Tryptone Bile Agar

M961

Intended Use

Recommnended for rapid detection and enumeration of *Escherichia coli* in food using a modified direct plating method.

Composition**

Ingredients	Gms / Litre
Trypone	20.000
Bile salts mixture	1.500
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 36.5 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Tryptone Bile Agar was formulated by Anderson and Baird-Parker (1). The International Commission on the Microbiological Specifications for Foods (CMSF) (6) compared the Most Probable Number (MPN) and the Anderson-Baird-Parker Direct Plating Method (DPM) and observed that DPM was superior to MPN for enumeration of *Escherichia coli* from raw meats. Superiority of DPM method was noticed by the organization on the basis of less variability, better recovery from frozen samples, greater rapidity and the smaller quantity of medium required. The DPM enumerates both anaerogenic and late lactose fermenting strains of *E. coli* which could be missed by the MPN method (about 10%)(3). This formulation is recommended by ISO committee for the enumeration of *E. coli* (7). Holbrook et al (5) modified the DPM for detection and enumeration of sublethally damaged cells of *E. coli* in frozen, dried, heat processed or acid foods and found that resuscitation step reduces the high concentration of sugar present in the inoculum to a level which does not interfere with the production of indole as the synthesis of tryptophanase is inhibited by high sugar concentrations (2).

Certain organisms breakdown the amino acid tryptophan with the help of enzymes that mediate the production of indole by hydrolytic activity (10). 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 alcohol layer. The alcohol layer extracts and concentrates the red colour complex. The indole positive organisms other than *E. coli* are inhibited by bile salts and elevated incubation temperature.

Type of specimen

Food samples: meat and meat product

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (11).

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.

Limitations :

1. Due to variable nutritional requirements, some strains show poor growth on this medium.

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.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Yellow coloured clear to slightly opalescent gel forms in Petri plates.

Reaction
Reaction of 3.65% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
Cultural characteristics observed after an incubation at 44°C for 24 hours.

Organism	Inoculum (CFU)	Growth	Recovery
# Klebsiella aerogenes ATCC 13048 (00175*)	>=10^4	inhibited	0%
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	>=50%

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 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder 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 (8,9).

Reference

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11. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

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