93745 TSC Agar (Tryptose Sulfite Cycloserine Agar, Perfringens Agar)

For the isolation and enumeration of vegetative forms as well as spores from *Clostridium perfringens* in food, clinical specimens and other material acc. to Harmon, et al. (1971).

Composition:

Ingredients	Grams/Litre
Meat peptone	15.0
Soya peptone	5.0
Yeast extract	5.0
Ammonium ferric citrate	1.0
Sodium disulfite	1.0
Agar	15.0
Final pH 7.6 +/- 0.2 at 25°C	

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

Appearance:	Faintly beige, homogeneous, free flowing powder.
Gelling:	Firm.
Colour and Clarity:	Light brownish-yellow coloured, clear gel forms in petri plates.

Directions:

Base Agar:

Dissolve 42 g in 1 litre distilled water. Sterilize by autoclaving at 121°C for 15 minutes. Cooling to approx. 50°C.

TSC Agar:

To 1 litre Base Agar add 400 mg rehydrated D-cycloserine (Sigma 30020) as a sterile filtered aqueous solution
or 2 vials of Perfringens T.S.C. Supplement (P9352) and 50 ml Egg Yolk Emulsion (17148). Mix well and pour into petri plates.

TSC Agar egg yolk free (also used as overlay agar):

Add 400 mg rehydrated D-cycloserine (Sigma 30020) or 2 vials of Perfringens T.S.C. Supplement (P9352)
to 1 litre Base Agar and mix well.

TSC Fluorescence Agar:

Add to 1 litre Base Agar 5.5 g rehydrated TSC Agar supplement (80548) as a sterile filtered aqueous solution. Mix well and pour plates.

SFP Agar (Shahidi-Ferguson Perfringens Agar):

To 1 litre Base Agar 2 vials of Perfringens S.F.P. Supplement (P9477) and 50 ml Egg Yolk Emulsion (17148) are added. Mix well and pour into sterile petri plates.

Principle and Interpretation:
Meat peptone, soya peptone and yeast extract provide essential nutrients and vitamins for the development of clostridia. The H_2S-positive bacteria reduce the sulfite (from Sodium disulfite) in the culture medium to sulfide, which forms a black salt with ammonium ferric citrate (FeS).
Egg yolk promotes the growth of *Cl. perfringens* but for counting of plates it can be an advantage to add no egg yolk and have smaller colonies [4,5,6].
No lecithinase activity can be detected without egg yolk but not all *Clostridium perfringens* strains show an opaque zone after overnight incubation [4]. So, both black lecithinase-positive and black lecithinase-negative colonies should be considered as presumptive *Clostridium perfringens* on TSC or SFP Agar and need further confirmatory tests like nitrate reduction, lactose fermentation, gelatin liquefaction and the absence of motility [8].
Cycloserine inhibits the accompanying bacterial flora. It is the reason why some inhibited colonies appear smaller. It also reduces the diffuse and disturbing blackening around the *Cl. perfringens* colonies. All this can simplify the counting of plates with high numbers of colonies. Higher counts have been demonstrated by using a pour plate technique. The differences are thought to be due to exposure of the *Cl. perfringens* cells to high oxygen tension in the surface plating procedure.
TSC Agar Supplement contains Sucrose, D-cycloserine and 4-Methylumbelliferylphosphate (MUP). *Clostridium perfringens* possess the enzyme phosphatase, which cleaves MUP and results in setting free the fluorogen 4-methylumbelliferone. The fluorogen can be detected under a long wavelength UV lamp (360 nm). Sucrose is a fermentable carbohydrate source for many clostridia. It supports the growth and germination of clostridia. With the addition of indicator e.g. 0.04 g/l bromocresol purple it is possible to differentiate further (Sucrose positives: *Cl. perfringens, Cl. baratii, Cl. paraputrificum, Cl. tertium*; Sucrose negatives: *Cl. biferentans, Cl. difficle, Cl. sporogenes*).

The addition of kanamycin sulfate (12 mg/l; 60615) and polymyxin B sulphate (30,000 IU/l ≈ 3.6 mg/l; 81334) present in the Perfringens S.F.P. Supplement gives a better recovery rate than with cycloserine but still a high degree of selectivity and specificity for *Cl. perfringens* [1,2,3]. It was observed that more unspecific colonies where found on SFP Agar

For incubation are diverse temperatures (35-45°C) recommended. A higher temperature increase the back ground growth but result in a lower recovery rate[13].

Cultural characteristics after 18-24 hours at 44°C (under anaerobic conditions).

Organisms (ATCC)	Growth	Black colony	Lecithinase activity
Clostridium perfringens (13124)	+++	+	+
Clostridium tetani (19406)	-/++	-	
Clostridium novyi (10543)	-/++	-	
Pseudomonas aeruginosa (27853)	-/+	-	
Bacillus cereus (11778)	-/+	-	

References:
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This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.