Perfringens (OPSP) Selective Supplement A and Pefringens (OPSP) Selective Supplement B

PRODUCT INFORMATION

P007-1MU - Polymyxin B Sulfate, Powder, 1MU

P007-10MU - Polymyxin B Sulfate, Powder, 10MU

P007-100MU - Polymyxin B Sulfate, Powder, 100MU

DESCRIPTION

Perfringens Agar (OPSP) with Perfringens (OPSP Selective Supplement A and Pefringens (OPSP) Selective Supplement B is a medium for the enumeration of *Clostridium perfringens* in foods.

BACKGROUND

Sulfadiazine is a sulfonamide antibiotic.

Oleandomycin is a macrolide antibiotic. It is synthesized from strains of *Streptomyces antibioticus*.

Polymyxin B is an antibiotic primarily used for resistant gram-negative infections. It is derived from the bacterium *Bacillus polymyxa.* Polymyxin B is a mixture of two closely related compounds, polymyxin B1 and polymyxin B2. It has a bactericidal action against almost all gram-negative bacilli except the Proteus group.

Mechanism of action

Polymyxins bind to the cell membrane and alter its structure, making it more permeable. The resulting water uptake leads to cell death.

APPLICATION IN PERFRINGENS AGAR (OPSP)

Perfringens Agar (OPSP), is based on the formulation developed by Handford. The medium utilises sulphadiazine (100 µg/ml), oleandomycin phosphate (0.5 µg/ml) and polymyxin B sulphate (10 IU/ml), presented as freeze-dried supplements to give a high degree of selectivity and specificity for *Clostridium perfringens*. Sodium metabisulphite and ammonium ferric citrate are used as an indicator of sulphite reduction by *Clostridium perfringens* which produces black colonies on this medium when using a pour plate technique. Tests for confirmation of *Clostridium perfringens* are described in a study initiated by the International Commission on Microbiological Specifications for Foods (I.C.M.S.F.).

Sulphite reducing bacteria other than *Clostridium perfringens* such as salmonellae, *Proteus* spp. and *Citrobacter freundii*, as well as staphylococci and *Bacillus* species, are inhibited on OPSP Agar. Perfringens Agar (OPSP), also, has the advantage of inhibiting growth of *Clostridium bifermentans* and *Clostridium butyricum*. These sulphite reducing organisms grow readily on Shahidi-Ferguson Perfringens Agar (SFP) and Tryptone-Sulphite-Neomycin Agar (TSN) as black colonies with a tendency to spread and obscure the whole surface of the medium.

Occasional strains of *enterococci* will grow on Perfringens Agar (OPSP) as white colonies, easily distinguished from the large black colonies of *Clostridium perfringens*.

Clostridium perfringens enumeration media which include egg yolk in order to detect lecithinase activity have not proved satisfactory partly because *Clostridium perfringens* colonies may frequently fail to produce haloes and thus appear falsely to be negative, and partly because counting is rendered impractical as the organism often grows in the form of large spreading colonies which completely blacken the medium.

Content concentrations

Typical Formula*	**mg/litre**
Perfringens Agar (OPSP)	
Tryptone	15
Yeast extract	5
Soya peptone	5
Liver extract	7
Ferric ammonium citrate	1
Sodium metabisulphite	1
Tris buffer	1.5
Agar	10
Final pH 7.3 ± 0.2 @ 25°C	
Perfringens (OPSP Selective Supplement A	
Sodium sulphadiazine	100
Pefringens (OPSP) Selective Supplement B	
Oleandomycin phosphate	0.5
Polymyxin B	10,000 IU
* Adjusted as required to meet performance standards	

Table 1 - Typical Formula for Perfringens Agar (OPSP), Perfringens (OPSP Selective Supplement A and Pefringens (OPSP) Selective Supplement B

METHOD

Preparation

Suspend appropriate amount of Perfringens Agar (OPSP) to distilled water and bring gently to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Allow to cool to 50°C and aseptically add the contents of Perfringens Agar (OPSP) supplements A and B which have been rehydrated as directed. Mix well and pour into sterile dishes.

Protocol

1. Make up the medium according to the preparation. Prepare pour plates, containing approximately 25 ml per plate, using 1 ml aliquots of a suitable series of dilutions of the homogenised test sample. Mix well before setting.

2. It is unlikely that colonies of *Clostridium perfringens* will blacken if plates are surface-inoculated unless the inoculum is covered with a layer of agar.

3. Incubate the plates at 35°C for 18-24 hours anaerobically.

4. *Clostridium perfringens* may be seen as large black colonies (2-4 mm diameter) within the depth of the agar. Occasional strains of Enterococcus faecalis which may grow on Perfringens Agar (OPSP) as small colourless colonies are easily distinguished from *Clostridium perfringens* .

Quality control

Positive control:

*Clostridium perfringens A*TCC® 13124: Good growth; black coloured colonies with opaque halo

Negative control:

Escherichia coli ATCC® 25922: Inhibited

REFERENCES

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