Pneumocystis carinii Delanoe and Delanoe

PRA-159™

Description

Pneumocystis carinii strain M167-6 was isolated in October 2003 from the lungs of an immunosuppressed Lewis male rat in Cincinnati, Ohio. This parasitic protozoan is cultivated in rats and has applications in opportunistic pathogen research.

Strain designation: M167-6

Type strain: Yes

Storage Conditions

Product format: Frozen

Storage conditions: -80°C or colder for 1 week, vapor phase of liquid nitrogen for long-term storage

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local

or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Host:

in vivo cultivation, rat

Handling Procedures

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 just sufficient to cover the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically remove the contents of the ampule with a syringe and inoculate an uninfected, immunosuppressed rat. Follow the protocol for maintenance *in vivo*. The course of infection may be longer or shorter than usual depending on percent recovery of the parasite from the frozen state.

Culture maintenance:

When the frozen ampule arrives, store it as indicated above until ready to use. The following directions for establishing an infection are adapted from: Boylan, C.J. and W.L. Current. 1992. Improved rat model of *Pneumocystis carinii pneumonia*: induced laboratory infections in *Pneumocystis*-free animals. Infect. Immun. 60: 1589-1597. They must be followed carefully to assure success.

1. Seven days prior to thawing the frozen ampule, immunosuppress rats (viral antibody-negative male or female rats weighing 120-140 grams each, any of several strains, e.g., Lewis, Sprague-Dawley, Fischer 344) by administering one injection of 4 mg of methylprednisolone acetate (Upjohn Co., Kalamazoo, MI).
2. On day seven, thaw the frozen ampule rapidly in a 35°C water bath as indicated above.
3. Transfer the thawed contents to a centrifuge tube and add an equal volume of RPMI 1640 medium (GIBCO 31800-022) containing 20% (v/v) heat-inactivated fetal bovine serum.
4. Centrifuge at 1000 x g for 5 minutes, remove supernatant and resuspend the pellet with medium specified in step 3 to a volume such that the final concentration of the *P. carinii* nuclei is 10^7-10^8 per mL (the concentration of the nuclei will be specified on the certificate of analysis shipped with the frozen ampule).
5. Aspirate 0.1 mL of the well-mixed suspension into a 1.0 mL syringe fitted with a three-inch, 20-gauge curved stainless steel animal feeding tube (Popper and Sons, New Hyde Park, NY). Keep filled syringe on ice until ready to inoculate.
6. Lightly anesthetize rats by exposing them briefly to halothane.

7. Suspend anesthetized rats by their upper incisors on a wire loop at the top of a board held at a 60 degree incline. Pull tongue to one side of the lower incisors with a pair of forceps, insert the feeding tube prepared in step 5 and express 0.1 mL of inoculum followed by 0.4 mL of air into the trachea. Note: To assure correct placement of the inoculum into the trachea, direct the feeding tube along the back of the tongue into the larynx while palpating the trachea.
8. Continue weekly injections of 4 mg of methylprednisolone into inoculated rats.
9. Six weeks post-inoculation, check for infection by killing a rat, removing the lungs and preparing impression smears.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Pneumocystis carinii* Delanoe and Delanoe (ATCC PRA-159)

References

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

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