Dehydrated Culture Media

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PERFRINGENS AGAR BASE (TSC AND SFP)

Code: CM0587

A basal medium for use either on its own or with selective agents to make Tryptose Sulphite (TS) agar, Tryptose Sulphite Cycloserine (TSC) agar or Shahadi Ferguson Perfringens (SFP) agar for the presumptive identification and enumeration of Clostridium perfringens.

Typical Formula*	**gm/litre**
Tryptose	15.0
Soya peptone	5.0
Yeast extract	5.0
Sodium metabisulphite	1.0
Ferric ammonium citrate	1.0
Agar	19.0

pH 7.6 ± 0.2 @ 25°C

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

PERFRINGENS (SFP) SELECTIVE SUPPLEMENT

Code: SR0093

	SR0093E	
Vial contents (1 vial per 500ml medium)	**per litre**	
Kanamycin sulphate	6.0mg	12.0mg
Polymyxin B	15,000IU	30,000IU

PERFRINGENS (TSC) SELECTIVE SUPPLEMENT

Code: SR0088

	SR0088E	
Vial contents (1 vial per 500ml medium)	**per litre**	
D-cycloserine	200.0mg	400.0mg

Directions

To Prepare the Agar Base

Suspend 23g in 500ml of distilled water and heat gently until the agar is completely dissolved. Sterilise by autoclaving at 121°C for 10 minutes. Allow the medium to cool to 50°C.

To Prepare Tryptose Sulphite Cycloserine Agar (TSC Agar)

To 500ml of Agar base cooled to 50°C add the rehydrated contents of 1 vial of TSC supplement (SR0088) and 25ml of egg yolk emulsion (SR0047). Mix well and pour into sterile Petri dishes.

To Prepare Egg Yolk Free TSC Agar

To 500 ml of Agar base cooled to 50°C add the rehydrated contents of 1 vial of TSC supplement (SR0088). Mix well and pour into sterile Petri dishes.

To Prepare Shahidi-Ferguson Perfringens Agar (SFP Agar)

To 500 ml of Agar base cooled to 50°C add the rehydrated contents of 1 vial of SFP supplement

(SR0093) and 25ml of egg yolk emulsion (SR0047) mix well and pour into sterile Petri dishes.

To Prepare Agar for an Overlay
For TSC or SFP Agar used as an overlay, the egg yolk emulsion (SR0047) is omitted. Its inclusion does not improve the lecithinase reaction and diminishes the visibility of the colonies.

Description
Perfringens Agar Base (TSC and SFP) is a nutrient medium to which egg yolk emulsion (SR0047) and the appropriate antibiotic supplement can be added, to make either Shahidi-Ferguson Perfringens (SFP)[1] Agar or Tryptose Sulphite Cycloserine (TSC)[2] Agar. An egg yolk free TSC Agar had been described[4,5] which has the advantage that smaller colonies are formed. This can simplify the counting of plates with high numbers of colonies.

Higher counts have been demonstrated by using it with a pour-plate technique. The differences were thought to be due to exposure of the *Clostridium perfringens* cells to high oxygen tension in the surface plating procedure[4].

Shahidi-Ferguson Perfringens Agar is based on the formulation developed by Shahidi and Ferguson[1]. The medium utilises kanamycin sulphate (12mg/l) and polymyxin B sulphate (30,000IU/l) as the selective agents, to give a high degree of selectivity and specificity for *Clostridium perfringens*.
Tryptose Sulphite Cycloserine Agar was developed using the same basal medium as SFP Agar[2] but with 400mg/l of D-cycloserine as the selective agent. Sodium metabisulphite and ferric ammonium citrate are used as an indicator of sulphite reduction by *Clostridium perfringens* which produces black colonies in both media.

Trials[3] have indicated that polymyxin B and kanamycin sulphate used in SFP Agar allow a greater recovery of both vegetative cells and spores of *Clostridium perfringens* than either polymyxin B and sulphadiazine used in Sulphite Polymyxin Sulphadiazine Agar, or neomycin used in Tryptone Sulphite Neomycin Agar. However, a greater number of non-specific colonies were found on SFP Agar.

In another study[2], *Serratia marcescens* and *Streptococcus lactis* were the only facultative anaerobes to grow on TSC Agar, whereas SFP Agar also allowed the growth of *Enterococcus, Proteus* and *Enterobacter* strains. However, it also allowed a slightly higher rate of recovery of *Clostridium perfringens* than TSC Agar. Both SFP Agar and TSC Agar permitted growth of other sulphite-reducing *Clostridium* species tested.

Some strains of *Cl. perfringens* may produce an opaque zone around the colony due to lecithinase activity, but this is not considered to be universal for all *Clostridium perfringens* strains after overnight incubation[4], and both black, lecithinase positive and black, lecithinase negative colonies should be considered as presumptive *Clostridium perfringens* on TSC or SFP Agars and confirmatory tests carried out. Lecithinase positive, facultative anaerobes may grow on SFP Agar to produce completely opaque plates that mask the egg yolk reaction of *Clostridium perfringens*.

Technique

1. Make up the medium according to the directions and prepare plates containing approximately 20ml of a basal layer of TSC or SFP Agar containing egg yolk.
2. Prepare 0.1ml aliquots of a suitable series of dilutions of the homogenised test sample and spread over the surface of the basal layer using a sterile swab.Overlay with an additional 10ml of egg yolk free TSC or SFP Agar. Cultures which are not overlaid with agar are unlikely to grow as black colonies.
3. Incubate the plates at 35°C for 18-24 hours with an anaerobic Gas Generating Kit (BR0038) in a gas-jar. Alternatively, use AnaeroGen (AN0025 or AN0035). AnaeroGen does not require the addition of water or a catalyst.
4. Alternatively, pour-plates using approximately 25ml per plate of TSC or SFP Agar containing egg yolk may be prepared using 1ml aliquots of a suitable series of dilutions of the homogenised test sample. Mix the plates well before the agar gels. With this technique, lecithinase activity of *Clostridium perfringens* colonies is more difficult to see. *Clostridium perfringens* colonies may be seen as large, black (2-4mm diameter) colonies within the depth of the agar.

Egg yolk free TSC Agar is used with the techniques described above. *Clostridium perfringens* colonies are black, but, in the absence of egg yolk, no lecithinase activity can be detected.

Tests for confirmation are described in a study initiated by the International Commission on Microbiological Specifications for Foods[6] involving nitrate reduction, lactose fermentation, gelatin

liquefaction and the absence of motility. All black colonies growing on TSC or SFP Agars should be tested.

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/green coloured gel

Quality control

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

Escherichia coli ATCC® 25922 *
Inhibited

* This organism is available as a Culti-Loop®

Precautions
Black colonies appearing on these two media may be organisms other than *Clostridium perfringens*.

References
1. Shahidi S. A. and Ferguson A. R. (1971) *Appl. Microbiol*. 21. 500-506.
2. Harmon S. M., Kauttar D. A. and Peeler J. T. (1971) *Appl. Micobiol*. 22. 688-692.
3. Harmon S. M., Kautter D. A. and Peeler J. T. (1971) *Appl. Microbiol*. 21. 922-927.
4. Hauschild A. H. W. and Hilsheimar R. (1974) *Appl. Microbiol*. 27. 78-82.
5. Hauschild A. H. W. and Hilsheimar R. (1974) *Appl. Microbiol*. 27. 521-526.
6. Hauschild A. H. W., Gilbert R. J., Harmon S. M., O'Keefe M. F. and Vahlfeld R. (1977) *Can. J. Microbiol*. 23. 884-892.