



Letheen Broth, Modified(Modified Letheen Broth)

M976

Intended Use:

Recommended for screening cosmetic products for microbial contamination

Composition**

Ingredients	Gms / Litre
Peptone	20.000
Tryptone	5.000
HM peptone B#	5.000
Yeast extract	2.000
Sodium chloride	5.000
Sodium bisulphite	0.100
Lecithin	0.700
Polysorbate 80	5.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

Suspend 42.8 grams in 1000 ml purified / distilled water. Heat if necessary to ensure complete dissolution. Dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C.

Principle And Interpretation

In the early 40s, Weber and Black recommended the use of lecithin and polysorbates to neutralize the antimicrobial action of the quaternary ammonium compounds (7). In 1965, the methodology was accepted by AOAC for the antimicrobial assays and extended their use to all the cationic detergents. In 1978, the FDA incorporated it as pre-enrichment medium for every microbial examination of cosmetics.

Letheen Broth, Modified is prepared as per FDA (1) for screening cosmetic products for microbial contamination. There are great chances of altering the chemical composition of cosmetics by the metabolism of organisms thereby spoiling and causing harm to the users (2,6,8). Direct colony counts and enrichment culturing are the methods of choice for isolating microorganisms from cosmetic products. The word Letheen represents a combination of lecithin and polysorbate (tween) 80. Peptone, tryptone, HM peptone B and yeast extract provide nitrogenous nutrients, carbon compounds, long chain amino acids and trace elements to the microorganisms. Incorporation of lecithin and polysorbate 80 to the medium enables the recovery of bacteria from materials containing residues of disinfectant compounds or preservatives used in cosmetics. Polysorbate 80 is added to nullify phenolic compounds, hexachlorophene, formalin and along with lecithin neutralizes ethyl alcohol (3). Lecithin also neutralizes quaternary ammonium compounds present in the cosmetics. Sodium chloride maintains the osmotic balance of the medium.

Enrichment in this medium should be done for 7 days at 30-32°C and then subcultured on Letheen Agar, Modified (M946) and/or MacConkey Agar (M081).

Type of specimen

Cosmetic samples.

Specimen Collection and Handling

For cosmetics samples follow appropriate techniques for handling specimens as per established guidelines (4,5).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Further biochemical and serological test must be carried out for further identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Yellow coloured, clear solution in tubes

Reaction

Reaction of 4.28% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	luxuriant
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	50-100	luxuriant
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 6538 (00032*)	50-100	good-luxuriant

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use.

Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

1. Bacteriological Analytical Manual, 1995, Food and Drug Administration, 8th Ed., AOAC International, Gaithersburg, MD, U.S.A.
2. Dunningan A. P., 1968, Drug Cosmet. Ind., 102:43.
3. Favero (Chm.), 1967, A State of the Art Report, Biological Contamination Control Committee, American Association for Contamination Control.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
6. Smart R. and Spooner D. F., 1972, J. Soc. Cosmet. Chem., 23:721.
7. Weber and Black, 1948, Soap Sanitary Chem., 24:134-139
8. Wilson L. A. and Ahearn D. G., 1977, Am. J. Ophthalmol., 84:112.

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