



Lactose Gelatin Medium, Modified

M987I

Intended Use:

Recommended for detection and enumeration of *Clostridium perfringens* from food samples. The composition and performance criteria are in accordance with ISO 7937:2004 and ISO 11133: 2014, Amd 2 : 2020.

Composition**

ISO Specification-Lactose-gelatin medium		M987I : Lactose Gelatin Medium, Modified	
Ingredients	g / L	Ingredients	g / L
Enzymatic digest of casein	15.000	Tryptose#	15.000
Yeast extract	10.000	Yeast extract	10.000
Lactose	10.000	Lactose	10.000
Gelatin	120.000	Gelatin	120.000
Phenol red	0.050	Phenol red	0.050
Final pH (at 25°C)	7.5±0.2	Final pH (at 25°C)	7.5±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Enzymatic digest of casein

Directions

Suspend 15.5 grams in 100 ml warm purified/distilled water. Heat to boiling to dissolve the medium completely and dispense 10 ml amounts in 15X150 mm screwcapped 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

Lactose Gelatin Medium, Modified was prepared in accordance with APHA (1) and is recommended by F.D.A for detection and colony count of *Clostridium perfringens* in foods (2) . This formulations is also recommended by ISO committee for enumeration of *Clostridium perfringens* (3,4).

Tryptose and yeast extract 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. The 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 culture or isolates from Tryptose sulphite cycloserine (TSC) agar plate.

Type of specimen

Food samples

Specimen Collection and Handling

Processing : (3,4)

Pre-enrichment : Incubate under anaerobic conditions at 37 °C for 18 h to 24 h. Streak the colonies on Perfringens Agar Base (T.S.C.) (M837I), and add an overlay of 10 ml of the Perfringens Agar Base (T.S.C.). Allow to solidify and incubate anaerobically at 37°C for 18-24 h. Select from each plate at least one characteristic and well-separated colony. If necessary, repeat the streaking and inoculation on Perfringens Agar Base (T.S.C.) plates until well-isolated, characteristic black colonies are obtained.

Confirmation: Inoculation and reading of lactose-gelatin medium. Inoculate each selected colony into the freshly deaerated lactose-gelatin medium. Incubate under anaerobic conditions at 37°C for 24 h. Examine the tubes of the lactose-gelatin medium for the presence of gas and a yellow colour (due to acid formation) indicating fermentation of lactose. Chill the tubes for 1 h at 5°C and check for gelatin liquefaction. If the medium has solidified, re-incubate for an additional 24 h and again check for gelatin liquefaction. After incubation, transfer with no delay 5 drops of the thioglycollate culture to the Lactose Sulphite Broth Base (M1287) by means of a sterile pipette. Incubate aerobically at 46°C for 18-24 h in the water bath.

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. Discard unused medium 3 weeks after preparation.

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

Beige to reddish pink homogeneous coarse powder

Gelling

Semisolid, comparable with 12% Gelatin gel.

Colour and Clarity of prepared medium

Red coloured clear to slightly opalescent gel forms in tubes

Reaction

Reaction of 15.5% w/v aqueous solution at 25°C. pH : 7.5±0.2

pH

7.30-7.70

Cultural Response

Cultural characteristics observed after an incubation at 30 - 35°C for 24 - 48 hours .

Organism	Growth	Gelatin liquefaction
<i>Clostridium perfringens</i> ATCC 13124 (00007*)	good	Positive reaction
<i>Clostridium perfringens</i> ATCC 12924	luxuriant with acid and gas production.	Positive reaction

Key : *Corresponding WDCM numbers.

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. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
2. Bacteriological analytical manual, 1995, 8th ed., Food and Drug Administration, AOAC International, U.S.A.
3. International Organization for Standardization (ISO), 1985, enumeration of *Clostridium perfringens* Draft ISO/DIS 7937.
4. Microbiology of food, animal feeding stuffs and water- Preparation, production, storage and performance testing of culture
5. media, EN ISO 11133:2014(E) /Amd.: 2020 .
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
7. 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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