S.F.P. Agar Base

M1005F

S.F.P. Agar Base with the addition of selective supplement and enrichment is used for the presumptive identification and enumeration of *Clostridium perfringens* in foods in accordance with FDA BAM, 1998.

Composition**

Ingredients	Gms / Litre
Tryptose	15.000
Yeast extract	5.000
Papaic digest of soyabean meal	5.000
Ferric ammonium citrate	1.000
Sodium metabisulfite	1.000
Agar	20.000
Final pH (at 25°C)	7.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 23.5 grams in 460 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C. Add 40 ml of Egg Yolk Emulsion (FD045F) and reconstituted contents of 1 vial of T.S.C Supplement (FD014). Mix well before pouring into sterile Petri plates.

Principle And Interpretation

Clostridium is a large genus of gram-positive spore bearing anaerobes. *Clostridium perfringens* poisoning is one of the common type of food borne illness. Cooked meat or poultry products are considered to be the causative agents of the *C.perfringens* infection (1). Spores of this species in general are resistant to heat and they germinate and grow, during cooking (2). Diarrheoa is the major symptom of *C.perfringens* poisoning, the major etiological agent being a heat-labile Enterotoxin produced by the sporulating cells (3). Enumerating the microorganism in food samples plays an important role in the epidemiological investigation of outbreaks of foodborne illness. S.F.P Agar Base can be used for the presumptive identification and enumeration of *Clostridium perfringens* in foods in accordance with FDA BAM, 1998 (4).

The medium along with the egg yolk emulsion and D-cycloserine which acts as the selective agents give high degree of selectivity for *C.perfringens*. Tryptose, Soya peptone and yeast extract supply nitrogenous compounds, carbon , sulphur, of sulphite reduction by *C. perfringens* , indicated by black colonies. D-cycloserine (FD014) used in the medium inhibit competitive bacteria and thus allowing a better recovery of *C. perfringens* (1).

Mix 25 g sample with 225 ml peptone dilution fluid under aseptic conditions to get uniform suspension (1:10). Make appropriate dilutions up to 10-6 using peptone dilution fluid. Make a layer of S.F.P agar base without egg yolk using 6-7 ml of the medium into 100 x 15 mm Petri dishes. 1 ml of each dilution of the suspension is transferred to the center of duplicate agar plates. Pour additional 15 ml S.F.P agar base without egg yolk or S.F.P agar base with 50% egg yolk emulsion into this and mix with inoculum by gentle rotation. Alternatively, another plate method has also been preferred for foods containing other types of sulfate reducing organisms wherein 0.1 ml of each dilution is spread over pored plates of TSC containing egg yolk emulsion. After inoculum has been absorbed (about 5 min), overlay plates with 10 ml TSC agar without egg yolk emulsion. Solidify and incubate anaerobicaly as per the BAM protocol. Count those plates with 20-200 colonies that are black in colour. *C. perfringens* colonies in egg yolk medium are black with a 2-4 mm opaque white zone surrounding the colony as a result of lecithinase activity. Lecithinase positive facultative anaerobes may grow on S.F.P. Agar making the plates completely opaque and thus may mask the egg yolk reaction of *C. perfringens* . Organisms other than *C. perfringens* may produce black colonies. Therefore presumptive *C. perfringens* colonies need to be further confirmed by motility test, nitrate reduction and gelatin liquefaction.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 2.0% Agar gel.

Colour and Clarity of prepared medium
Basal medium yields amber coloured slightly opalescent gel. With addition of Egg Yolk Emulsion, yellow coloured opaque gel forms in Petri plates.

Reaction
Reaction of the medium (4.7gm in 95 ml distilled water) at 25°C. pH : 7.6±0.2

pH
7.40-7.80

Cultural Response
M1005F: Cultural characteristics observed after an incubation at 35-37°C for 40-48 hours under anaerobic condition with added Egg Yolk Emulsion (FD045) and S.F.P. Supplement (FD013).

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony	Lecithinase
Cultural Response					
Clostridium perfringens ATCC 12924	50-100	luxuriant	>=50%	black	positive reaction, opaque zone around colony
Escherichia coli ATCC 25922	>=10^3	inhibited	0%	-	negative reaction

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

Reference

1.Downes, F.P. and Ito, K. 2001. Methods For The Microbiological Examination of Foods. APHA, Food 4 ed. Washington, D.C.
2.Harmon, S.M., Kauttar, D.A. and Peiler, J.T. 1971. Appl. Microbiol., 22.
3.Duncan, C. L. 1973. J. Bacteriol, 113.
4.FDA, U.S. 1998. Bacteriological Analytical Manual. 8 ed. Gaithersburg, MD: AOAC International.

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