Listeria Selective Supplement

PRODUCT INFORMATION

C001-1g - Cycloheximide Powder, 1g

C001-5g - Cycloheximide, Powder, 5g

C039-100mg - Colistin Sulfate, Powder, 100mg

C039-1g - Colistin Sulfate, Powder, 1g

F013-1g - Fosfomycin Sodium, Powder, 1g

F013-5g - Fosfomycin Sodium, Powder, 5g

F013-50g - Fosfomycin Sodium, Powder, 50g

DESCRIPTION

Listeria Selective Agar with Listeria Selective Supplement is a selective and diagnostic medium for the detection of *Listeria monocytogenes.*

BACKGROUND

Cycloheximide is widely used in biomedical research to inhibit protein synthesis in eukaryotic cells studied in vitro (i.e. outside of organisms). Its effects are rapidly reversed by simply removing it from the culture medium.

Colistin is a polymyxin antibiotic produced by certain strains of *Bacillus polymyxa* var. *colistinus*. Colistin is a mixture of cyclic polypeptides colistin A and B. Colistin is effective against most Gram-negative bacilli and is used as a polypeptide antibiotic.

Cefotetan is an injectable antibiotic of the cephamycin type for prophylaxis and treatment of bacterial infections.

Fosfomycin is a broad-spectrum antibiotic produced by certain *Streptomyces* species.

Mechanism of action

Cycloheximide is an inhibitor of protein biosynthesis in eukaryotic organisms, produced by the bacterium Streptomyces griseus. Cycloheximide exerts its effect by interfering with the translocation step in protein synthesis (movement of two tRNA molecules and mRNA in relation to the ribosome) thus blocking translational elongation.

Fosfomycin inhibits bacterial cell wall biogenesis by inactivating the enzyme UDP-N-acetylglucosamine-3-enolpyruvyltransferase, also known as MurA. This enzyme catalyzes the committed step in peptidoglycan biosynthesis, namely the ligation of phosphoenolpyruvate (PEP) to the 3'-hydroxyl group of UDP-N-acetylglucosamine. This pyruvate moiety provides the linker that bridges the glycan and peptide portion of peptidoglycan. Fosfomycin is a PEP analog that inhibits MurA by alkylating an active site cysteine residue (Cys 115 in the Escherichia coli enzyme).

APPLICATION IN LISTERIA SELECTIVE AGAR

Foodborne infection by *Listeria monocytogenes* has prompted increased concern for detecting this organism in foods, in the environment and in pathological specimens from both human and animal subjects.

Most infections in adult humans are symptomless and result in intestinal, vaginal and cervical carriage. Infection during pregnancy may cause abortion, premature delivery and neonatal infection. The possibility of listeriosis should be considered in any woman with unexplained recurrent miscarriage, premature labour or foetal death. The organism should be sought in blood cultures and genital-tract swabs.

The most common clinical manifestation in both adults and neonates is meningitis. Widely disseminated infection, abscesses, sub-acute bacterial endocarditis and opportunistic infections in immunosuppressed patients occur less frequently.

Birds, fish and other animals are all susceptible to infection with *Listeria*. It is of particular importance in domestic farm animals. In the Federal Republic of Germany reporting of listeriosis in animals is compulsory and meat inspection law in the same country requires examination for *Listeria* because of its significance in meat hygiene.

Listeria monocytogenes is very widespread in the environment. Isolation has been reported from milk, cheese, sewage and riverwater, and silage. Because *Listeria* is so widespread sources of infections are numerous. Uncooked vegetable foods have been implicated; an episode associated with consumption of coleslaw 7 was linked with cabbage from a farm using sewage fertiliser. In outbreaks caused by dairy products, cattle

with mastitis may be the source of the organism. Of great importance to veterinarians is the considerable increase amongst sheep of infection manifesting as abortion or encephalitis due largely to changing practices in silage manufacture.

The ability to isolate the organism has been impeded in the past by lack of an effective selective medium, as *Listeria monocytogenes* can be easily and completely overgrown by competing flora.

Listeria Selective Medium is based on the formulation described by Curtis et al. and is recommended for the detection of *Listeria monocytogenes* from clinical and food specimens.

The medium utilises

(i) the selective inhibitory components lithium chloride, acriflavine, colistin sulphate, cefotetan, cycloheximide or amphotericin B and fosfomycin,

(ii) the indicator system aesculin and ferrous iron for the isolation or differentiation of *Listeria monocytogenes*.

Listeria monocytogenes hydrolyses aesculin, producing black zones around the colonies due to the formation of black iron phenolic compounds derived from the aglucon. Gram-negative bacteria are completely inhibited. Most unwanted Gram-positive species are suppressed, but some strains of *enterococci* grow poorly and exhibit a weak aesculin reaction, usually after 40 hours incubation. Some *staphylococci* may grow as aesculin-negative colonies.

Typical *Listeria monocytogenes* colonies are almost always visible after 24 hours, but incubation should be continued for a further 24 hours to detect slow-growing strains.

Techniques for isolation vary with the author and the material under examination. For all specimens selective enrichment and cold enrichment have been shown to increase isolation rates significantly. The efficacy of *Listeria* Selective Medium has been confirmed for various foods following the methodology and using selective enrichment media described in the literature.

Content concentrations

Typical Formula*	mg/litre
Listeria Selective Agar	
Columbia Blood Agar Base	39
Aesculin	1
Ferric ammonium citrate	0.5
Lithium chloride	15
Final pH 7.0 ± 0.2 @ 25°C	
Listeria Selective Supplement	
Cycloheximide	400
Colistin sulphate	20
Acriflavine	5
Cefotetan	2
Fosfomycin	10
* Adjusted as required to meet performance standards	

Table 1 - Typical Formula for Listeria Selective Agar and Listeria Selective Supplement

METHOD

Preparation

Suspend appreciate amount of Listeria Selective Agar in distilled water. Bring gently to the boil to dissolve. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add the contents of Listeria Selective Supplement reconstituted with 5 ml of 70% ethanol. Mix well and pour into sterile Petri dishes.

Protocol

Faecal and biological specimens:

The sample is homogenised in 0.1% peptone water (1 part to 9 parts peptone water).

Direct surface plate method

1. Inoculate 0.1 ml of the homogenised specimen onto the Listeria Selective Medium plates.

2. Incubate at 35°C for up to 48 hours.

3. Examine for typical colonies of Listeria after 24 and 48 hours incubation.

Selective Enrichment Method:

1. Add the homogenised specimen to the selective enrichment broth and incubate at 30°C for up to 7 days.

2. Inoculate 0.1 ml of the selective enrichment broth, after 24 hours, 48 hours and 7 days, onto the Listeria Selective Medium plates.

3. Incubate the plates at 35°C for up to 48 hours.

4. Examine for typical colonies of *Listeria* after 24 and 48 hours incubation.

Food and Environmental Samples:

Protocols for isolation vary with the author, material and authorities. For detection of *Listeria monocytogenes* when present in small numbers, the test samples must be inoculated into an enrichment broth to allow multiplication before isolation and identification. Depend-

ing on the type of sample under test, an appropriate method and selective enrichment broth should be chosen prior to inoculation onto the Listeria Selective Medium plates.

1. Inoculate 0.1 ml of the selective enrichment broth onto the Listeria Selective Medium plates.

2. Incubate at 35°C for up to 48 hours.

3. Examine for typical colonies after 24 and 48 hours incubation.

Colonies presumptively identified as Listeria monocytogenes must be confirmed by biochemical and serological testing.

Note: Differences in susceptibility of *Listeria monocytogenes, Listeria seeligeri* and *Listeria ivanovii* to b-lactam antibiotics and fosfomycin have been observed dependent on whether incubation is at 30°C or 35-37°C.

Quality control

Positive control:

Listeria monocytogenes ATCC® 7644: Good growth; brown coloured colonies with aesculin hydrolysis

Negative control:

Entercoccus feacalis ATCC® 29212 *: No growth

REFERENCES

1. Lancet (1985 [2]) August 17. 364-365.

2. Hayes et al (1986) Appl. Env. Microbiol. 50. 438-440.

3. Fernandez Garayzabal J.F. et al (1986) Can. J. Microbiol. 32. 149-150.

4. James S.M., Ferrin S.L. and Agee B.A. (1985) MMWR 34. 357-359.

5. Watkins J. and Sleath K.P. (1981).

6. Gitter M. (1983) Vet. Rec. 112, 314.

7. Schlech W.F., Lavigne P.M. and Bortolussi R.A. (1983) N. Eng. J. Med. 308. 203-206.

8. Appleyard W. (1986) Communicable Diseases, Scotland. April 1986. CDS 86/13.

9. Curtis G.D.W., Mitchell R.G., King A. F. and Griffin E.J. (1989) Letters in Appl. Microbiol. 8. 95-98.

10. van Netten P., van de Ven A., Perales I. and Mossel D.A.A. (1988) Int. J. Food Microbiol. 6. 187-198.

11. Prentice G.A. and Neaves P. (1988) Bulletin of the International Dairy Federation No. 223.

12. Hayes P.S., Feeley J.C. Graves L.M., Ajello G.W. and Fleming D.W (1986) Appl. & Environ. Microbiol. 51. 438-440.

13. Garayzabal J.F.F. Rodriquez L.D., Boland J.A.V. Cancelo J.L.B. and Fernendez G.S. (1986) Can. J. Microbiol. 32. 149-150.

14. Doyle M.P., Meske L.M. and Marth E.H. (1985) J. of Food Protection, 48. 740-742.

15. Crowther J.S. (1988) Personal Communication, Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford, U.K.

16. Neaves P. and Prentice G.A. (1988) Personal Communication, Technical Division, Milk Marketing Board, Thames Ditton, Surrey.

17. Lovett J., Francis D.W. and Hunt J.M. (1987) J. Food Prot. 50. 188-192.

18. Donelly C.W. and Baigent G.J. (1986) Appl. and Environ. Microbiol. 52. 689-695.

19. Hammer P., Hahn G. and Heeschen W. (1988) Deutsch Mock-Zeit. 50. 1700-1706.

20. Food and Drug Administration (FDA) Bacteriological Analytical Manual 7th Edition 1992, AOAC Int. Publishers Arlington V.A.

21. Foodborne Pathogens. Monograph Number 2 -- Listeria, page 7. Oxoid Ltd, Wade Road, Basingstoke, Hampshire, U.K.

22. Al-Zoreki N. and Sandine W.E. (1990) Appl. Env. Microbiol. 56. 3154-3157.

23. Bille J. and Doyle M.P. (1991) ``Listeria and Erysipelothrix", 287-295 in Balows A., Hauster W.J. Jnr., Herrman K.L., Isenberg H.D. and Shadomy H. J. (Editors), Manual of Clinical Microbiology, 5th Edition, American Society for Microbiology, Washington, D.C.

24. Curtis G.D.W., Nichols W.W. and Falla T.J. (1989) Letters in Appl. Microbiol. 8. 169-172.