Technical Information

S.F.P. Agar Base

Product Code: DM 2005

Application: - 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.

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

C. perfringens is found in raw meats, poultry, dehydrated soups and sauces, raw vegetables and other foods and food ingredients, but occurrences of foodborne illness are usually associated with cooked meat or poultry products [2]. Spores of some strains that may resist heat during cooking germinate and grow in foods that are not properly refrigerated [3]. A heat-labile enterotoxin produced only by sporulating cells [5] induces the major symptom of diarrhea in perfringens food poisoning. The foods in which conditions are favorable for sporulation may contain enterotoxin. Enumerating the microorganism in food samples and fasces from patient plays a role in the epidemiological investigation &contimation of outbreaks of foodborne illness [2].

Shahidi Ferguson Perfringens (S.F.P.) Agar Base is prepared according to the formulation of Shahidi and Ferguson [1]. Lecithinase and sulphite reactions can be identified on this medium. The medium along with the egg yolk emulsion and the supplement containing kanamycin and polymyxin B as the selective agents give high degree of selectivity for C. perfringens .Tryptose, papaic digest of soyabean meal and yeast extract supply nitrogenous compounds, carbon, sulphur, vitamin B complex etc. necessary for the growth of Clostridia . Sodium bisulphite and ferric ammonium citrate are the indicators of sulphite reduction by C. perfringens, which thereby produces black colonies. Kanamycin and polymyxin B (MS2013) used in the medium inhibit competitive bacteria and thus allowing a better recovery of vegetative cells and spores of C. perfringens than either polymyxin B or sulphadiazine alone [2]. Some strains of C. perfringens may form an opaque zone around the colony due to their 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 tests.

For the isolation and enumeration of C. perfringens from foodstuffs, inoculate the surface of the medium with 0.1 ml of decimal dilutions of the specimen in Peptone Water (DM1028). Allow the media surface to dry for 5-10 minutes. Cover the surface with 10 ml of agar without egg yolk emulsion and solidify [4]. Incubate at 37°C for 20-24 hours in anaerobic conditions. For presumptive count, select and count those colonies, which are larger black and surrounded by an opaque zone.

Methodology

Suspend 23.5 grams of powder media in 475 ml distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C. Add 25 ml of Egg Yolk Emulsion (MS2045) and reconstituted contents of 1 vial of S.F.P. Supplement (MS2013). Mix well before pouring into sterile Petri plates.

Quality Control

Physical 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 range 7.40-7.80

Cultural Response/Characteristics
DM 2005: Cultural characteristics observed after an incubation at 35-37°C for 40-48 hours under anaerobic condition with added Egg Yolk Emulsion (MS2045) and S.F.P. Supplement (MS2013).

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony	Lecithinase
Clostridium perfringens ATCC 12924	50-100	luxuriant	>=50%	Black	Positive reaction opaque zone around colony
Escherichia coli ATCC25922	>=10^3	inhibited	0%	-	Negative reaction

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

1. Shahidi S. A. and Ferguson A. R., 1971, Appl. Microbiol. 21:500.
2. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.
3. Harmon S. M., Kautter D. A. and Peeler J. T., 1971, Appl. Microbiol., 21:922.
4. ICMSF 1978, Microorganisms in food; Their Significance and Methods of Enumeration. University of Toronto Press.264-273.
5. Duncan C. L., 1973, J. Bacteriol., 113:932.

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