Ready Prepared Media

Technical Information

Perfringens Agar Plate

Product Code: PM 1579

Application : Recommended for selective isolation and enumeration of *Clostridium perfringens* in food .

Composition**

Ingredients	Gms / Litre
Tryptone	15.000
Soya peptone	5.000
Yeast extract	5.000
HL extract #	7.000
Ferric ammonium citrate	1.000
Sodium metabisulphite	1.000
Tris buffer	1.500
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Liver extract

Principle & Interpretation

Clostridial species are one of the major causes of food poisoning/ gastrointestinal illnesses. They are gram-positive spore- forming rods that occur naturally in the soil (1). Foods commonly contaminated with *Clostridium perfringens* include meat, meat pies, poultry, stews and gravy. Among the family are: *Clostridium botulinum* which produces one of the most potent toxins in existence; *Clostridium tetani*, causative agent of tetanus; and *C. 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. The major virulence factor of *C. perfringens* is the CPE enterotoxin, which is secreted upon invasion of the host gut, and contributesto food poisoning and other gastrointestinal illnesses (1).

Perfringens Agar (O.P.S.P.) is based on the formula developed by Handford (2) and is used as a selective medium forisolation and enumeration of *C. perfringens* in foods (3).

Tryptone, yeast extract, Soya peptone and HL extract supply most of the essential nitrogenous nutrients, vitamin B complex and trace ingredients for the growth of *C.perfringens*. Sodium metabisulphite and ferric ammonium citrate are used as indicators of sulphate reduction by *C. perfringens*, which produces black colonies. Tris buffer helps in maintaining buffering action. The antibiotics sulphadiazine, oleandomycin and polymyxin B make the medium highly selective inhibiting sulphite-reducing bacteria other than *C. perfringens* such as *Salmonella*, *Bacillus* species, *Proteus* species, Staphylococci etc.

Prepare 10 fold dilution of a 10 % homogenate of the food sample in 0.1 % Peptone Water (M028). Viable counts of *C.perfringens* bacilli or spores are obtained by plating 0.1 ml of different dilutions onto duplicate plates of blood agar containing 5 mg/lit of gentamicin/lt. Incubate at 37°C for 18-24 hours in two sets, one anaerobically and another aerobically. Alternatively incorporate 1 ml of the dilution into 25 ml of molten and cooled Perfringens Agar (O.P.S.P.) containing supplements. Incubate anaerobically for 18-24 hours at 37°C. Perfringens Agar with supplements gives high degree of selectivity and specificity.

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Type of specimen

Food samples

Specimen Collection and Handling

Prepare 10 fold dilution of a 10 % homogenate of the food sample in 0.1 % Peptone Water (M028). Viable counts of *C. perfringens* bacilli or spores are obtained by plating 0.1 ml of different dilutions onto duplicate plates of blood agar containing 5 mg/lit of gentamicin/lt. Incubate at 37°C for 18-24 hours in two sets, one anaerobically and another aerobically. Alternatively incorporate 1 ml of the dilution into 25 ml of molten and cooled Perfringens Agar (O.P.S.P.) containing supplements. Incubate anaerobically for 18-24 hours at 37°C. Perfringens Agar with supplements gives high degree of selectivity and specificity.

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 guidleines should be followed while handling specimens. Saftey guidelines may be referred in individual safety data sheets

Limitations

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. Further biochemical and serological tests must be carried out for further identification.

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.

Methodology

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

Quality Control

Appearance
Sterile Perfringens Agar Plate in 90 mm disposable plates.
Colour of medium
Amber coloured medium
Quantity of medium
25 ml of medium in 90 mm disposable plates.
Reaction
7.10-7.50
Sterility Test
Passes release criteria
Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours

Oragnism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Bacillus subtilis subsp.spizizenii ATCC 6633 (00003*)	>=10^4	inhibited	0%	
Clostridium bifermentans ATCC 17837	>=10^4	inhibited	0%	
Clostridium butyricum ATCC 13732	>=10^4	inhibited	0%	
Clostridium perfringensATCC 12924	50-100	luxuriant	>=50%	black
Enterococcus faecalis ATCC29212 (00087*)	50-100	non-poor	<=10%	white ,if any
Proteus vulgaris ATCC13315	>=10^4	inhibited	0%	
Salmonella Typhi ATCC 6539	>=10^4	inhibited	0%	
Staphylococcus aureussubsp. aureus ATCC 25923 (00034*)	>=10^4	inhibited	0%	

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

- On receipt store between 2-8°C Use before expiry date on the label.
- 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 (4,5).

Further Reading

1. Czeczulin J. R., Hanna P. C., Mcclane B. A., 1993, Infect. Immun. 61: 3429-3439.
2. Handford P. M., 1974, J. Appl. Bacteriol., 37: 559.
3. Hauschild A. H. W. et al, 1977, ICMSF Methods Studies VIII, Can. J. Microbiol., 23:884.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. 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.
6. 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.

Disclaimer

- User must ensure suitability of the product(s) in their application prior to use.
- The product conform solely to the technical information provided in this booklet and to the best of knowledge research and developmentwork carried at **CDH** is true and accurate
- **Central Drug House Pvt. Ltd.** reserves the right to make changes to specifications and information related to the products at any time.
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- of diagnostic reagents extra.
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- Do not use the products if it fails to meet specificatons for identity and performens parameters.