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

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

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