



Lactose Gelatin Medium, Modified

M987

Lactose Gelatin Medium, Modified is recommended for detection and presumptive identification of *Clostridium perfringens* from foods 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 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 (1). 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 (3) and a slight modification of this medium is recommended by APHA for detection of *Clostridium perfringens* in foods (4).

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 (3).

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

M987: 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
<i>Clostridium perfringens</i> ATCC 12924	50-100	luxuriant	acid and gas production	positive reaction
<i>Clostridium paraperfringens</i> ATCC 27639	50-100	good	acid production	positive reaction

Storage and Shelf Life

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

Reference

1. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Tenover F. C., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, 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. FDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, DC.
4. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.

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