Lauryl Sulphate Broth (Lauryl Tryptose Broth)

M080

Intended use

Recommended for the detection of coliforms in water, wastewater, dairy products and other food samples.

Composition**

Ingredients	Gms / Litre
Tryptose	20.000
Lactose	5.000
Sodium chloride	5.000
Dipotassium hydrogen phosphate	2.750
Potassium dihydrogen phosphate	2.750
Sodium lauryl sulphate (SLS)	0.100
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 35.6 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Distribute into tubes containing inverted Durhams tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. For inoculum of 1 ml or less, use single strength medium. For inocula of 10 ml or more, double strength or proportionate medium should be prepared.

Principle And Interpretation

Coliforms are considered to be members of *Enterobacteriaceae*, which grow in the presence of bile salts and produce acid and gas from lactose within 48 hours at 37°C (1). These bacteria can also be defined as, members of *Enterobacteriaceae* capable of growing at 37°C, that normally possess β-galactosidase (2). Lauryl Sulphate Broth is used for the detection of coliforms in water, dairy products and other foods, as recommended by APHA (3, 4, 5). It can also be used for the presumptive detection of coliforms in water, effluent or sewage by the MPN test (3). Lauryl Sulphate Broth was developed by Mallmann and Darby (6). Cowls (7) demonstrated that inclusion of sodium lauryl sulphate makes the medium selective for coliform bacteria. It was later investigated that Lauryl Sulphate Broth gave a higher colon index than the confirmatory standard methods media and also that gas production in Lauryl Sulphate Broth not only acts as a presumptive test but also as a confirmatory test for the presence of coliforms, in the routine testing of water (6). Lauryl Sulphate Broth is also recommended by the ISO Committee for the detection of coliforms (8).

Lauryl Sulphate Broth is designed to obtain rich growth and substantial amount of gas from small inocula of coliform organisms. Aerobic spore-bearers are completely inhibited in this medium. Tryptose provides essential growth substances, such as nitrogen and carbon compounds, sulphate and trace ingredients. The potassium phosphates provide buffering system, while sodium chloride maintains osmotic equilibrium. Sodium lauryl sulphate inhibits organisms other than coliforms.For inoculum of 1 ml or less, use single strength medium. For inocula of 10 ml or more, double strength or proportionate medium should be prepared. After inoculation, incubate the tubes at 37°C for 24 to 48 hours. For every tube showing fermentation (primary fermentation), inoculate two tubes of Lauryl Tryptose Broth from the tube showing primary fermentation and incubate these tubes at 37°C and 44°C respectively. If there is fermentation in the tube incubated at 44°C after 8 to 24 hours, perform indole test by adding Kovacs reagent. A positive indole test in a broth tube showing gas production at 44°C indicates the presence of *Escherichia coli*. If no fermentation occurs in the tube incubated at 37°C after 24 hours, the primary fermentation is assumed to be due to organisms other than coliforms. Broth becomes cloudy if stored at 2-8°C, but it gets cleared at room temperature.Refer appropriate references for standard procedures (3, 4, 5).

Type of specimen

Food and dairy samples ; Water samples

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,2,8). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(3) 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets

Limitations :

1. Due to poor nutritional variations, some strains may show poor growth.

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 without any precipitate

Reaction

Reaction of 3.56% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

M080: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Gas Production	Indole production (44°C)
Cultural Response				
Escherichia coli ATCC 25922 (00013)*	50-100	luxuriant	positive reaction	positive reaction, red ring at the interface of the medium
# Klebsiella aerogenes ATCC 13048 (00175)*	50-100	luxuriant	positive reaction	negative reaction, no colour development / cloudy ring
Enterococcus faecalis ATCC 29212 (00087)*	>=10^3	inhibited		
Salmonella Typhimurium ATCC 14028	50-100	luxuriant	negative reaction	negative reaction, no colour development / cloudy ring
Staphylococcus aureus ATCC 25923	>=10^3	inhibited		

Key : # Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. Department of Environment, Department of Health and Social Security, Public Health Laboratory Service, 1982, Methods for the Examination of Water and Associated Materials, The Bacteriological Examination of Drinking Water Supplies, 1982, Her Majestys Stationary Office, London.
2. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill, Livingstone
3. Eaton A. D., Clesceri L. S., Rice E. W. and Greenberg A. W., (Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st Ed., APHA, Washington, D.C.
4. Marshall R. T., (Ed.), 1992, Standard Methods for the Examination of Dairy Products, APHA, Washington, D.C.
5. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.
6. Mallmann W. C. and Darby C. W., 1941, Am. J. Public Health, 31:127
7. Cowls P. B., 1938, J. Am. Water Works Assoc., 30:979.
8. International Organization for Standardization (ISO), 1991, Draft ISO/DIS 4831.

Revision : 1 / 2011