LETHEEN BROTH AOAC

Dehydrated culture medium

1 - INTENDED USE

Liquid medium for determining the phenol coefficient of cationic surface-active materials.

2 - COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Beef extract	5.0 g
Peptone	10.0 g
Sodium chloride	5.0 g
Lecithin	0.7 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Quisno, Gibby and Foter[1] found that the addition of lecithin and polysorbate 80 to a liquid medium resulted in a neutralisation of high concentrations of quaternary ammonium salts. This new medium was called "letheen broth", an expression of the contracted name of the main components: lecithin and tween. The medium was originally developed as a subculture broth for the neutralization of quaternary ammonium salts. Letheen Broth AOAC is used for determining the phenol coefficient of cationic surface-active materials[2] according to AOAC procedures.[3] It is recommended also for recovering bacteria from the solutions containing residues of sanitizers from food utensils and equipment.

Phenol coefficient is the measure of the disinfecting power of a substance, determined by dividing the figure indicating the degree of dilution of the disinfectant that kills a microorganism within a given time by that indicating the degree of dilution of phenol killing the microorganism under similar conditions.

Letheen Broth contains beef extract and a meat peptone which provide nitrogen, carbon, minerals and amino acids for the microbial growth. Sodium chloride is a source of electrolytes and maintains the osmotic equilibrium. Lecithin and polysorbate 80 neutralize quaternary ammonium compounds, phenols, hexachlorophene, formalin.

4 – DIRECTIONS FOR MEDIUM PREPARATION

Suspend 20.7 g in 1000 ml of cold purified water, add 5 g of polysorbate 80 (Tween® 80 REF 42120502), heat to boiling with frequent agitation to completely dissolve the powder. Distribute and sterilize by autoclaving at 121°C for 15 minutes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	pale yellow, limpid
Final pH at 20-25 °C	7.0 ± 0.2

6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Letheen Broth AOAC	Dehydrated medium	4015912	500 g (24.2 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops, swabs and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, Tween® 80 (REF 42120502) ancillary culture media and reagents.

8 - SPECIMENS

Disinfectants and products containing disinfectants. Refer to applicable International Standards and regulations and operate in accordance with good laboratory practice for sample collection, storage and transport to the laboratory.

9 - TEST PROCEDURE, READING AND INTERPRETATION

Consult the current edition of the appropriate references[3,4] for the recommended procedure for sample preparation, inoculation, analysis and interpretation of results.

10 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. aureus ATCC 25923	35-37°C / 18-24H / A	good growth
P. aeruginosa ATCC 27853	35-37°C / 18-24H / A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

11 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated Letheen Broth AOAC supplemented with 5 g/L of polysorbate 80 (Test Batch: TB), is tested for productivity and neutralising properties of a quaternary ammonium base by comparing the results with a previously approved Reference Batch (RB) and with a general-purpose medium without neutralising properties (Tryptic Soy Broth).

The above characteristics are tested with the following strains: *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923. After incubation at 37°C for 24 hours, good growth of the tested organisms is obtained with Letheen Broth AOAC supplemented with quaternary ammonium base, whereas growth is totally or partially inhibited in Tryptic Soy Broth supplemented in the same way.

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12 – LIMITATIONS OF THE METHOD

The effectiveness of preservative neutralization with this medium depends on both the type and concentration of the preservative(s).

13 - PRECAUTIONS AND WARNINGS

- This product is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the preparation process of tubed or bottled media.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).
The user is responsible for the manufacturing and quality control processes of prepared media and for the validation of the period of validity of the finished products, according to the type (tubes/bottles), and the storage method applied (temperature and packaging). According to MacFaddin the self-prepared medium may be stored at 2-8°C for 4 weeks.[2]

15 - REFERENCES

1. Quisno R, Gibby IW, Foter MJ. A neutralizing medium for evaluating the germicidal potency of the quaternary ammonium salts. Am J Pharm Sci Support Public Health 1946; 118:320-323
2. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985
3. AOAC International. Official Methods of Analysis 21st Edition; 2019.
4. US Environmental Protection Agency Office of Pesticide Programs. Standard Operating Procedure for AOAC Use Dilution Method for Testing Disinfectants. Online Revision 01-17-20.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	Store in a dry place	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/08

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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