



Perfringens Agar Base (O.P.S.P.)

M579

Intended Use:

Recommended for selective isolation and enumeration of *Clostridium perfringens* from 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

Directions

Suspend 25.25 grams in 500 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add rehydrated contents of 1 vial of Perfringens Supplement-I (FD011) and Perfringens Supplement-II (FD012) each. Mix well before pouring into sterile Petri plates.

Principle And 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 contributes to 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 for isolation 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/lit. 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.

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/lit. 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 guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets

Limitations :

1. 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.

Quality Control

Appearance

Cream to brownish yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Amber coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.05% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours with added Perfringens Supplement I(FD011) and Perfringens Supplement II(FD012).

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
<i>Bacillus subtilis subsp. spizizenii</i> ATCC 6633 (00003*)	$\geq 10^4$	inhibited	0%	
<i>Clostridium bifermentans</i> ATCC 17837	$\geq 10^4$	inhibited	0%	
<i>Clostridium butyricum</i> ATCC 13732	$\geq 10^4$	inhibited	0%	
<i>Clostridium perfringens</i> ATCC 12924	50-100	luxuriant	$\geq 50\%$	black
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	50-100	none-poor	$\leq 10\%$	white, if any
<i>Proteus vulgaris</i> ATCC 13315	$\geq 10^4$	inhibited	0%	
<i>Salmonella</i> Typhi ATCC 6539	$\geq 10^4$	inhibited	0%	
<i>Staphylococcus aureus subsp. aureus</i> ATCC 25923 (00034*)	$\geq 10^4$	inhibited	0%	

Key : *Corresponding WDCM numbers.

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

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.

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Disclaimer :

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