

## Formula

### Difco™ Maximum Recovery Diluent

Approximate Formula\* Per Liter

Peptone .....	1.0	g
Sodium Chloride .....	8.5	g

\*Adjusted and/or supplemented as required to meet performance criteria.

## Directions for Preparation from Dehydrated Product

1. Dissolve 9.5 g of the powder in 1 L of purified water.
2. Dispense into final containers and cap loosely.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

## Procedure

Consult appropriate references for dilution procedures when testing foods.<sup>1-4</sup>

## Expected Results

Refer to appropriate references and procedures for results.

## References

1. DeMello, Danielson and Kiser. 1951. J. Lab. Clin. Med. 37:579.
2. Gunter. 1954. J. Bacteriol. 67:628.
3. Straka and Stokes. 1957. Appl. Microbiol. 5:21.
4. Patterson and Cassells. 1963. J. Appl. Bacteriol. 26:493.

## Availability

### Difco™ Maximum Recovery Diluent

Cat. No. 218971 Dehydrated – 500 g

# McBride Listeria Agar

## Intended Use

McBride Listeria Agar is used for isolating *Listeria monocytogenes* with or without the addition of blood.

## Summary and Explanation

First described in 1926 by Murray, Webb and Swann,<sup>1</sup> *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.<sup>2</sup> The first reported food-borne outbreak of listeriosis was in 1985<sup>3</sup> and, 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 *L. monocytogenes*.<sup>4</sup>

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

*Listeria* species grow over a pH range of 4.4-9.6 and survive in food products with pH levels outside of these parameters.<sup>7</sup> *Listeria* species 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*.<sup>8</sup>

Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization and serological confirmation.

McBride Listeria Agar is prepared according to the formulation of McBride and Girard,<sup>9</sup> who originally described the medium and its use in the selective isolation of *Listeria* from experimentally mixed cultures. The medium has been recommended for isolating *Listeria* from clinical specimens,<sup>10</sup> raw milk<sup>11,12</sup> and food samples.<sup>7</sup>

When the medium is used as a blood agar, a narrow zone of beta-hemolysis may be evident around and under *Listeria* colonies.

## Principles of the Procedure

Peptone and beef extract provide nitrogen, vitamins and minerals. Sodium chloride maintains the osmotic balance of the medium. Partial selectivity is provided by lithium chloride, glycine and phenylethanol, which aid in suppressing both

## User Quality Control

### Identity Specifications

#### Difco™ McBride Listeria Agar

Dehydrated Appearance:	Light tan, homogeneous, may form soft lumps.
Solution:	4.6% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Without Blood – Light to medium amber, slightly opalescent. With Blood – Cherry red, opaque.
Reaction of 4.6% Solution at 25°C:	pH 7.3 ± 0.2

### Cultural Response

#### Difco™ McBride Listeria Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours. Observe the colonies under oblique transmitted light.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Escherichia coli</i>	25922	10 <sup>3</sup> -2×10 <sup>3</sup>	Inhibition
<i>Lactobacillus rhamnosus</i>	9595	10 <sup>3</sup> -2×10 <sup>3</sup>	Inhibition
<i>Listeria monocytogenes</i>	19114	10 <sup>2</sup> -10 <sup>3</sup>	Good*
<i>Listeria monocytogenes</i>	19115	10 <sup>2</sup> -10 <sup>3</sup>	Good*

\**Listeria* colonies gray to blue color with a ground glass appearance.

gram-positive and gram-negative bacteria other than *Listeria*. If increased inhibition of fungi is also needed, cycloheximide can be added after autoclaving. Agar is the solidifying agent.

## Formula

### Difco™ McBride Listeria Agar

Approximate Formula* Per Liter		
Tryptose .....	10.0	g
Beef Extract .....	3.0	g
Sodium Chloride .....	5.0	g
Glycine .....	10.0	g
Lithium Chloride .....	0.5	g
Phenylethanol .....	2.5	g
Agar .....	15.0	g

\*Adjusted and/or supplemented as required to meet performance criteria.

## Directions for Preparation from Dehydrated Product

1. Suspend 46 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. To enhance selectivity and/or differentiation, aseptically add 0.2 g/L cycloheximide and/or sterile defibrinated blood to the medium at 45-50°C. Mix well.
5. Test samples of the finished product for performance using stable, typical control cultures.

## Procedure

When testing clinical specimens for *Listeria*, inoculate directly onto primary plating media and McBride Listeria Agar.<sup>10</sup>

When isolating *Listeria* from raw milk and food samples, refer to appropriate references.<sup>7,12</sup>

## Expected Results

Observe colonies under oblique transmitted light. *Listeria* colonies should display a gray to blue color with a ground glass appearance.

## References

1. Murray, Webb and Swann. 1926. J. Pathol. Bacteriol. 29:407.
2. Monk, Clavero, Beuchat, Doyle and Brackett. 1994. J. Food Prot. 57:969.
3. Wehr. 1987. J. Assoc. Off. Anal. Chem. 70:769.
4. Bremer and Osborne. 1995. J. Food Prot. 58:604.
5. Grau and Vanderlinde. 1992. J. Food Prot. 55:4.
6. Patel, Hwang, Beuchat, Doyle and Brackett. 1995. J. Food Prot. 58:244.
7. Ryser and Donnelly. 2001. In Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
8. Kramer and Jones. 1969. J. Appl. Bacteriol. 32:381.
9. McBride and Girard. 1960. J. Lab. Clin. Med. 55:153.
10. Pezzlo. 1992. In Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
11. Hayes, Feeley, Graves, Ajello and Fleming. 1986. Appl. Environ. Microbiol. 51:438.
12. Flowers, Andrews, Donnelly and Koenig. 1993. In Marshall (ed.), Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

## Availability

### Difco™ McBride Listeria Agar

COMPF SMD

Cat. No. 212156 Dehydrated – 500 g\*

\*Store at 2-8°C.

# McClung Toabe Agar Base Egg Yolk Enrichment 50%

## Intended Use

McClung Toabe Agar Base is used with Egg Yolk Enrichment 50% for isolating and detecting *Clostridium perfringens* in foods based on the lecithinase reaction.

## Summary and Explanation

McClung and Toabe<sup>1</sup> formulated a medium for isolating *C. perfringens* from foods. With the addition of 50% egg yolk emulsion, *C. perfringens* and a few other *Clostridium* species show the lecithinase reaction.

*C. perfringens* is found in raw meats, poultry, dehydrated soups and sauces, raw vegetables and other foods and food ingredients, but occurrences of food borne illness are usually associated with cooked meat or poultry products.<sup>2</sup> Spores of some strains that may resist heat during cooking germinate and grow in foods that are not adequately refrigerated.<sup>3</sup> Enumerating the microorganism in food samples plays a role in epidemiological investigation of outbreaks of food borne illness.<sup>2</sup>

## Principles of the Procedure

McClung Toabe Agar Base contains peptone as a source of carbon, nitrogen, vitamins and minerals. Dextrose is the carbohydrate source. Sodium chloride provides essential ions. Magnesium sulfate provides divalent cations and sulfate.

Disodium phosphate and monopotassium phosphate maintain pH balance and provide a source of phosphates. Agar is the solidifying agent. Egg Yolk Enrichment 50% provides egg yolk lecithin. Lecithinase-producing clostridia, such as *C. perfringens*, hydrolyze the lecithin and produce opaque halos.

## Formulae

### Difco™ McClung Toabe Agar Base

Approximate Formula* Per Liter		
Proteose Peptone .....	40.0	g
Dextrose .....	2.0	g
Disodium Phosphate .....	5.0	g
Monopotassium Phosphate .....	1.0	g
Sodium Chloride .....	2.0	g
Magnesium Sulfate .....	0.1	g
Agar .....	25.0	g

### Difco™ Egg Yolk Enrichment 50%

Sterile concentrated egg yolk emulsion.

\*Adjusted and/or supplemented as required to meet performance criteria.

## Directions for Preparation from Dehydrated Product

### Difco™ McClung Toabe Agar Base

1. Suspend 75 g of the powder in 1 L of purified water. Mix thoroughly.