Letheen Agar Base Modified without Tween (NCM0130)

Intended Use
Letheen Agar Base Modified (without Tween) is used with Tween 80 (Polysorbate 80) for the isolation of microorganisms from cosmetics in a laboratory setting. Letheen Agar Base Modified is not intended for use in the diagnosis of disease or other conditions in humans.

Description
In 1948, Weber and Black described the value of a highly nutritional solid medium containing neutralizing agents for quaternary ammonium compounds in sanitizers. The addition of Lecithin and Tween 80 (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. Letheen Agar Base Modified is recommended by the FDA for use in the microbiological testing of cosmetics.

Typical Formulation

Letheen Agar Base	25.0 g/L
Enzymatic Digest of Casein	10.0 g/L
Enzymatic Digest of Animal Tissue	10.0 g/L
Yeast Extract	2.0 g/L
Sodium Chloride	5.0 g/L
Sodium Bisulfite	0.1 g/L

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

Supplement / Liter (7992)
Tween 80 (Polysorbate 80), 7 mL
Note: Equivalence for Tween 80 in grams to mL is @ 1:1

Precaution
Refer to SDS

Preparation
1. Suspend 52.1 g of the medium and 7 mL of Tween 80 (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.
4. Cool to 45-50°C.

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 incubated aerobically at 35 ± 2°C and examined for growth after 18 - 24 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results
Enterococcus faecalis ATCC® 29212	10 - 300	Growth
Escherichia coli ATCC® 25922	10 - 300	Growth
Pseudomonas aeruginosa ATCC® 27853	10 - 300	Growth
Salmonella typhimurium ATCC® 14028	10 - 300	Growth
Staphylococcus aureus ATCC® 25923	10 - 300	Growth

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

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Test Procedure

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).

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if it is not free flowing, or if medium 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.

Storage

Store dehydrated culture media at 2-30°C away from direct sunlight. 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.

References

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