

09136 Tryptophan Broth (Tryptophan Medium)

Medium to perform the indole test to confirm *E. coli* in water acc. EN ISO 9308-1:2000 and *E. coli* O157 acc. ISO 16654:2001

Composition:

Ingredients	Grams/Litre
Casein enzymic hydrolysate	10.0
Sodium chloride	5.0
DL-Tryptophan	1.0
Final pH 7.5 +/- 0.2 at 25°C	

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C. Use before expiry date on the label.

Appearance: Faintly yellow coloured, homogeneous, free flowing powder.

Color and Clarity: Light yellow clear solution.

Directions:

Dissolve 16g Tryptophan Broth in one liter water by heating. Dispense 3 ml per test tube. Close the tubes with cotton plugs, plastic or metal caps. Autoclave for 15 min at 121 +/- 3°C.

Principle and Interpretation:

Enterohemorrhagic *Escherichia coli* (EHEC) are *E. coli* which can produce Shiga-like (vero) toxin. EHEC infections originate from fecal contamination, then made its way to humans via meat, milk, water or vegetable products. There are several serotypes of EHEC but *E. coli* O157:H7 is the most described strain. EHEC victims displaying symptoms such as bloody diarrhea, abdominal cramping, vomiting and renal failure. (1, 2, 4). Tryptophan Medium is prepared as recommended by ISO Committee (5, 6), the formulation is a modification of original formula of APHA where the medium is devoid of tryptophan (7).

Casein enzymic hydrolysate provides carbonaceous and nitrogenous nutrients required for the growth of microorganisms. Sodium chloride maintains the osmotic equilibrium. The amino acid tryptophan is a substrate to detect tryptophanase by the indole reaction. Certain microorganisms can cleave tryptophan by the enzyme tryptophanase to pyruvate, indole and ammonia. With Kovac's reagents the indole production can be detected by building of a colored complex in a hydrophobic upper layer (alcohol phase).

E. coli O157 test principle acc. ISO 16654:2001:

The test sample is enriched in Modified Tryptone Soya Broth (08069) by incubating at 42°C for 18-24 hours. *E. coli* O157:H7 is then isolated on MacConkey Sorbitol Agar (88902). 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 Broth.



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

Organisms (ATCC)	Growth	Indole production
<i>Escherichia coli</i> O157:H7 (25922)	+++	Positive indole reaction, red ring at the upper layer
<i>Escherichia coli</i> O157:H7 (NCTC 12900)	+++	Positive indole reaction, red ring at the upper layer
<i>Enterobacter aerogenes</i> (13048)	+++	Negative indole reaction, no colour development / cloudy ring

Reference:

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5. Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Escherichia coli* O157, ISO 16654:2001
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9. S.M. Finegold, E.J. Baron, Bailey and Scotts Diagnostic Microbiology, 7th Ed., The C.V. Mosby Co., St. Louis (1986)

Precautions and Disclaimer

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