**Designation:** IN-23-G1-C2 [NCTC 11986]

**Deposited Name:** Legionella pneumophila subsp. pneumophila Brenner et al.

**Antigenic Properties:** Serogroup 9

**Medium**

ATCC® Medium 1099: CYE (Charcoal Yeast Extract) Buffered Medium

**Growth Conditions**

- **Temperature:** 37.0°C
- **Atmosphere:** 5% CO₂

**Propagation Procedure**

1. Open vial according to enclosed instructions.
2. Using a single tube of #1099 broth (5 to 6 ml), withdraw approximately 0.5 to 1.0 ml with a Pasteur or 1.0 ml pipette. Rehydrate the entire pellet.
3. Aseptically transfer this aliquot back into the broth tube. Mix well.
4. Use several drops of the suspension to inoculate a #1099 agar slant and/or plate.
5. Incubate the tubes and plate at 37°C for 48-72 hours in an atmosphere of 5% CO₂.

Two colony types observed on #1099 agar are a) small, entire, glistening, circular, smooth, and raised; and b) large, entire, glistening, circular, smooth, white, low convex.

Strain shows growth by turbidity in the supernatant portion of the broth. Growth on solid medium is indicated by the formation of white colonies.

Legionella species are nutritionally fastidious and are inhibited by media of poor caliber, so strict attention should be paid to the quality of the ingredients. The pH of the medium should be checked when cool, and is most critical. Unbuffered medium should not be used. Exposure of medium to light, particularly when hot, may also result in the accumulation of peroxides which can be inhibitory to the bacteria.

Before rehydration, vials may be stored at +2 to +8°C for extended periods with minimal loss of viability. Subculturing to maintain culture is discouraged because of the possibility of mutation, selection of variants, or contamination. To minimize changes, it is recommended that cells be harvested and stored at 70°C or below.

Additional information on this culture is available on the ATCC® web site at [www.atcc.org](http://www.atcc.org).

**References**

References and other information relating to this product are available online at [www.atcc.org](http://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.

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