)*, strain CDC:V615 is a clinical isolate collected in 2009 from the cerebrospinal fluid of a 22-year-old male patient in the United States.[1]

N. fowleri is a free-living pathogenic amoeboflagellate that feeds mainly on bacteria, yeast and algae. It is the causative agent of primary amoebic meningoencephalitis, a water-borne disease of the central nervous system in humans and is associated with recreational activities in contaminated waters.[2-8] Infection occurs when the amoeba enters the human nasal cavity where it attaches to the nasal mucosa and travels along olfactory nerves, eventually entering the brain, where it causes extensive tissue damage, inflammation, and hemorrhagic necrosis.[5] *N. fowleri* is a moderate thermophile and can tolerate temperatures up to 45°C. It has been isolated worldwide from soil and fresh waters naturally heated by the sun, including lakes, ponds, well water, geothermal springs and in areas thermally-polluted by industries.[2-8]

The *N. fowleri* life cycle consists of three morphological stages: a dividing, feeding, infective amoeboid trophozoite; a transitory pear-shaped di-flagellate formed from the amoeba during conditions of nutrient deprivation in water; and, a resistant cyst that forms during adverse environmental conditions such as food deprivation, crowding, ionic changes and the presence of toxin-producing bacteria.[5,6,8] Eight genotypes of *N. fowleri* have been identified using the internal transcribed spacer regions and 5.8S ribosomal RNA gene sequences.[2-5]

Material Provided:

Each vial of NR-46501 contains approximately 0.5 mL of culture in cryopreservative [7.5% dimethylsulfoxide (DMSO)]. Please refer to the Certificate of Analysis for the specific culture media used for each lot and refer to Appendix I for cryopreservation instructions.

Packaging/Storage:

NR-46501 was packaged aseptically in screw-capped plastic cryovials and is provided frozen on dry ice. The product should be stored at cryogenic temperature (-130°C or colder), preferably in the vapor phase of a liquid nitrogen freezer. If liquid nitrogen storage facilities are not available, frozen cryovials may be stored at -70°C or colder for approximately one week.

Note: Do not under any circumstances store vials at temperatures warmer than -70°C. Storage under these conditions will result in the death of the culture.

To insure the highest level of viability, the culture should be initiated immediately upon receipt. Any warming of the product during shipping and transfer must be avoided, as this will adversely affect the viability of the product. For transfer between freezers and for shipping, the product may be placed on dry ice for brief periods, although use of a portable liquid nitrogen carrier is preferred. Please read the following recommendations prior to using this material.

Growth Conditions:

Modified Peptone - Yeast Extract - Nucleic Acid - Folic Acid - Hemin (PYNFH) medium (ATCC® Medium 1034) supplemented with 10% heat-inactivated fetal bovine serum (Appendix II)
Incubation:
Temperature: 35°C
Atmosphere: Aerobic
Propagation:

1. Place the frozen vial in a 35°C to 37°C water bath and thaw for approximately 2 to 3 minutes. Do not agitate the vial. Do not leave the vial in the water bath after it is thawed.
2. Immediately after thawing, aseptically transfer the contents of the vial to a T-25 tissue culture flask containing 5 to 10 mL modified PYNFH medium.
3. Screw the cap on tightly and incubate the tube or flask at 35°C.

Maintenance:

1. When the culture is at or near peak density, vigorously agitate or scrape the surface of the flask to detach adherent cells.
2. Transfer approximately 0.25 mL to a new flask containing 5 to 10 mL of freshly made modified PYNFH medium.
3. Screw the caps on tightly and incubate at 35°C.
4. The amoeba will form an almost continuous sheet of cells on the bottom surface of the flask. Repeat steps 1 through 3 every 7 to 10 days.

Please refer to Appendix I for cryopreservation instructions.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Naegleria fowleri*, Strain CDC:V615, NR-46501."

Biosafety Level: 2

Appropriate safety procedures should always be used with this

material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

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References:

1. Visvesvara, G. S., Personal Communication.
2. Zhou, L., et al. "Genetic Variations in the Internal Transcribed Spacer and Mitochondrial Small Subunit rRNA Gene of *Naegleria* Spp." J. Eukaryot. Microbiol. 50 (2003): 522-526. PubMed: 14736150.
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6. Marciano-Cabral, F. "Biology of *Naegleria* Spp." Microbiol. Rev. 52 (1988): 114-133. PubMed: 3280964.
7. De Jonckheere, J. F. "A Century of Research on the Amoeboflagellate Genus *Naegleria*." Acta Protozool. 41 (2002): 309-342.
8. Visvesvara, G. S., H. Moura and F. L. Schuster. "Pathogenic and Opportunistic Free-Living Amoebae: *Acanthamoeba* Spp., *Balamuthia mandrillaris, Naegleria fowleri,* and *Sappinia dipoloidea*." FEMS Immunol. Med. Microbiol. 50 (2007): 1-26. PubMed: 17428307.
9. Martinez-Castillo, M., et al. "*Naegleria fowleri* after 50 Years: Is it a Neglected Pathogen?" J. Med. Microbiol. 65 (2016): 885-896. PubMed: 27381464.

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APPENDIX I: CRYOPRESERVATION

1. Harvest *Naegleria* from multiple flasks by scraping the surface of the flask with a cell scraper to detach adhering trophozoites.
2. Transfer the cell suspensions to 15 mL or 50 mL plastic centrifuge tubes.
3. Adjust the cell concentration to 1 × 10^6 to 2 × 10^7 cells/mL with fresh modified PYNFH medium.
   Note: If the concentration of cells is too low, centrifuge at 1300 × g for 10 minutes and resuspend in a smaller volume of fresh medium to yield the desired cell concentration.
4. Mix equal volumes of cell suspension and fresh medium containing 15% DMSO to yield a final concentration of 1 × 10^6 to 2 × 10^7 cells/mL in 7.5% DMSO. The freezing process should start 15 to 30 minutes following the addition of cryoprotective solution to the cell suspension.
5. Dispense 0.5 mL aliquots into 1 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°C/min to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through this phase. At -40°C, plunge vials into liquid nitrogen. Alternatively, place the vials in a Nalgene 1°C freezing container. Place the container at -80°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°C or colder).

APPENDIX II: Modified Peptone - Yeast Extract - Nucleic Acid - Folic Acid - Hemin (PYNFH) Medium (ATCC® MEDIUM 1034)

1. Prepare the Basal medium (see recipe below), autoclave for 20 minutes at 121°C, and allow to cool.
2. Prepare the Buffer solution (see recipe below) and filter sterilize.
3. Aseptically prepare the PYNFH medium (see recipe below), mix thoroughly and store at 4°C:

Basal Medium	
Peptone	10.0 g
Yeast Extract	10.0 g
Yeast Nucleic Acid	1.0 g
Folic Acid	15.0 mg
Hemin	1.0 mg
Distilled water	880.0 mL

Buffer Solution	
KH_2PO_4	18.1 g
Na_2HPO_4	25.0 g
Distilled Water	1.0 L

4. Aseptically prepare the PYNFH medium (see recipe below), mix thoroughly and store at 4°C:

PYNFH Medium	
Basal medium	880.0 mL
Buffer Solution	20.0 mL

5. Aseptically supplement PYNFH medium with 100.0 mL heat-inactivated fetal bovine serum prior to use.

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