Tryptose Yeast Extract Broth (AOAC)

M988

Tryptose Yeast Extract Broth (AOAC) with the addition of salicin or raffinose and an acid base indicator (Phenol Red) is recommended by AOAC for detection of *Clostridium perfringens* in foods.

Composition**

Ingredients	Gms / Litre
Tryptose	20.000
Yeast extract	5.000
Sodium chloride	5.000
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30 grams in 1000 ml distilled water. Heat if necessary, to dissolve the medium completely. Dispense in flasks or tubes. Add Salicin (1%) or Raffinose (1%) as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Incubate media for 24 hours at 35°C transfer 1 ml culture to test tube and add 1-2 drops 0.04% phenol red. Yellow colour indicates acid production from salicin. Re-incubate media for additional 48 hours.

Principle And Interpretation

Clostridium perfringens produces a heat-resistance enterotoxin which causes food-poisoning if ingested. In perfringens poisoning, the vehicle is almost always an improperly cooked meat (1). The heat resistance of its spores often allows *C. perfringens* to survive incomplete cooking of food, with the surviving bacteria then able to cause food poisoning (2). This makes detection and isolation of these organisms from food important. Tryptose Yeast Extract Broth is recommended by AOAC (3) for the confirmation of *C. perfringens* in foods.

Tryptose and yeast extract provide necessary nutrients to the organisms. Sodium chloride maintains the osmotic equilibrium. Addition of salicin or raffinose helps in distinguishing clostridia species. Salicin is usually not fermented by *C. perfringens* but is rapidly fermented with production of acid and gas by other species. Acid is usually produced from raffinose by *C. perfringens* but not by other species. Addition of 1-2 drops of 0.04% phenol red to the culture after incubation helps in the detection of acid production (yellow colour).

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 3.0% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH
6.70-7.10

Cultural Response
M988: Cultural characteristics observed when incubated anaerobically, after an incubation at 35-37°C for 18-72 hours, after addition of phenol red indicator.

Organism	Inoculum (CFU)	Growth	Acid from Salicin	Acid from Raffinose
Clostridium perfringens ATCC 12924	50-100	luxuriant	negative reaction, no colour change or red	positive reaction, yellow colour

Clostridium sporogenes ATCC 11437	50-100	luxuriant	negative reaction, no colour change or red	negative reaction, no colour change or red

Storage and Shelf Life

Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

Reference

1. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
2. Doyle M. P., Beuchat L. R. and Montville T. J., Food Microbiology, Fundamentals and Frontiers, ASM Press, Washington D.C.
3. Horwitz W., (Ed.), 2000, Official Methods of Analysis of AOAC International, 17th Ed., AOAC International, Gaithersburg, Maryland.

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