Lauryl Sulfate Broth (Lauryl Tryptose Broth LTB) ISO

Cat. 1310

For the detection of coliforms in waters

Practical information

Aplications	Categories
Selective enumeration	Coliforms
Selective enumeration	Escherichia coli
Detection	Coliforms
Detection	Escherichia coli

Industry: Food / Dairy products

Regulations: ISO 11133 / ISO 4831 / ISO 7251

Principles and uses

Lauryl Sulfate Broth (Lauryl Tryptose Broth – LTB) is a selective medium recommended for the enumeration of coliforms in water and dairy products as well as for confirmatory tests of lactose fermentation with gas production by coliforms in foods. Another advantage of this medium is that the indole test can be performed directly in the tube.

APHA recommends the use of Lauryl Tryptose Broth for the Most Probable Number Presumptive Test of coliforms in waters, effluent or sewage, also as a confirmatory test of lactose fermentation with gas production for milk samples, and for the detection of coliforms in foods. This broth was elaborated to promote a rich growth and high gas production from small inocula of coliform organisms.

Lauryl Sulfate Broth is recommended by the norm ISO 4831 and ISO 7251 for the detection and enumeration of coliforms and E. coli respectively, by the most probable number technique.

The coliform group is both aerobic and anaerobic, Gram-negative, non spore forming, and ferment lactose producing acid and gas at 35°C within 48 hours.

Tryptose in a 2% concentration improves the early growth phase of coliforms when compared to Casein peptone. The buffered broth allows slow lactose fermenters to increase gas production in a shorter time.

Tryptose provides nitrogen, vitamins, minerals and amino acids essential for growth. Lactose is a fermentable complex carbohydrate energy source. Potassium phosphates are the buffering agents, and Sodium chloride supplies essential electrolytes for transport and osmotic balance. Sodium lauryl sulfate is the selective agent used to inhibit organisms other than coliforms. Sporulating aerobic bacteria are completely inhibited.

Formula in g/L

Lactose	5	Potassium dihydrogen phosphate	2,75
Sodium chloride	5	Sodium lauryl sulfate	0,1
Enzymatic Digest of Plants & Animal Tissue	20	Dipotassium hydrogen phosphate	2,75

Preparation

Suspend 35,6 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes with Durham gas collecting tubes for gas detection. Sterilize in autoclave at 121°C for 15 minutes.

Instructions for use

For the detection and enumeration of coliforms according to ISO 4831:
- Inoculate the tubes of selective enrichment broth (Lauryl Tryptose Broth Cat. 1310) with the test portion.
- For inoculum of 1 ml or less, use single strength LTB medium.
- For inoculum of 10 ml or more, use double strength LTB medium.
- Incubate at 30 ºC or 37 ºC (as agreed) for 24 h (double strength medium) or, for 24 h and another additional 24 h if neither gas formation nor opacity preventing the detection of gas formation is not observed at this stage (single strength medium).
- From the incubated tubes of double strength LTB and incubated tubes showing gas formation or opacity of single strength LTB, inoculate a tube of

confirmation medium (Brilliant Green Bile Broth 2% Cat.1228) and observe after 24 or 48 h.
- A tube with gas formation is a positive tube.
- Count the total number of positive tubes.

For the detection and enumeration of presumptive E. coli according to ISO 7251:
- Inoculate the tubes of selective enrichment broth (Lauryl Tryptose Broth Cat. 1310) with the initial suspension.
- For inoculum of 1 ml or less, use single strength LTB medium.
- For inoculum of 10 ml or more, use double strength LTB medium.
- Incubate the tubes of LTB at 37 ºC for up to 48 h, and examine the gas production after 24 h and 48 h.
- Each tube of double-strength LTB that has given rise to opacity, cloudiness or gaseous emission, and each tube of single-strength LTB that has given rise to gaseous emission, is subcultured to a tube containing EC Broth (Cat. 1522).
- Incubate the tubes of Ec Broth at 44 ºC for up to 48 h, and examine the gas production after 24 h and 48 h.
- Each tube of EC Broth that has given rise to gaseous emission is subcultured to a tube containing Indole-Free Peptone Water (Cat. 1403) and incubated at 44º C for 48 h.
- Tubes showing opacity, cloudiness or gas production in LTB and whose subcultures have produced gas in EC broth and indole in Peptone water, are considered as positive tubes that containing presumptive E coli.
- Count the total E. coli by the MPN method.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Pale beige	Clear amber	6,8 ± 0,2

Microbiological test

According to ISO 11133:

- For detection of coliforms ISO 4831:
Incubation conditions: Productivity, Selectivity (24±2 h to 48±2 h) / 30±1 ºC).
Inoculation conditions: Productivity qualitative (<100 CFU) / Selectivity (10^4-10^6 CFU).

- For detection of E. coli ISO 7251:
Incubation conditions: Productivity, Selectivity (24±2 h to 48±2 h) / 37±1 ºC).
Inoculation conditions: Productivity qualitative (<100 CFU) / Selectivity (10^4-10^6 CFU).

Microorganisms	Specification	Characteristic reaction
Enterococcus faecalis ATCC 19433	No growth	
Escherichia coli ATCC 25922	Turbidity (2) and gas in Durham tube	Gas production and turbidity
Enterococcus faecalis ATCC 29212	No growth	
Citrobacter freundii ATCC 43864	Turbidity (2) and gas in Durham tube	Gas production and turbidity
Escherichia coli ATCC 8739	Turbidity (2) and gas in Durham tube	Gas production and turbidity

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

ISO 4831 Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of coliforms — Most probable number technique
APHA 1998. Standard Methods for the examination of water and wastewater, 20th Edition.
Association of Official Analitical Chemist. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD. Association of Official Analytical Chemists. 1995. Oficial methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
ISO 7251 Microbiology of food and animal feeding stuffs-Horizontal method for the detection and enumeration of presumptive Escherichia coli- Most probable number technique.