

## Lethen Broth with Tween, Modified (NCM0104)

### Intended Use

Lethen Broth with Tween, Modified is used for the recovery of microorganisms from cosmetics in a laboratory setting and 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 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 as disinfectant residues can result in a false negative (no-growth) test result.

Lethen Broth with Tween, Modified is based on the formula described in FDA Bacteriological Analytical Manual, and a modification of Lethen Broth Base. This medium is recommended by the FDA for use in the microbiological testing of cosmetics. Lethen Broth with Tween, Modified has Polysorbate 80 (Tween) incorporated into the medium.

### Typical Formulation

Lethen Broth Base	20.7 g/L
Enzymatic Digest of Casein	5.0 g/L
Enzymatic Digest of Animal Tissue	10.0 g/L
Yeast Extract	2.0 g/L
Sodium Bisulfite	0.1 g/L
Polysorbate 80	5.0 g/L

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

### Precaution

Refer to SDS

### Preparation

1. Dissolve 42.8 g of the medium in one liter of purified water.
2. Heat with frequent agitation to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

### Quality Control Specifications

**Dehydrated Appearance:** Powder is homogeneous, lumpy, and tan.

**Prepared Appearance:** Prepared medium is light to medium amber, and clear to moderate haze.

**Expected Cultural Response:** Cultural response in Lethen Broth with Tween, Modified incubated aerobically at 35 ± 2°C and examined for growth after 18 - 24 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results
<i>Enterococcus faecalis</i> ATCC® 29212	10 - 300	Growth
<i>Escherichia coli</i> ATCC® 25922	10 - 300	Growth
<i>Pseudomonas aeruginosa</i> ATCC® 27853	10 - 300	Growth
<i>Staphylococcus aureus</i> ATCC® 25923	10 - 300	Growth

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

## Test Procedure

1. Prepare and dilute samples in Lethen Broth with Tween, Modified in accordance with established guidelines. Proceed with procedure using Lethen Agar Base Modified without Tween, Product No. NCM0130.
2. Using the spread plate technique, inoculate in duplicate 0.1 mL of the diluted samples onto Lethen Agar Base, Modified, Potato Dextrose Agar containing Chlortetracycline, Baird Parker Agar, Anaerobic Agar, and a second set of Lethen Agar Base, Modified without Tween plates.
3. Incubate one set of Lethen 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 Lethen Agar Base, Modified only if there is no growth on the primary Lethen Agar Base, Modified.

## Results

Refer to appropriate references for results, or in Lethen Agar Base, Modified NCM0130, Product Information Sheet.

## 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.

## Limitation of the Procedure

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

## Storage

Store sealed bottle containing the dehydrated medium at 2 - 8°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.

## References

1. Weber, G. R., and L. A. Black. 1948. Relative efficiency of quaternary inhibitors. Soap and Sanit. Chem. 24:134-139.
2. [www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalytical manualBAM/default.htm](http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalytical manualBAM/default.htm).
3. 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.
4. 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.
5. Brummer, B. 1976. Influence of possible disinfectant transfer on *Staphylococcus aureus* plate counts after contact sampling. Appl. Environ. Microbiol. 32:80-84.
6. Favero (chm.). 1967. Microbiological sampling of surfaces-a state of the art report. Biological Contamination Control Committee, American Association for Contamination Control.