PRODUCT SPECIFICATION SHEET

Perfringens Agar Base (T.S.C./S.F.P. Agar Base) (DM566)

Intended Use

Perfringens Agar Base (T.S.C./S.F.P. Agar Base) (DM566) is recommended for presumptive identification and enumeration of *Clostridium perfringens*, with addition of selective supplements and enrichment.

Product Summary and Explanation

Perfringens Agar Base is a nutritious base medium used in the preparation of S.F.P Agar and T.S.C Agar. Depending upon the formula, supplements are added to increase the selectivity of the medium. Tryptose Sulphite Cycloserine Agar (T.S.C) was originally formulated by Harmon et al[1] for the enumeration of *C.perfringens* from food. T.S.C Agar has been documented as one of the most useful media for the quantitative recovery of *C. perfringens* while suppressing growth of other facultative anaerobes.[2] Shahidi-Ferguson Perfringens (S.F.P) Agar is based on the formula by Shahidi and Ferguson.[3] If desired, Egg Yolk Emulsion can be added to either formula. Perfringens Agar Base is also recommended by APHA.[4] Perfringens Agar Base (with 20.0 g/l agar) can be made selective either by addition of D-cycloserine (MS055)[1, 2] or kanamycin and polymyxin B (MS056).[3] T.S.C Agar Base (with MS055) or S.F.P Agar Base (with MS056) is comparable in performance for isolation of *C. perfringens*.[5,6]

Principles of the Procedure

Perfringens Agar Base tryptose, papaic digest of soyabean meal, yeast extract, beef extract which provides nitrogenous compounds, carbon, sulphur, vitamin B complex and trace elements essential for clostridial growth. Sodium metabisulphite increases the aerotolerance of *Clostridium perfringens* by acting as oxygen scavengers. Ferric ammonium citrate acts as an indicator of sulphite reduction, indicated by black coloured colonies. D-Cycloserine (MS055), kanamycin and polymyxin B (MS056) help in the selective isolation of *C. perfringens* by inhibiting accompanying flora. The addition of Egg Yolk Emulsion may produce lecithinase activity, causing an opaque zone around the colony. Homogenized food samples can be directly streaked on the surface of plates or can be pre-enriched in Cooked Meat Medium (DM248) before streaking.

Formula / Liter

Ingredients	Gms / Liter
Tryptose	15.00
Beef extract	5.00
Papaic digest of soyabean meal	5.00
Yeast extract	5.00
Sodium metabisulphite	1.00
Ferric ammonium citrate	1.00
Agar	15.00
Final pH:(at 25°C) 7.6±0.2	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

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Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 23.50 grams of the medium in 475 ml of distilled water.
2. Heat to boiling, to dissolve the medium completely. Distribute into tubes as desired.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Cool to 50°C. Add 25 ml of Egg Yolk Emulsion (MS038) and rehydrated contents of 1 vial of Perfringens S.F.P. Supplement (MS055) / Perfringens T.S.C. Supplement (MS056).
5. Alternatively if fluorogenic detection is desired add rehydrated contents of Clostridium perfringens supplements (MS078) instead of MS055 / MS056.
6. Mix well before pouring into sterile Petri plates.

Quality Control Specifications

Dehydrated Appearance	Light yellow to brownish yellow homogeneous free flowing powder
Prepared Medium	Basal medium : Amber coloured clear to slightly opalescent gel After Addition of Egg Yolk Emlusion (MS038) : Yellow coloured opaque gel forms in Petri plates
Reaction of 4.7% w/v aqueous solution	pH 7.6 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response: Cultural characteristics observed under anaerobic condition with added T.S.C Supplement (MS056)/S.F.P Supplement (MS055) and Egg Yolk Emulsion (MS038), after an incubation at 35-37°C for 18-24 hours.

Sr. No.	Organisms	Results to be achieved					
		Inoculum (CFU)	Growth	Recovery	Sulphite Reduction	Lecithinase/ Haloes	Fluorescence
1.	*Clostridium perfringens ATCC 12924*	50-100	Luxuriant	>=50%	positive, blackening of medium	positive reaction, opaque zone around the colony	positive reaction
2.	*Clostridium sordellii ATCC 9714*	>=10^3	inhibited	0%	-	-	-

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures for a complete discussion on the isolation and identification of *C. perfringens* and other anaerobic bacteria.

Results

1. *Clostridium perfringens* produce black colonies on T.S.C Agar and S.F.P Agar.
2. If Egg Yolk Emulsion is added, colonies may have an opaque halo around the black colony due to lecithinase activity.
3. All black colonies should be confirmed.
4. Cultures which are not overlaid with agar are unlikely to produce black colonies.

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Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Both black lecithinase-positive and black lecithinase-negative colonies should be considered as presumptive *Clostridium perfringens* on T.S.C or S.F.P Agars. Perform confirmatory tests.
2. Egg yolk positive facultative anaerobes may grow on S.F.P Agar producing completely opaque plates, and covering up the egg yolk reaction of *Clostridium perfringens*.

Packaging

Product Name : Perfringens Agar Base (T.S.C./S.F.P. Agar Base)
Product Code : DM566
Available Pack sizes : 500gm

References

1. Harmon S. M., Kauttar D.A. and Peiler J. T., 1971, Appl. Microbiol., 22:688-692.
2. Harmon S. M. and Kautter D.A., 1987, J. Asso. Off. Anal. Chem., 70: 994.
3. Shahidi S. A. and Ferguson A R., 1971, Appl. Microbiol., 21,500
4. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th ed., American Public Health Association, Washington, D.C.
5. Horwitz, (Ed.), Official Methods of Analysis of AOAC International, 17th Ed., AOAC International, Gaithersburg, Md.
6. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.

Further Information

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DM566PSS, QAD/FR/024,Rev.00/01.01.2018

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		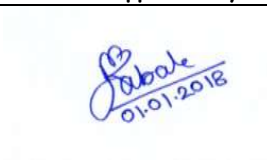
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