

PEPTONE-TRYPTONE WATER

Ready to use tubes



Peptone Tryptone Water - from left:
uninoculated tube, *E.coli* Ind+,
E.aerogenes Ind-

INTENDED USE

Ready to use liquid medium to test the production of indole by microorganisms and for the detection of *E.coli* in food and water samples based on indole production.

TYPICAL FORMULA (g/l)

Tryptone	10
Sodium Chloride	5

Final pH 7.3 ± 0.2

DESCRIPTION

Peptone-Tryptone Water is recommended for the detection of indole production by microorganisms, especially *Enterobacteriaceae*, because of its high content of tryptophan. The formulation meets the requirement of Tryptone Water recommended by ISO 7251.

Organisms possessing the enzyme tryptophanase degrade the tryptophan to produce indole and other metabolic products. The indole production can be determined by the production of a red-violet colour complex upon application of Kovacs Reagent (cat. No.19171000). If present, indole reacts with the aldehyde group of p-dimethyleminobenzaldehyde with the development of a red-violet colour.

TECHNIQUE

Indole determination using pure culture:

Inoculate a tube and incubate at 37°C for 24 +/- 3 hours. Incubation at 44°C for 24 hours is advisable for detecting indole production in the confirmation test for faecal coliforms or *E.coli*. After incubation add 1ml of Kovacs Reagent. The formation of a red-violet ring indicates a positive reaction. A yellow-brown ring indicates a negative reaction.

Faecal coliforms

For the enumeration of faecal coliform bacteria with the most probable number technique proceed as following:

From each of the positive tubes of MacConkey Broth, inoculate, with a loop, a tube of Brilliant Green Bile Broth 2% and a tube of Peptone Tryptone Water and incubate at 44 °C. Observe for gas

production in Brilliant Green Bile Broth 2%, after 24 and 48 hours of incubation and test the indole production in Peptone Water after 24 hour of incubation. The MacConkey Broth tubes, which are positive to gas production in Brilliant Green Bile Broth 2% and are indole positive must be considered positive for faecal coliforms.

E.coli (MPN procedure)

ISO 7251 recommends the following method for the enumeration of *E.coli* with MPN procedure:

1. Prepare the test sample and the decimal dilution in accordance with the specific Laboratory method using Maximum Recovery Diluent (Cat. N° 401691) or another suitable diluent.
2. Take three tubes of double-strength Lauryl Pepto Bios Broth and three tubes of single-strength Lauryl Pepto Bios Broth and by means of a sterile pipette transfer to each tube 10ml or 1ml respectively of the test sample, if liquid or 10ml or 1ml of the initial suspension in the case of other products.
3. Repeat the inoculation of the single strength and of the double strength liquid medium for each of the further decimal dilutions, using a fresh pipette for each dilution.
4. Incubate the tubes at 35- 37°C for 24 +/- 2 hours
5. From each of the incubated tubes showing gas production inoculate with a loop a tube of EC Broth and incubate at 45°C for 24 +/- 2 hours.
6. From each of the EC Broth tubes showing gas production inoculate with a loop a tube of Peptone-Tryptone Water and incubate at 45°C for 48 hours.
7. Carry on the indole test by adding 0.5 ml of Kovacs' Reagent
8. Express the results as the Most Probable Number of *E.coli* on the basis of gas production in the EC Broth and the positive result to indole test, in Peptone Water.

PRECAUTIONS

For *in vitro* diagnostic use only. Observe approved biohazard precautions and aseptic techniques. To be used only by adequately trained and qualified laboratory personnel. Sterilize all biohazard waste before disposal.

STORAGE

Store at 2-8° away from direct light - When stored as directed the tubed media remain stable until the expiry date shown on the label. Do not use beyond stated expiry date. Media should not be used if there are any signs of deterioration (shrinking, cracking, discoloration) or contamination.

REFERENCES

- ISO 7251. Microbiology-General Guidance for enumeration of *E. coli* - Most Probable Number Technique. 1993-12-15
- Mackenzie, E.F.W., Taylor, E.W. & Gilbert. W.E. (1948) J. Gen. Microbiol., 2, 197.

PACKAGING**551891****Peptone Tryptone Water****20 x 9 ml ready to use tubes**