

Lethen Broth, Modified

LQ131D

Intended use

Recommended for determination of bacterial activity of quaternary ammonium compounds using *Escherichia coli* or *Staphylococcus aureus*.

Composition**

Ingredients	g / L
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

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

Label the ready to use LQ131D bottle. Inoculate the sample and incubate at specified temperature and time.

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 (1). 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 Broth, Modified is prepared as per FDA (2) 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 (3,4,5). Direct colony counts and enrichment culturing are the methods of choice for isolating microorganisms from cosmetic products. The word Lethen 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 (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 Lethen 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 (7,8). 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 complete 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

Sterile clear Leethen broth modified in glass bottle.

Colour

Light yellow coloured clear solution

Quantity of Medium

500 ml of medium in glass bottle.

pH

6.80- 7.20

Sterility Check

Passes release criteria

Cultural response

Cultural characteristics was observed after incubation at 35-37°C for 18-48 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 15-30°C. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (7,8).

Reference

1. Weber and Black, 1948, Soap Sanitary Chem., 24:134-139
2. Bacteriological Analytical Manual, 1995, Food and Drug Administration, 8th Ed., AOAC International, Gaithersburg, MD, U.S.A.
3. Dunningan A. P., 1968, Drug Cosmet. Ind., 102:43.
4. 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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