

Technical Information

Tryptone Broth (Tryptone Water)

Product Code: DM 1463

Application: Tryptone Broth is used for the detection of indole production by coliforms.

Composition **

Ingredients	Gms / Litre
Casein enzymic hydrolysate	10.000
Sodium chloride	5.000
Final pH (at 25°C)	7.5±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Tryptone Water is recommended by APHA ⁽¹⁾ for detection of indole production by coliforms, which is an important feature in differentiation of bacteria. A slight modification of Tryptone Water (DM1463I) is recommended by ISO committee ⁽²⁾ for the same purpose. This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole which accumulates in the medium ⁽³⁾.

Casein enzymic hydrolysate is a good substrate for indole production because of its high tryptophan content. Certain organisms breakdown the amino acid tryptophan enzymatically that mediate the production of indole by hydrolytic activity ⁽⁴⁾. The indole produced can be detected by either Kovacs or Ehrlich's reagent ⁽⁵⁾. Indole combines with the aldehyde present in the above reagent to give red colour in the alcoholic layer. The alcohol layer extracts and concentrates the red colour complex.

Tryptone Water is used side by side with Brilliant Green Bile Broth 2% (DM1121) to determine the most probable number (MPN) of *E. coli* in food sample. Growth and gas production in DM1121 and indole production in Tryptone Water following incubation of both media at 44 ± 1°C is used as the basis for the presumptive *E. coli* test. For determination of indole, inoculate the medium with inoculum of an 18-24 hours pure culture. Incubate the tubes at 35 ± 2°C for 18-24 hours. Add 0.5 ml of Kovacs indole reagent (025046) directly to the tube and agitate. Allow the tubes to stand for 5-10 minutes. Formation of red ring at the top of the tube indicates indole production.

Indole testing is recommended as an aid in the differentiation of microorganisms based on indole production. For complete identification of the organisms, further biochemical confirmation is necessary.

Methodology

Suspend 15 grams of powder media in 1000 ml distilled water. Shake well & heat if necessary to dissolve the medium completely. Dispense into tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Quality Control

Physical Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Yellow coloured clear solution without any precipitate

Reaction

Reaction of 1.5% w/v aqueous solution at 25°C. pH : 7.5±0.2

pH Range:-

7.30-7.70

Cultural Response/Characteristics

DM 1463: Cultural characteristics observed after incubation at 35-37°C for 18-24 hours. Add 0.2 to 0.3ml of Kovacs' Indole Reagent (025046) to each tube after incubation.



Dehydrated Culture Media
Bases / Media Supplements

Organism	Inoculum (CFU)	Growth	Indole reaction
<i>Escherichia coli</i> ATCC 25922	50-100	luxuriant	positive reaction, red ring at the interface of the medium
<i>Enterobacter aerogenes</i> ATCC 13048	50-100	luxuriant	negative reaction, no colour development / cloudy ring
<i>Klebsiella pneumoniae</i> ATCC 13883	50-100	luxuriant	negative reaction, no colour development / cloudy ring

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.

Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

1. Greenberg A. E., Clesceri L. S. and Eaton A. D., (Eds.), 1998, Standard Methods for the Examination of Water and Wastewater, 20th Ed., APHA, Washington, D.C.
2. International Organization for Standardization (ISO), 1990, Draft ISO/DIS 7251:1993.
3. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone.
4. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Williams and Wilkins, Baltimore. 5. Finegold S. M. and Baron E. J., 1986, Bailey and Scotts Diagnostic Microbiology, 7th Ed., The C.V. Mosby Co., St. Louis

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