LISTERIA-SELECTIVE AGAR

INTENDED USE

Listeria-selective agar is for isolating and differentiating *Listeria monocytogenes*.

SUMMARY AND EXPLANATION

First described in 1926 by Murray, Webb and Swann,[1] *Listeria monocytogenes* is a widespread problem in public health and the food industries. This organism can cause human illness and death, particularly in immunocompromised individuals and pregnant women.[2] The first reported foodborne outbreak of listeriosis was in 1985.[3] Since then, microbiological and epidemiological evidence from both sporadic and epidemic cases of listeriosis has shown that the principal route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.[4]

Implicated vehicles of transmission include turkey frankfurters,[5] coleslaw, pasteurized milk, Mexican-style cheese, paté and pickled pork tongue. The organism has been isolated from commercial dairy and other food processing plants and is ubiquitous in nature, being present in a wide range of unprocessed foods and in soil, sewage, silage and river water.[6]

Listeria spp. grow over a pH range of 4.4-9.6 and survive in food products with pH levels outside these parameters.[7] *Listeria* spp. are microaerophilic, gram-positive, asporogenous, non-encapsulated, non-branching, regular, short, motile rods. Motility is most pronounced at 20°C.

The most common contaminating bacteria found in food sources potentially containing *Listeria* are streptococci, especially the enterococci, micrococci and *Bacillus* species, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*.[8]

Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization and serological confirmation. Listeria-selective agar is prepared according to the formulation of Curtis et al.[9] who originally described the medium and its use in the selective isolation of *Listeria* from mixed cultures.

PRINCIPLE

Peptones and beef heart digest provide nitrogen, carbon, amino acids and vitamins. Agar is the solidifying agent. Sodium chloride maintains the osmotic balance.

Ferric ammonium citrate aids in the differentiation of *Listeria* spp. Since all *Listeria* spp. hydrolyze esculin, the addition of ferric ions to the medium will detect the reaction. A blackening of the colony and

surrounding medium in cultures containing esculin-hydrolyzing bacteria results from the formation of 6,7-dihydroxycoumarin which reacts with the ferric ions.[10]

Selectivity is provided by the presence of lithium chloride in the formula. The high salt tolerance of *Listeria* is used as a means to markedly inhibit growth of enterococci.

Selectivity is increased by adding various antimicrobial agents to the base. Incorporating these agents into Listeria-selective agar will completely inhibit gram-negative organisms and most gram-positive organisms after 24 hours of incubation.

Listeria-selective agar is recommended for isolating and identifying *Listeria monocytogenes* from processed meat and poultry products.

REAGENTS (FORMULA)

Pancreatic Digest of Casein	8.9	g
Proteose Peptone No. 3	4.4	g
Yeast Extract	4.4	g
Tryptic Digest of Beef Heart	2.7	g
Starch	0.9	g
Sodium Chloride	4.4	g
Esculin	1.0	g
Ferric Ammonium Citrate	0.5	g
Lithium Chloride	15.0	g
Agar	15.3	g
Deionized Water	1000.0	ml

PROCEDURE

The USDA method involves enrichment of the food sample in UVM Modified Listeria Enrichment Broth (one part sample to nine parts broth) at 30°C. After incubation, a portion of the enrichment mixture is plated onto Listeria-selective agar.

The FDA method involves adding 25 mL of liquid or 25 g of solid material to 225 mL Listeria Enrichment Broth and incubating at 30°C for 2 days. After enrichment, the broth is plated onto Listeria-selective agar.

For further information when testing food samples or clinical specimens for *Listeria*, consult appropriate references.

EXPECTED RESULTS

Select esculin-positive colonies and confirm their identity by further biochemical testing. Use macroscopic tube and rapid slide tests for definitive serological identification. For additional information, refer to appropriate references.

QUALITY CONTROL

All lot numbers have been tested and have been found to be acceptable. Customers can test products using the following quality control organisms. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, sample results should not be reported.

Organisms	**Incubation**	**Results**
Escherichia coli ATCC 25922	35 ± 2°C for 18-48 hours	Complete Inhibition
Listeria monocytogenes ATCC 19114	35 ± 2°C for 18-48 hours	Growth, Black Colonies

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