

# **Tryptophan Medium**

M1339

## **Intended Use:**

Recommended for detection of indole reaction. The composition and performance criteria of this medium are as per specifications laid down in ISO/DIS 16654.

### **Composition\*\***

ISO 16654-2001 Specification-Tryptophan Medium		M1339-Tryptophan Medium	
Ingredients	g / L	Ingredients	g / L
Tryptone	10.000	Tryptone	10.000
Sodium chloride	5.000	Sodium chloride	5.000
DL-Tryptophan	1.000	DL-Tryptophan	1.000
Final pH ( at 25°C)	7.5±0.2	Final pH ( at 25°C)	7.5±0.2

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

### Directions

Suspend 16.0 grams in 1000 ml purified/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

Tryptone 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.

### **Type of specimen**

Clinical and non-clinical samples, Water samples

### **Specimen Collection and Handling:**

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). After use, contaminated materials must be sterilized by autoclaving before discarding.

### Warning and Precautions :

In Vitro diagnostic use. For professional use only. 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. Well isolated colonies must be used for erroneous results.

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

#### pН

7.30-7.70

#### **Cultural Response**

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

Organism	Inoculum	Growth	Indole production negative
#Klebsiella aerogenes ATCC 13048 (00175*)	50-100	luxuriant	reaction, no colour development cloudy
<i>Escherichia coli</i> 0157:H7 NCTC 12900 (00014*)	50-100	luxuriant	ring positive reaction, red ring at the interface of
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	the medium positive reaction, red ring at the interface of the medium
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 15-30°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 (9,10).

#### Reference

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