LETHEEN AGAR BASE, MODIFIED (7495)

Intended Use

Letheen Agar Base, Modified is used with Polysorbate 80 for the isolation of microorganisms from cosmetics.

Product Summary and Explanation

In 1948, Weber and Black described the value of a highly nutritional solid medium containing neutralizing agents for quaternary ammonium compounds in sanitizers.[1] The addition of Lecithin and Polysorbate 80 to Tryptone Glucose Extract (TGE) Agar resulted in a medium that effectively neutralizes quaternary ammonium compounds in testing of germicidal activity. Total neutralization of disinfectants is critical. Disinfectant residues can result in a false negative (no-growth) test.

Letheen Agar Base, Modified is based on the formula described in FDA Bacteriological Analytical Manual, and a modification of Letheen Agar Base.[2] Letheen Agar Base, Modified is recommended by the FDA for use in the microbiological testing of cosmetics.[3]

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide nitrogen and carbon required for good growth of a wide variety of bacteria and fungi. The nitrogen level was increased in Letheen Agar Base, Modified to provide better growth. Yeast Extract provides vitamins and cofactors in this medium. Sodium Chloride maintains the osmotic balance. Sodium Bisulfite, Polysorbate 80, and Lecithin (present in Letheen Agar Base) neutralizes quaternary ammonium compounds. Polysorbate 80 neutralizes phenols, hexachlorophene, formalin, and with Lecithin, ethanol.[4-7] These preservatives are commonly used in the cosmetic industry. Agar is the solidifying agent.

Formula / Liter	
Letheen Agar Base	25 g
Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue	10 g
Yeast Extract	2 g
Sodium Chloride	5 g
Sodium Bisulfite	0.1 g
Agar	15 g

Supplement / Liter
Polysorbate 80, 7 g

Final pH: 7.2 ± 0.2 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. HARMFUL. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 52.1 g of the medium and 7 g of Polysorbate 80 in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is light to medium yellow and trace to moderately hazy.

Expected Cultural Response: Cultural response on Letheen Agar Base, Modified at 35°C after 18 - 24 hours incubation.

Microorganism	Response
Enterococcus faecalis ATCC® 29212	growth
Escherichia coli ATCC® 25922	growth
Pseudomonas aeruginosa ATCC® 27853	growth
Salmonella typhimurium ATCC® 14028	growth
Staphylococcus aureus ATCC® 25923	growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure[3]

1. Prepare and dilute samples in Letheen Broth Base, Modified in accordance with established guidelines.
2. Using the spread plate technique, inoculate in duplicate 0.1 mL of the diluted samples onto Letheen Agar Base, Modified, Potato Dextrose Agar containing Chlortetracycline, Baird Parker Agar, Anaerobic Agar, and a second set of Letheen Agar Base, Modified plates.
3. Incubate one set of Letheen Agar Base, Modified at 30°C for 48 hours and the other set at 35°C under anaerobic conditions for 2 - 4 days. Incubate the Potato Dextrose Agar plates at 30°C for 7 days and the Baird Parker Agar plates, if inoculated, at 35°C for 48 hours.
4. Incubate diluted samples from Step 1 at 35°C for 7 days. Subculture enriched samples onto Letheen Agar Base, Modified only if there is no growth on the primary Letheen Agar Base, Modified.

Results

Examine plates for evidence of growth and characteristic colonial morphology. Determine colony counts and subculture each colony type onto Letheen Agar Base, Modified and MacConkey Agar (also Baird Parker if used in Step 2).

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.

Packaging

Letheen Agar Base, Modified	**Code No.**	**7495A**	**500 g**
		7495B	**2 kg**
		7495C	**10 kg**

References

1. **Weber, G. R., and L. A. Black.** 1948. Relative efficiency of quaternary inhibitors. Soap and Sanit. Chem. **24:**134-139.
2. **Tomlinson, L. (ed.).** 1992. FDA Bacteriological Analytical Manual, 7th ed. AOAC International, Arlington, VA.
3. **Hitchins, A. D., T. T. Tran, and J. E. McCarron.** 1992. *In* Tomlinson, L. A. (ed.). FDA Bacteriological Analytical Manual, 7th ed. AOAC International, Arlington, VA.
4. **Quisno, R., I. W. Gibby, and M. J. Foter.** 1946. A neutralizing medium for evaluating the germicidal potency of the quaternary ammonium salts. Am. J. Pharm. **118:**320-323.
5. **Erlandson, A. L., Jr., and C. A. Lawrence.** 1953. Inactivating medium for hexachlorophene (G-11) types of compounds and some substituted phenolic disinfectants. Science. **118:**274-276.
6. **Brummer, B.** 1976. Influence of possible disinfectant transfer on *Staphylococcus aureus* plate counts after contact sampling. Appl. Environ. Microbiol. **32:**80-84.
7. **Favero (chm.).** 1967. Microbiological sampling of surfaces-a state of the art report. Biological Contamination Control Committee, American Association for Contamination Control.

Technical Information

Contact Acumedia Manufacturers, Inc. at TEL (800)783-3213 in the US/Canada or (410)780-5120 and FAX (800)875-8563 in the US/Canada or (410)780-5470 for Technical Service on questions involving dehydrated culture media preparation or performance.