



DEV Tryptophan Broth

M1901

Intended Use:

Recommended for subcultivation of coliform, differentiation and for indole testing in the bacteriological examination of water.

Composition**

Ingredients	Gms / Litre
HM peptone #	10.000
Sodium chloride	5.000
DL-Tryptophan	1.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Meat peptone

Directions

Suspend 16 grams in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and dispense into tubes or flasks as desired.

Principle And Interpretation

Tryptone Water is recommended by APHA (5) for detection of indole production by coliforms, which is a key feature in differentiation of bacteria. This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole which accumulates in the medium (2). 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. Certain microorganisms breakdown tryptophan with the help of the enzyme tryptophanase that mediate the production of indole by hydrolytic activity (8). The indole produced can be detected by Kovacs or Ehrlichs reagent (4). Indole combines with the aldehyde present in the above reagent to give red colour in the alcohol layer. The alcohol layer extracts and concentrates the red colour complex.

It contains HM peptone which provides necessary nitrogen, carbon sources, vitamins, growth factors and also trace ingredients to nonfastidious organisms. Sodium chloride maintains osmotic equilibrium of the medium. DL-Tryptophan is an amino acid, which serves as a substrate to study indole reaction.

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 :

1.This medium is general purpose medium and may not support the growth of fastidious organisms.

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 to slightly opalescent solution .

Reaction

Reaction of 1.6% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

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 (00097*)	50-100	luxuriant	negative reaction, no colour development / cloudy ring

Key : (*) Corresponding WDCM numbers.

(#) Formerly known as *Enterobacter aerogenes*

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.

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. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone.
4. Finegold S. M. and Baron E. J., 1986, Bailey and Scotts Diagnostic Microbiology, 7th Ed., The C.V. Mosby Co., St. Louis.
5. 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.

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.
8. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.

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