



## Tryptophan Medium

M1339

Tryptophan Medium is recommended for detection of indole production.

### Composition\*\*

Ingredients	Gms / Litre
Casein enzymic hydrolysate	10.000
Sodium chloride	5.000
DL-Tryptophan	1.000
Final pH ( at 25°C)	7.5±0.2

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 16 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

### Principle And Interpretation

Enterohemorrhagic *Escherichia coli* (EHEC) is a defined subset of Shiga-like (vero) toxin-producing *E. coli*. EHEC infections are waterborne or food borne. EHEC is ingested most commonly with undercooked ground beef (1, 2, 3). There are more than 50 serotypes of EHEC. However, *E. coli* O157:H7 is the prototype EHEC.

*E. coli* O157:H7 can cause an asymptomatic infection, mild diarrhea, or a diarrheal illness that is characterized by non-bloody (progressing to bloody) diarrhea and abdominal cramps (together known as hemorrhagic colitis), few leukocytes in stools and lack of significant fever (1, 2, 4). Tryptophan Medium is prepared as per the formula approved by ISO Committee (5), that is a modification of original formula of APHA where the medium is devoid of tryptophan (6). This medium is useful for the detection of indole production by *Escherichia coli* O157: H7, which is a key feature in differentiation of coliforms.

Casein enzymic hydrolysate provides carbonaceous and nitrogenous sources required for the growth of microorganisms. Tryptophan is an amino acid, which serves as a substrate to study indole reaction. Certain microorganisms breakdown tryptophan with the help of the enzyme tryptophanase that mediate the production of indole by hydrolytic activity (7). The indole produced can be detected by Kovacs or Ehrlichs reagent (8). 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.

The test sample is enriched in Modified Soyabean Bile Broth Base (M1286I) by incubating at 42°C for 18-24 hours. *E. coli* O157:H7 is then isolated on MacConkey Sorbitol Agar Base (M298I). Pale coloured colonies obtained on incubation at 35-37°C for 18-24 hours are reported as presumptive *E. coli* O157:H7. Presumptive colonies are subjected to indole test that makes the use of Tryptophan Medium (M1339).

### 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 1.6% aqueous solution at 25°C. pH : 7.5±0.2

#### pH

7.30-7.70

#### Cultural Response

M1339: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum	Growth	Indole production
----------	----------	--------	-------------------

**Cultural Response**

<i>Enterobacter aerogenes</i> ATCC 13048	50-100	luxuriant	negative reaction, no colour development / cloudy ring
<i>Escherichia coli</i> 0157:H7 NCTC 12900	50-100	luxuriant	positive reaction, red ring at the interface of the medium
<i>Escherichia coli</i> ATCC 25922	50-100	luxuriant	positive reaction, red ring at the interface of the medium

**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**

- Centers for Disease Control and Prevention, 1993, Morbid. Mortal. Weekly Rep. 42: 257:253.
- Griffin P. M. and Tauxe R. V., 1991, Epidemiol. Rev. 13: 60-91
- Kay B. A., Griffin P. M., Strockbine N. A. and Wells J. G., 1994, Clin. Microbiol., Newsletter, 16:17-19.
- Murray P. R., Baron J. H., Pfaller M. A., Tenover J. C. and Tenover F. C., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
- International Organization for Standardisation (ISO) Draft: ISO/DIS 16654:1999.
- 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.
- MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
- Finegold S. M. and Baron E. J., 1986, Bailey and Scotts Diagnostic Microbiology, 7th Ed., The C.V. Mosby Co., St. Louis.

Revision : 1 / 2011

**Disclaimer :**

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.