Peptone water, Indole Free

M463AI

Intended use

Recommended for detection and enumeration of presumptive *Escherichia coli.* The composition and performance criteria of this medium are as per the specifications laid down in ISO 7251:2005.

Composition**

ISO 7251:2005 Specification- Peptone water, Indole Free

Ingredients	g / L
Tryptone #	10.000
Sodium chloride	5.000
Final pH (after sterilization)	7.3±0.2

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**Formula adjusted, standardized to suit performance parameters
Enzymatic digest of casein

Directions

Dissolve 15 grams in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes or flasks as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Peptone Water is recommended by ISO Committee (1) for detection of indole production by coliforms, which is a key feature in differentiation of bacteria. This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole which accumulates in the medium (2).

Tryptone is a good substrate for indole production because of its high tryptophan content. Certain organisms breakdown the amino acid tryptophan with the help of enzymes that mediate the production of indole by hydrolytic activity (3). The indole produced can be detected by either Kovacs or Ehrlichs reagent (4). Indole combines with the aldehyde present in the above reagent to give red colour in the alcoholic layer. The alcohol layer extracts and concentrates the red colour complex. Tryptone Water is used in conjunction with Broth 2 % (M127I) to determine the most probable number (MPN) of *E.coli* in water and food sample. Tubes showing opacity, cloudiness or gas production in the liquid selective enrichment medium and whose subcultures have produced gas in th EC broth and indole in the peptone water at 44°C are considered to contain presumptive *Escherichia coli.* For determination of indole, inoculate the medium with inoculum of an 18-24 hours pure culture. Incubate the tubes at 35 ± 2°C for 18-24 hours. Add 0.5 ml of indole reagent (R008) directly to the tube and agitate. Allow the tubes to stand for 1 minute. Formation of red ring at the top of the tube indicates indole production. Indole testing is recommended as an aid in the differentiation of microorganisms based on indole production.

Type of specimen

Food and animal feeding stuffs

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (1). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

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.

Limitations :

1. For complete identification of the organisms, further biochemical confirmation is necessary.

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 1.5% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Cultural characteristics observed after an incubation at 44 °C for 48 h ± 2 h. Add 0.5ml kovac's indole reagent (R008) to each tube after incubation.

Organism	Growth	Indole reaction
Escherichia coli ATCC 25922 (00013*)	luxuriant	positive reaction, red ring at the interface of the medium
# Klebsiella aerogenes ATCC 13048 (00175*)	luxuriant	negative reaction, no colour development / cloudy ring

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 15-25°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 (5,6).

Reference

1. International Organization for Standardization (ISO), ISO/DIS 7251:2005(E).
2. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone.
3. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Williams and Wilkins, Baltimore.
4. Finegold S. M. and Baron E. J., 1986, Bailey and Scotts Diagnostic Microbiology, 7th Ed., The C.V. Mosby Co., St. Louis.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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