Tryptone Water

Intended Use

Tryptone Water is recommended for use in the detection of *Escherichia coli* in food and water samples based on indole production.

Summary and Explanation

Tryptone Water is based on the Tryptone Water formula described in ISO standard 7251.[1] In this procedure, Tryptone Water is used with Lauryl Tryptose (or Sulfate) Broth and EC Broth to determine the most probable number (MPN) of *E. coli* present in the sample. Gas production in both media and indole production in Tryptone Water is used as the basis for this presumptive *E. coli* test.

Tryptone Water may also be used for differentiation of other bacteria based on indole production.

Principles of the Procedure

Tryptone Water contains both tryptone (1%) and sodium chloride. Due to its high tryptophan content, tryptone is suitable for use in detecting indole production by bacteria. Tryptophan is hydrolyzed and deaminated to produce indole, pyruvic acid and ammonia.[2] Indole can then be detected by the addition of either Kovacs' or Ehrlich's Reagent, which contain an aldehyde group. The aldehyde group combines with indole to produce a red color in the alcohol layer. Sodium chloride is added to the medium to provide a suitable osmotic environment.

User Quality Control

Identity Specifications

Difco™ Tryptone Water

Dehydrated Appearance:	White to light beige, free flowing, homogeneous.
Solution:	1.5% solution, soluble in purified water. Solution is pale to medium amber, clear to slightly opalescent.
Prepared Appearance:	Light to medium amber, clear to slightly opalescent.
Reaction of 1.5% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Difco™ Tryptone Water

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours. Add 0.5 mL Indole Reagent (Kovacs) to the tubes to test for indole production. Formation of a red color denotes a positive indole test.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	INDOLE PRODUCTION
Enterobacter cloacae	13047	10^2-3×10^2	Good	–
Escherichia coli	25922	10^2-3×10^2	Good	+

Formula

Difco™ Tryptone Water

Approximate Formula* Per Liter		
Tryptone	10.0	g
Sodium Chloride	5.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve 15 g of the powder in 1 L of purified water.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Test For Enumeration of Presumptive *E. coli*

1. Suspend one part sample in 9 parts diluent. Homogenize sample.
2. Prepare serial 10-fold dilutions to 10^{-6} using 1 mL of homogenate and 9 mL of diluent. Mix each dilution thoroughly.
3. Transfer 10 mL of test sample or initial suspension to each of 3 tubes of double-strength Lauryl Tryptose Broth (LTB). Repeat using 3 tubes of single-strength LTB. Mix well.
4. For each of the serial 10-fold dilutions, transfer 10 mL of test sample to each of 3 tubes of double-strength LTB. Repeat using 3 tubes of single-strength LTB. Mix well.
5. Incubate all tubes of LTB at 35-37°C for 24 ± 2 hours and up to 48 hours, if necessary, observing tubes for gas formation.
6. Inoculate one 3-mm loopful of broth from each tube in Step 5 showing gas formation to 10 mL of EC Broth warmed to 45°C.
7. Incubate the EC Broth tubes in a water bath at 45°C for 24 ± 2 hours (up to 48 hours if necessary), observing for gas formation.
8. Inoculate one 3-mm loopful of broth from each tube in Step 7 showing gas formation to 5-10 mL of Tryptone Water warmed to 45°C.

9. Incubate Tryptone Water tubes in a water bath at 45°C for 48 hours.
10. Add 0.5 mL of Indole Reagent to Tryptone Water tubes, mix well and examine after 1 minute.

Indole Determination Using Pure Cultures

1. Inoculate Tryptone Water using a light inoculum of an 18-24 hour pure culture.
2. Incubate the tubes at 35 ± 2°C with loosened caps for 18-24 hours.
3. Add 0.5 mL of Indole Reagent (Kovacs) directly to the tube and agitate. Allow tubes to stand for 5-10 minutes.

Expected Results

Test For Enumeration of Presumptive *E. coli*

For each dilution, record tubes as positive if a red ring forms at the top of the medium indicating indole production. Determine the MPN (Most Probable Number) of *E. coli* present in the sample based on the number of tubes that are positive for both gas and indole. Consult the appropriate 3-tube MPN table.[1]

Indole Determination Using Pure Cultures

Examine tubes for the formation of a red ring at the top of the tube indicating indole production.

Limitations of the Procedure

1. Detection of *E. coli* in meats using Tryptone Water is a presumptive test. If confirmatory testing is required, please consult appropriate references.
2. Indole testing is recommended as an aid in the differentiation of microorganisms based on indole production. For complete identification of the organism, further biochemical evaluation is necessary.

References

1. International Organization for Standardization. 1993. Microbiology – general guidance for enumeration of presumptive *E. coli* – most probable number technique. ISO 7251, 1993-12-15, 2nd ed. ISO, Geneva, Switzerland.
2. MacFaddin. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Baltimore, Md.

Availability

Difco™ Tryptone Water

ISO

Cat. No. 264410 Dehydrated – 500 g

Difco™/BBL™ Indole Reagent

Cat. No. 261185 Droppers – 50 × 0.5 mL