BD Modified Letheen Broth •
BD Modified Letheen Broth with 5% Polysorbate 80

INTENDED USE

BD Modified Letheen Broth is used for microbiological testing of cosmetics. **BD Modified Letheen Broth with 5% Polysorbate 80** is used for testing materials with a high lipid or oil content.

These products may be available in different containers, volumes, and packaging units. This document applies to all of them.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Letheen Broth was developed as a subculture medium for the neutralization of quaternary ammonium compounds in disinfectant testing. Quisno, Gibby and Foter found that the addition of lecithin and Tween 80 to F.D.A. Broth resulted in a medium that neutralized high concentrations of quaternary ammonium salts.[2] 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 by the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC) for use with disinfectants containing cationic surface active materials.[3] Letheen Broth is specified for use by the American Society for Testing Materials (ASTM) in the Standard Test Method for Preservatives in Water Containing Cosmetics.[4]

Modified Letheen Broth is based on Letheen Broth, Modified as described in the 7th edition of the U.S. FDA Bacteriological Analytical Manual.[5] Letheen Broth, Modified is recommended by the FDA for use in the microbiological testing of cosmetics.[6]

BD Modified Letheen Broth consists of Letheen Broth which contains Peptamin and Beef Extract which provide the carbon and nitrogen sources necessary for growth supplemented with peptones, yeast extract, and bisulfite for improved growth and for neutralization of the preservatives included in cosmetics.[6-9] Sodium Chloride is included to maintain osmotic balance.

BD Modified Letheen Broth with 5% Polysorbate 80 contains an increased concentration of polysorbate 80 to dissolve or emulsify materials with a high oil or lipid content.

REAGENTS

Approximate Formulas* Per Liter Purified Water

BD Modified Letheen Broth	
Letheen Broth	25.7 g
Tryptone	5.0
Proteose Peptone No. 3	10.0
Yeast Extract	2.0
Sodium Bisulfite	0.1
pH 7.2 +/- 0.2	

BD Modified Letheen Broth with 5% Polysorbate 80	
Letheen Broth	25.7 g
Tryptone	5.0
Proteose Peptone No. 3	10.0
Yeast Extract	2.0
Sodium Bisulfite	0.1
Polysorbate 80	50,0
pH 7.2 +/- 0.2	

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

Sterility Information

The products mentioned in this document are sterilized by autoclaving in their final containers. For many of these products, a sterility claim is available on the Certificate of Analysis (http://regdocs.bd.com or http://www.bd.com/europe/regulatory/).

PRECAUTIONS
For Laboratory Use Only.
Do not use containers if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

STORAGE AND SHELF LIFE
On receipt, store containers in the dark at 5 to 25° C, until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date and incubated for the recommended incubation times.

USER QUALITY CONTROL
Inoculate samples of the medium with the strains mentioned below. An inoculum of 10 to 100 CFU should be used per 100 ml of medium. Incubate as indicated in the footnote. Good to excellent growth is indicated by heavy turbidity. In case of doubt, subculture 10 to 50 µl onto **Trypticase**™ Soy Agar plates.

Risk of secondary contamination: Before inoculation, the outer surface of the bottles, especially the lid, cap, and/or stopper should be disinfected using a sporocidal disinfectant . Twist-off screw caps of Wide Mouth jars must be opened in a Laminar Airflow cabinet. Wear gloves that have been disinfected before opening the lid!

Test Strain	Expected Results
Staphylococcus aureus ATCC 6538	Growth good to excellent
Bacillus subtilis ATCC 6633	Growth good to excellent
Pseudomonas aeruginosa ATCC 9027	Growth good to excellent
Escherichia coli ATCC 8739	Growth good to excellent
Salmonella Abony DSM 4224	Growth good to excellent
* *Candida albicans* ATCC 10231	Growth good to excellent
* *Aspergillus niger* ATCC 16404	Growth good to excellent

Incubation: 18-24h, 36°C ± 1°C aerob ; * 1-5d, 22,5°C ± 2,5°C aerob

Appearance:
BD Modified Letheen Broth and **BD Modified Letheen Broth with 5% Polysorbate 80**: Medium to amber, slightly opalecent, may have a fine precipitate.

PROCEDURE
Materials Provided
BD Modified Letheen Broth or **BD Modified Letheen Broth with 5% Polysorbate 80** (ready-to use bottled media)

Materials Not Provided
Ancillary culture media, reagents and laboratory equipment as required.

Test Procedure
BD Modified Letheen Broth and **BD Modified Letheen Broth with 5% Polysorbate 80** are used in a variety of procedures. Consult the references for further information.

Risk of secondary contamination: Before inoculation, the outer surface of the bottles, especially the lid, cap, and/or stopper should be disinfected using a sporocidal disinfectant . Twist-off screw caps of Wide Mouth jars must be opened in a Laminar Airflow cabinet. Wear gloves that have been disinfected before opening the lid!

Results
Growth of microorganisms is shown by turbidity. Subculture to solid media is necessary for confirmation and further identification. Refer to appropriate references and procedures.[3-5,9]

REFERENCES

1. Weber, G. R., and L. A. Black. 1948. Relative efficiency of quaternary inhibitors. Soap and Sanit. Chem. 24:134-139.
2. Quisno, R., I. W. Gibby, and M. J. Foter. 1946. A neutralizing medium for evaluating the germicidal potency of the quaternary ammonium salts. Am. J. Pharm. 118:320-323.
3. Association of Official Analytical Chemists. 1995. Official methods of analysis, 16th ed. Association of Official Analytical Chemists, Washington, D.C.
4. American Society for Testing Materials. 1991. Standard test method for preservatives in water-containing cosmetics, E 640-78. Annual Book of ASTM Standards, Philadelphia, PA.
5. Tomlinson, L. (ed.). 1992. FDA Bacteriological Analytical Manual, 7th ed. AOAC International, Arlington, VA.
6. Hitchins, A. D, T. T. Tran, and J. E. McCarron. 1992. *In* L.A. Tomlinson (ed.), FDA Bacteriological Analytical Manual, 7th Ed. AOAC International, Arlington, VA.
7. Erlandson, A. L., Jr., and C. A. Lawrence. 1953. Inactivating medium for hexachlorophene (G-11) types of compounds and some substituted phenolic disinfectants. Science 118:274-276.
8. Brummer, B. 1976. Influence of possible disinfectant transfer on *Staphylococcus aureus* plate counts after contact sampling. Appl. Environ. Microbiol. 32:80-84.
9. Favero (chm.). 1967. Microbiological sampling of surfaces-a state of the art report. Biological Contamination Control Committee, American Association for Contamination Control.

PACKAGING/AVAILABILITY

For container types, fill volumes, package sizes, and for availability of these products, please contact your local BD representative.

FURTHER INFORMATION

For further information please contact your local BD representative.

Becton Dickinson GmbH
Tullastrasse 8 – 12
D-69126 Heidelberg/Germany
Phone: +49-62 21-30 50 Fax: +49-62 21-30 52 16
Reception_Germany@europe.bd.com

http://www.bd.com
http://www.bd.com/europe/regulatory/

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