



PRODUCT SPECIFICATION SHEET

Lactose Gelatin Medium (DM509)

Intended Use

Lactose Gelatin Medium (DM509) is recommended for detection of *Clostridium* species from food samples.

Product Summary and Explanation

Clostridia are widely distributed 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⁾ Some very potent pathogens of the genus *Clostridium* are *Clostridium botulinum*, *Clostridium tetani* and *Clostridium perfringens*, which are able to produce highly toxic toxins. *Clostridium botulinum*, 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 is recommended from AOAC⁽³⁾ and APHA for detection of *Clostridium perfringens* in foods.⁽⁴⁾

Principles of the Procedure

Lactose Gelatin Medium contains lactose which is fermented by the *Clostridium* species, mainly by *Clostridium perfringens* yielding acid and gas. Phenol red is the pH indicator which turns yellow at acidic pH. Gelatin is a protein derived by the hydrolysis of collagen, and which is usually liquefied by *Clostridium perfringens* within 24-48 hours. Disodium phosphate buffers the medium.

Formula / Liter

| Ingredients | Gms / Liter |
|--|-------------|
| Lactose | 10.00 |
| Disodium phosphate | 5.00 |
| Gelatin | 120.00 |
| Phenol red | 0.05 |
| Final pH: 7.5 ± 0.2 at 25°C | |
| Formula may be adjusted and/or supplemented as required to meet performance specifications | |

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 135 grams of the medium in one liter warm distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Dispense 10 ml amounts in 15x150 mm screw capped tubes.
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
5. Just before use, heat to boiling to remove dissolved oxygen and cool rapidly to incubation temperature.

Quality Control Specifications

| | |
|----------------------------|---|
| Dehydrated Appearance | Light yellow to pink coloured homogeneous free flowing slightly coarse powder |
| Prepared Medium | Red coloured clear to slightly opalescent gel forms in tubes |
| Reaction of 13.5% Solution | pH : 7.5 ± 0.2 at 25°C |
| Gel Strength | Firm, comparable with 1.5% Agar gel |



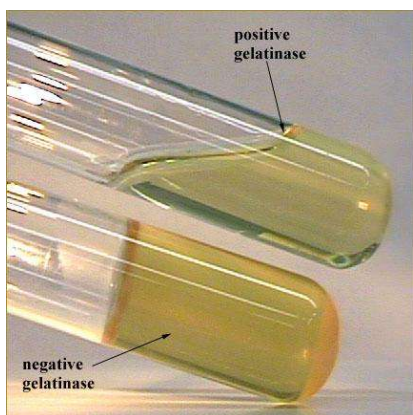


PRODUCT SPECIFICATION SHEET

Expected Cultural Response: Cultural characteristics observed under an anaerobic condition after an incubation at 35-37°C for 48-72 hours

| Sr. No. | Organisms | Results to be achieved | |
|---------|---|--|----------------------|
| | | Lactose Fermentation | Gelatin liquefaction |
| 1. | <i>Clostridium perfringens</i> ATCC 12924 | positive reaction, yellow colour | positive reaction |
| 2. | <i>Clostridium sporogenes</i> ATCC 11437 | negative reaction, no colour change or red | positive reaction |

The organisms listed are the minimum that should be used for quality control testing.



Test Procedure

1. Inoculate each selected colony into the Lactose Gelatin Medium.
2. Incubate under anaerobic conditions for 24-48 hours at 35-37°C.
3. Examine the tubes of Lactose Gelatine Medium for the presence of gas and a yellow colour due to acid formation indicating fermentation of lactose.
4. Chill the tubes for 1 hour at $5 \pm 3^\circ\text{C}$ and check for gelatine liquefaction.
5. If the medium has solidified, re-incubate for an additional 24 hours and again check for gelatine liquefaction.

Results

1. To read gelatinase, refrigerate until well chilled and compare to non-inoculated tubes. Tubes positive for gelatinase will remain liquid.
2. The isolated colonies which are not-motile, reduce nitrate, ferment lactose producing acid and gas, thereby producing liquefaction of gelatin in 48 hours are presumptively identified as *Clostridium perfringens*.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.





PRODUCT SPECIFICATION SHEET

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Lactose Gelatin Medium

Product Code : DM509

Available Pack sizes : 500gm

References

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

Further Information

For further information please contact your local MICROMASTER Representative.



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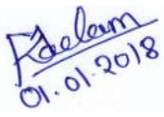


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