



Tryptone Broth (Tryptone Water)

M463

Intended use

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

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 15.0 grams in 1000 ml purified/ distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation Composition**

Tryptone Water is recommended by APHA (1) for detection of indole production by coliforms, which is a key feature in differentiation of bacteria. A slight modification of Tryptone Water (M463I) 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).

Tryptone is a good substrate for indole production because of its high tryptophan content. Certain organisms breakdown the amino acid tryptophan with the help of enzymes that mediate the production of indole by hydrolytic activity (4). The indole produced can be detected by either Kovacs or Ehrlichs 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 in conjunction with Brilliant Green Bile Broth 2% (M121) to determine the most probable number (MPN) of E.coli in food sample. Growth and gas production in M121 and indole production in Tryptone Water following incubation of both media at $44 \pm 1^\circ\text{C}$ 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 $35 \pm 2^\circ\text{C}$ for 18-24 hours. Add 0.5 ml of indole reagent (R008) 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.

Type of specimen

Water samples

Specimen Collection and Handling

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

Limitations :

Other biochemical tests must be carried out for confirmation.

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

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

7.30-7.70

Cultural Response

M463: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours. Add 0.2 to 0.3ml of Kovac's Indole Reagent(R008) to each tube after incubation.

Organism	Inoculum (CFU)	Growth	Indole reaction
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	luxuriant	positive reaction, red ring at the interface of the medium
# <i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	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

Key * -Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Use before expiry date on the label.

Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Reference

1. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd 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
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
7. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S. and Warnock, D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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