

## Technical Information

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

#### Product Code: DM1579

**Application:** 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

#### Principle & Interpretation

Clostridial species are gram-positive spore-forming rods that occur naturally in the soil <sup>(1)</sup>. They are one of the major causes of food poisoning/gastrointestinal illnesses. Foods commonly contaminated with *Clostridium perfringens* include meat, meat pies, poultry, stews and gravy. Among the family *Clostridium botulinum* produces one of the most potent toxins following *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 adopted 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 <sup>(1)</sup>.

Perfringens Agar (O.P.S.P.) is based on the formula devised by Handford <sup>(2)</sup> and is used as a selective medium for isolation and enumeration of *C. perfringens* in foods <sup>(3)</sup>.

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 % homogenate of the food sample in 0.1 % Peptone Water (MS2028) to make 10 fold dilutions. 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 different 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.

#### Methodology

Suspend 25.25 grams of powder media in 500 ml distilled water. Shake well and heat 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 (MS2011) and Perfringens Supplement-II (MS2012) each. Mix well before pouring into sterile Petri plates.

## Quality Control

### Physical 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 Range** 7.10-7.50

### Cultural Response/ characteristics

DM 1579: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours with added Perfringens Supplement I(MS2011) and Perfringens Supplement II(MS2012).

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

## Storage and Shelf Life

**Dried Media:** Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.

**Prepared Media:** 2-8° in sealable plastic bags for 2-5 days.

## 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.

## Disclaimer :

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