Letheen Agar • Letheen Broth

Intended Use

Letheen Agar is used to inactivate quarternary ammonium compounds and other preservatives when determining the number of bacteria present in cosmetics and other materials.

Letheen Broth is used for determining the phenol coefficient of cationic surface-active materials.

Summary and Explanation

The value of a highly nutritional solid medium containing neutralizing agents for quaternary ammonium compounds in sanitizers was described by Weber and Black[1] in 1948. The addition of lecithin and polysorbate 80 (Tween™* 80) to Tryptone Glucose Extract (TGE) agar resulted in a medium that effectively neutralizes quaternary ammonium compounds in the testing of germicidal activity. Letheen Agar is a modification of TGE agar with the addition of lecithin and polysorbate 80.

*Tween is a trademark of ICI Americas, Inc.

Letheen Broth was developed as a subculture medium for the neutralization of quaternary ammonium compounds in disinfectant testing. Quisno, Gibby and Foter[2] found that the addition of lecithin and polysorbate 80 to F.D.A. Broth resulted in a medium that neutralized high concentrations of quaternary ammonium salts. The resulting medium, termed "Letheen" (a combination of Lecithin and Tween), was easy to prepare and clear in appearance, which aided in visual inspection for growth. Letheen Broth is recommended in the *Official Methods of Analysis of AOAC International*[3] for use with disinfectants containing cationic surface active materials.

Letheen Agar and Letheen Broth are specified for use by the American Society for Testing and Materials (ASTM) in the Standard Test Method for Preservatives in Water-Containing Cosmetics.[4]

User Quality Control

Identity Specifications

Difco™ Letheen Agar

Dehydrated Appearance:	Tan, moist appearance, with a few clumps.
Solution:	3.2% solution, soluble in purified water upon boiling. Solution is light to medium amber, clear to slightly opalescent, may have a slight, fine precipitate (opalescent immediately after autoclaving).
Prepared Appearance:	Light to medium amber, slightly opalescent, may have a slight precipitate.
Reaction of 3.2% Solution at 25°C:	pH 7.0 ± 0.2

Difco™ Letheen Broth

Dehydrated Appearance:	Tan, appears moist, with a tendency to clump.
Solution:	2.57% solution, soluble in purified water upon boiling. Solution is light amber, clear to slightly opalescent (opalescent when hot). May have a very slight precipitate.
Prepared Appearance:	Light to medium amber, clear to slightly opalescent, may have a slight precipitate.
Reaction of 2.57% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ Letheen Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	11229	10^2-10^3	Good
Staphylococcus aureus	6538	10^2-10^3	Good

Difco™ Letheen Broth

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ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	11229	10^2-10^3	Good
Salmonella enterica subsp. *enterica* serotype Typhi	6539	10^2-10^3	Good
Staphylococcus aureus	6538	10^2-10^3	Good

Principles of the Procedure

Letheen Agar contains beef extract and peptone which provide the carbon and nitrogen sources required for growth of a wide variety of organisms. Dextrose is provided as a source of fermentable carbohydrate. Agar is the solidifying agent. Lecithin and polysorbate 80 are added to neutralize surface disinfectants.[2,5,6] Lecithin is added to neutralize quaternary ammonium compounds and polysorbate 80 is incorporated to neutralize phenols, hexachlorophene, formalin and, with lecithin, ethanol.[7]

Letheen Broth contains peptone and beef extract which provide the carbon and nitrogen sources necessary for growth. Lecithin and polysorbate 80 are added as surface active disinfectant neutralizing agents.[2,5,6] Sodium chloride is included to maintain osmotic balance.

Formulae

Difco™ Letheen Agar

Approximate Formula* Per Liter

Beef Extract	3.0	g
Pancreatic Digest of Casein	5.0	g
Dextrose	1.0	g
Agar	15.0	g
Polysorbate 80	7.0	g
Lecithin	1.0	g

Difco™ Letheen Broth

Approximate Formula* Per Liter

Beef Extract	5.0	g
Proteose Peptone No. 3	10.0	g
Polysorbate 80	5.0	g
Lecithin	0.7	g
Sodium Chloride	5.0	g

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

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ Letheen Agar – 32 g;
   Difco™ Letheen Broth – 25.7 g.
   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. Test samples of the finished product for performance using stable, typical control cultures.

NOTE: The dehydrated Letheen Agar has a characteristic "brown sugar" appearance and may seem moist. This does not indicate deterioration.

Procedure

Letheen Agar and Letheen Broth are used in a variety of procedures. Consult appropriate references for further information.[3,4]

Expected Results

Refer to appropriate references and procedures for results.[3,4]

References

1. Weber and Black. 1948. Soap and Sanit. Chem. *24*:134.
2. Quisno, Gibby and Foter. 1946. Am. J. Pharm. *118*:320.
3. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
4. American Society for Testing and Materials. 1991. Standard test method for preservatives in water-containing cosmetics, E 640-78. Annual Book of ASTM Standards. ASTM, Philadelphia, Pa.
5. Erlandson and Lawrence. 1953. Science *118*:274.
6. Brummer. 1976. Appl. Environ. Microbiol. *32*:80.
7. Favero (chm.). 1967. Microbiological sampling of surfaces-a state of the art report. Biological Contamination Control Committee, American Association for Contamination Control.

Availability

Difco™ Letheen Agar

Cat. No. 268010 Dehydrated – 500 g*

Difco™ Letheen Broth

AOAC

Cat. No. 268110 Dehydrated – 500 g*

Europe

Cat. No. 257325 Prepared Bottles, 1000 mL – Pkg. of 4

**Store at 2-8°C.*