Dehydrated Culture Media

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PERFRINGENS AGAR (OPSP)

Code: CM0543

For the enumeration of Clostridium perfringens in foods.

Typical Formula*	**gm/litre**
Tryptone	15.0
Yeast extract	5.0
Soya peptone	5.0
Liver extract	7.0
Ferric ammonium citrate	1.0
Sodium metabisulphite	1.0
Tris buffer	1.5
Agar	10.0
pH 7.3 ± 0.2 @ 25°C	

*** Adjusted as required to meet performance standards**

PERFRINGENS (OPSP) SELECTIVE SUPPLEMENT A

Code: SR0076

Vial contents (each vial is sufficient for 500ml of medium)	**per vial**	**per litre**
Sodium sulphadiazine	50.0mg	100.0mg

PEFRINGENS (OPSP) SELECTIVE SUPPLEMENT B

Code: SR0077

Vial contents (each vial is sufficient for 500ml of medium)	**per vial**	**per litre**
Oleandomycin phosphate	0.25mg	0.5mg
Polymyxin B	5,000IU	10,000IU

Directions

Suspend 22.8g in 500ml of 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 one vial each of Perfringens Agar (OPSP) supplements A and B (SR0076 and SR0077) which have been rehydrated as directed. Mix well and pour into sterile dishes.

Description

Oxoid Perfringens Agar (OPSP), is based on the formulation developed by Handford[1]. The medium utilises sulphadiazine (100µg/ml), oleandomycin phosphate (0.5µg/ml) and polymyxin B sulphate (10IU/ml), presented as freeze-dried supplements (SR0076 and SR0077) 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.)[2].

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)[3] and Tryptone-Sulphite-Neomycin Agar (TSN)[4] 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[5].

Technique

1. Make up the medium according to the directions. Prepare pour plates, containing approximately 25ml per plate, using 1ml 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 with an anaerobic Gas Generating Kit pack (BR0038) in a conventional gas-jar. Alternatively use Anaerogen (AN0025/AN0035). Anaerogen does not require the addition of water or a catalyst.
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*.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.
Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder
Prepared medium: Straw coloured gel

Quality control

Positive control:
Expected results
Clostridium perfringens ATCC® 13124
Good growth; black coloured colonies
Negative control:

Clostridium sporogenes ATCC® 19404 *
No growth

*** This organism is available as a Culti-Loop®**

Precautions
The production of black colonies on this medium is a presumptive identification of *Clostridium perfringens*. Further identification tests must be carried out.

References
1. Handford P. M. (1974) *J. Appl. Bact*. 37. 559-570.
2. Hauschild A. H. W., Gilbert R. J., Harmon S. M., O'Keeffe M. F. and Vahlefeld R. (1977) *ICMSF Methods Studies VIII, Can. J. Microbiol.* 23. 884- 892.
3. Shahidi S. A. and Ferguson A. R. (1971) *Appl. Microbiol*. 21. 500-506.
4. Marshall R. S., Steenbergen J. F. and McClung L. S. (1965) *Appl. Microbiol*. 13. 559-562.
5. Hauschild A. H. W. and Hilsheimer R. (1974) *Appl. Microbiol*. 27. 78-82.