Lactose Gelatin Medium, Modified

M987

Intended Use:

Recommended for detection and presumptive identification of *Clostridium perfringens* from food in accordance with AOAC

Composition**

Ingredients	Gms / Litre
Tryptose	15.000
Yeast extract	10.000
Lactose	10.000
Disodium phosphate	5.000
Gelatin	120.000
Phenol red	0.050
Final pH (at 25°C)	7.5±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 16 grams in 100 ml warm purified / distilled water. Heat to boiling to dissolve the medium completely and dispense 10 ml amounts in 15x150 mm screw capped tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Just before use, heat to boiling to remove dissolved oxygen and cool rapidly to incubation temperature.

Principle And Interpretation

Members of the genus *Clostridium* are distributed widely in nature and are found in soil as well as in fresh water and marine water sediments throughout the world (7). Clostridial species are one of the major causes of food poisoning / gastro-intestinal illnesses. They are gram-positive, spore-forming rods that occur naturally in soil (2). Among the family are: *Clostridium botulinum*, which produces one of the most potent toxins in existence; *Clostridium tetani*, causative agent of tetanus; and *Clostridium perfringens*, commonly found in wound infections and diarrhoea cases. The use of toxins to damage the host is a method deployed by many bacterial pathogens including *Clostridium*. Lactose Gelatin Medium, Modified is prepared in accordance with AOAC (4) and a slight modification of this medium is recommended by APHA for detection of *Clostridium perfringens* in foods (3).

Tryptose and yeast extract in the medium provide essential growth nutrients. Lactose is the fermentable sugar and phenol red acts as fermentation indicator, which changes from red to yellow due to acid production. Following incubation the medium tube is chilled for 1 hour at 5°C, if medium gels; it should be incubated for an additional 24 hours to examine gelatin liquefaction. The medium is stab inoculated with pure Fluid Thioglycollate Medium (M009) culture or isolates from Tryptose Sulphite Cycloserine (TSC) Agar plate. Refer appropriate references for standard procedures (4).

Type of specimen

Food samples; Water samples

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (8).
For water 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.

Limitations :

1. Before use, heat to boiling to remove dissolved oxygen and cool rapidly to incubation temperature.

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
Light yellow to light pink coarse free flowing powder

Gelling
Semisolid, comparable with 12% Gelatin.

Colour and Clarity of prepared medium
Red coloured, clear to slightly opalescent gel forms in tubes as butts

Reaction
Reaction of 16.0% w/v aqueous solution at 25°C. pH : 7.5±0.1

pH
7.40-7.60

Cultural Response
Cultural characteristics observed under anaerobic conditions, after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Lactose fermentation	Gelatin liquefaction
Clostridium perfringens ATCC 12924	50-100	luxuriant	acid and gas production	positive reaction
Clostridium paraperfringens ATCC 27639	50-100	good	acid production	positive reaction

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 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. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.

2. Czeczulin J. R., Hanna P. C., Mcclane B. A., 1993, Cloning, nucleotide sequencing, and expression of the *Clostridium perfringens* enterotoxin gene in *Escherichia coli.* Infect. Immun. 61: 3429-3439.

3. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.

4. FDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, DC.

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.

7. 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.

8. 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.

Revision : 03 / 2019