



Lethen Agar, Modified

M946

Intended Use

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

Composition**

Ingredients	Gms / Litre
Peptone	10.000
Tryptone	10.000
HM Peptone B #	3.000
Yeast extract	2.000
Sodium chloride	5.000
Lecithin	1.000
Polysorbate 80	7.000
Dextrose (Glucose)	1.000
Sodium bisulphite	0.100
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

Suspend 54.1 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. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

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 (3). 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. Lethen Agar, Modified is used to partially inactivate the preservatives in cosmetics being analyzed for the microbial content (1). This medium was originally recommended by APHA for use in microbial testing of water (2).

Peptone, tryptone, HM Peptone B and yeast extract provide nitrogenous and carbonaceous compounds, long chain amino acids, vitamins 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 (4). Lecithin also neutralizes quaternary ammonium compounds present in the cosmetics. Sodium chloride maintains the osmotic balance of the medium.

Type of specimen

Cosmetic products

Specimen Collection and Handling

For cosmetic samples follow appropriate techniques for handling specimens as per established guidelines (3,4).

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 clinical specimens. Safety guidelines may be referred in individual safety data sheets

Limitations :

1. Further biochemical tests must be carried out for further confirmation.

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Yellow coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.4% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours .

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery
Cultural Response			
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	luxuriant	≥70%
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	50-100	luxuriant	≥70%
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 6538 (00032*)	50-100	good-luxuriant	≥70%

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°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. Use before expiry date on the label.

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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (7,8).

Reference

1. Madden J. M. and Dallas W. S., 1984, Bacteriological Analytical Manual, 6th Ed., AOAC, Arlington, Va.
2. APHA, 1960, Standard Methods for the Examination of Water and Wastewater, 11th Ed., American Public Health Association, New York.
3. Weber and Black, 1948, Soap Sanitary Chem., 24:134-139.
4. Dunningan A. P., 1968, Drug Cosmet. Ind., 102:43.
5. Smart R. and Spooner D. F., 1972, J. Soc. Cosmet. Chem., 23:721.
5. Wilson L. A. and Ahearn D. G., 1977, Am. J. Ophthalmol., 84:112.
6. Favero (Chm.), 1967, A State of the Art Report, Biological Contamination Control Committee, American Association for Contamination Control.
7. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
8. 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.

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