



Lethen Broth, AOAC

M165

Intended Use:

Recommended for determination of bacterial activity of quaternary ammonium compounds using *Escherichia coli* or *Staphylococcus aureus* ATCC 6538.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
HM peptone B #	5.000
Lecithin	0.700
Polysorbate 80 (Tween 80)	5.000
Sodium chloride	5.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef extract

Directions

Suspend 25.7 grams in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Lethen Broth was developed by Quisno, Gibby and Foter (7) by the addition of lecithin and Polysorbate 80 to FDA Broth. In the early 40s, Weber and Black recommended the use of lecithin and polysorbate to neutralize the antimicrobial action of the quaternary ammonium compounds (9). Lethen Broth is recommended by AOAC to determine the phenol coefficient of cationic surfactants (6). Lethen Medium is also recommended for testing of cosmetics (8). The medium is also recommended by APHA for microbial testing of water (1).

HM peptone B, Peptone, supply essential nutrients and other trace elements for the microbial growth. Lecithin and polysorbate 80 enables the recovery of bacteria from solutions containing residues of disinfectant used in sanitization of utensils and equipments. Lecithin neutralizes quaternary ammonium compounds and polysorbate 80 neutralizes phenolic disinfectants, hexachlorophene and formalin (2).

Type of specimen

Environmental samples; Water samples

Specimen Collection and Handling

For environmental samples, follow appropriate techniques for sample collection and processing as per guidelines (5). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (2). 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. This medium is general purpose medium and may not support the growth of fastidious organisms.
2. Some organism may show poor growth due to nutritional variations.

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

Light yellow coloured clear solution in tubes

Reaction

Reaction of 2.57% 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	good-luxuriant
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 6538 (00032*)	50-100	good-luxuriant
<i>Escherichia coli</i> ATCC 8739 (00012*)	50-100	Good-luxuriant
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	50-100	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 (3,4).

Reference

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- Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
- 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.
- MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
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- Weber and Black, 1948, Am. J. Public Health, 38:1405.

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