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

M579

Perfringens Agar Base (O.P.S.P.) with selective supplements is used as a selective medium for isolation and enumeration of *Clostridium perfringens* in foods.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	15.000
Papaic digest of soyabean meal	5.000
Yeast extract	5.000
Liver 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

Directions

Suspend 25.25 grams in 500 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 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).

Casein enzymic hydrolysate, yeast extract, papaic digest of soyabean meal and liver 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.

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
M579: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours with addded Perfringens Supplement I(FD011) and Perfringens Supplement II(FD012).

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Cultural Response				
Bacillus subtilis ATCC 6633	>=10^3	inhibited	0%	
Clostridium bifermentans ATCC 17837	>=10^3	inhibited	0%	
Clostridium butyricum ATCC 13732	>=10^3	inhibited	0%	
Clostridium perfringens ATCC 12924	50-100	luxuriant	>=50%	black
Enterococcus faecalis ATCC 29212	50-100	none-poor	<=10%	white, if any
Proteus vulgaris ATCC 13315	>=10^3	inhibited	0%	
Salmonella Typhi ATCC 6539	>=10^3	inhibited	0%	
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0%	

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

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.

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