



C. perfringens Sporulation Broth

M947

Intended Use:

Recommended for promoting sporulation in *Clostridium perfringens*.

Composition**

Ingredients	Gms / Litre
Tryptose	15.000
Yeast extract	3.000
Starch, soluble	3.000
Magnesium sulphate	0.100
Sodium thioglycollate	1.000
Disodium hydrogen phosphate	11.000
Final pH (at 25°C)	7.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 33.1 grams in 1000 ml purified / distilled water. Heat if necessary to ensure complete solution. Dispense 20 ml amounts in 20 x 150 mm screw capped test tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Just before use, heat the medium in flowing steam for 20 minutes.

Principle And Interpretation

Clostridium perfringens is ubiquitous in nature and can be found as a normal component of decaying vegetation, marine sediment, intestinal tract of humans and other vertebrates, insects, and soil. *C. perfringens* is commonly encountered in infections as a benign component of the normal flora (6). *C. perfringens* food poisoning is one of the most common types of human foodborne illnesses. A heat-labile enterotoxin produced only by sporulating cells (1) induces the major symptoms of diarrhea in perfringens infections.

C. perfringens Sporulation Broth is formulated as per APHA (5) for enhancing sporulation in *C. perfringens*. The medium contains ingredients like tryptose, yeast extract and starch, which not only support the growth of *C. perfringens* but also stimulate spore formation in presence of magnesium sulphate. Sodium thioglycollate in the medium helps to maintain anaerobic conditions. Magnesium sulphate and disodium phosphate provide ions to the organism and helps in maintaining buffering conditions in the medium.

Type of specimen

Food and animal feed samples.

Specimen Collection and Handling

For food and animal feed samples, follow appropriate techniques for sample collection and processing as per guidelines (2). 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. Further isolation and biochemical tests should be carried out for confirmation.

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

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Medium amber coloured, clear to slightly opalescent solution with a slight precipitate in tubes

Reaction

Reaction of 3.31% w/v aqueous solution at 25°C. pH : 7.8±0.2

pH

7.60-8.00

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Sporulation
<i>Clostridium perfringens</i> ATCC 12924	50-100	good-luxuriant	positive
<i>Clostridium sporogenes</i> ATCC 11437	50-100	good-luxuriant	negative

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. Duncan C. L., 1973, J. Bacteriol., 113:932-936.
2. International Organization for Standardization (ISO- 7937:2004) : Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of *Clostridium perfringens*- Colony count technique
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. 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.
5. Speck M. L., (Eds.), 1984, Compendium of Methods for the Microbiological Examination of Foods, 2nd Ed., APHA, Washington, D.C
6. Wells C. L., Wilkins T. D., 1996, Barrons Medical Microbiology (Barron S. et al, Eds.), 4th Ed., Univ. of Texas Medical Branch.

Revision : 02/ 2019

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