

Letheen Broth, Modified**M976**

This medium is recommended for screening cosmetic products for microbial contamination.

Composition***

Ingredients	Gms / Litre
Peptic digest of animal tissue	20.000
Casein enzymic hydrolysate	5.000
Beef extract	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

Directions

Suspend 42.8 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and dispense as desired.

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 (5). 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, 3, 4). 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.

Peptic digest of animal tissue, casein enzymic hydrolysate, beef extract and yeast extract provide nitrogenous nutrients, carbon compounds 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 (6). 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).

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

Cultural Response

M976: Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth				
<i>Escherichia coli</i> ATCC 25922	50-100	luxuriant				
<i>Staphylococcus aureus</i> ATCC 25923	50-100	luxuriant				

<i>Staphylococcus aureus</i> ATCC 6538	50-100	good- luxuriant				
---	--------	--------------------	--	--	--	--

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. Smart R. and Spooner D. F., 1972, J. Soc. Cosmet. Chem., 23:721.
4. Wilson L. A. and Ahearn D. G., 1977, Am. J. Ophthalmol., 84:112.
5. Weber and Black, 1948, Soap Sanitary Chem., 24:134-139
6. Favero (Chm.), 1967, A State of the Art Report, Biological Contamination Control Committee, American Association for Contamination Control.

Storage and Shelf Life

Store below 30°C and prepared medium at 2-8°C. Use before expiry period on the label.