Perfringens Agar Base (T.S.C.)

M837I

Intended use

Recommended for the enumeration of *Clostridium perfringens* from food. The composition and performance criteria of this media are as per the specifications laid down in ISO 7937:2004.

Composition**

ISO Specification-Perfringens Agar Base(T.S.C.)

Ingredients	g / L
Enzymatic digest of protein	15.000
Enzymatic digest of soya	5.000
Yeast extract	5.000
Disodium disulphite ($Na_2S_2O_5$),anhydrous	1.000
Ammonium iron(III) citrate	1.000
Agar	9.000-18.000
Final pH (at 25°C)	7.6±0.2

Supplements to be added after autoclaving	g/ L
D-Cycloserine	0.400

Perfringens Agar Base(T.S.C.) M837I

Ingredients	g / L
Tryptose#	15.000
Soya peptone##	5.000
Yeast extract	5.000
Sodium metabisulphite	1.000
Ferric ammonium citrate	1.000
Agar	15.000
Final pH (at 25°C)	7.6±0.2

FD014 - 2 vials

T.S.C. Selective Supplement

Ingredients	Concentration
D-Cycloserine	200mg

**Formula adjusted, standardized to suit performance parameters.

Key : # Equivalent to Enzymatic digest of protein ## Equivalent to Enzymatic digest of soya

Directions

Suspend 21.0 gram in 500 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45 - 50°C and add rehydrated contents of one vial of T.S.C. Selective Supplement (FD014). Alternatively if fluorogenic detection is desired add rehydrated contents of CMF Selective Supplement (FD243). Mix well before pouring into sterile Petri plates.

Principle And Interpretation

Tryptose Sulphite Cycloserine Agar (TSC) was originally formulated by Harmon et al (1) for the enumeration of *C.perfringens* from food. TSC 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). Perfringens Agar Base is also recommended by APHA (3) and ISO Committee(4,5) for enumeration of *C.perfringens* from foods.

Tryptose, soya peptone, yeast extract, provide nitrogenous and carbonaceous compounds, long chain amino acids, vitamin B complex and trace elements essential for clostridial growth. Sodium metabisulphite and ferric ammonium citrate act as an indicator of sulphite reduction, indicated by black coloured colonies. D-cycloserine (FD014) help in the selective isolation of *C.perfringens* by inhibiting accompanying flora. Homogenized food samples can be directly streaked on the surface of plates or can be pre-enriched in Cooked Meat Medium (M149) before streaking.

Type of specimen

Food and animal feed stuffs

Specimen Collection and Handling:

(4,5)

Processesing: Pour 10ml to 15 ml of the T.S.C. agar maintained at 44ºC to 47ºC in the water bath into the dish and mix well with the inoculum by gently rotating each dish. When the medium has solidified, add an overlayer of 10ml of the same T.S.C. agar. Allow to solidify. Place the plates in modifies atmosphere jars or other suitable containers and incubate under anaerobic conditions at 37ºC for 20 h ± 2h. Longer incubation may result in excess blackening of the plates. **Counting and selection of colonies:** After the specified period of incubation select all plates containing less than 150 colonies. From these, select if possible, plates representing successive dilutions.

Count on each plate the characteristic colonies of presumptive *C. perfringens*.

Select five characteristic colonies and confirm them using one of the techniques described in biochemical confirmation.

Biochemical Confirmation:

I: Confirmation technique using Lactose Sulphite Broth Base (M1287):

Inoculation and Incubation:

Inoculate each selected colony into the Fluid Thioglycollate Medium (M009). Incubate under anaerobic conditions at 37ºC for 18 h to 24 h.

After incubation, tranfer with no delay 5 drops of the thioglycollate culture to the Lactose Sulphite Broth Base (M1287) by means of a sterile pipette. Incubate aerobically at 46ºC for 18 h to 24 h in the water bath.

Interpretation of the result:

Examine the tubes of Lactose Sulphite Broth Base (M1287) for the production of gas and the presence of a black color (iron sulfite precipitate). Durham tubes more than one-quater full of gas and tubes having a black precipitate are considered positive.

In case of doubt, when the Durham tube in a blackened medium is less than one-quater full of gas, transfer with no delay, using a sterile pipette, 5 drops of the previous growth in Lactose Sulphite Broth Base (M1287) to another tube of Lactose Sulphite Broth Base (M1287). Incubate in the water bath at 46ºC for 18 h to 24 h. Examine this tube as described above.

Bacteria which form characteristic colonies in the T.S.C. medium are considered as being *C. perfringens.* In all other cases, the tubes should be considered as negative.

II: Confirmation technique using Motility Nitrate Medium, Buffered (M630I):

Inoculation and reading of nitrate motility medium:

Stab-inoculate each selected colony into the freshly deaerated Motility Nitrate Medium, Buffered (M630I).

Incubate under anaerobic conditions at 37 °C for 24 h. Examine the tube of the nitrate motility medium for the type of growth along the stab line. Motility is evident from diffuse growth out into the medium away from the stab line.

Test for the presence of nitrite by adding, with the graduated pipette and the rubber bulb, 0.2 ml to 0.5 ml of the nitrite detection reagent (5.6) to each tube of nitrate motility medium.

The formation of a red colour confirms the reduction of nitrate to nitrite. If no red colour is formed within 15 min, add a small amount of zinc dust and allow to stand for 10 min. If a red colour is formed after the addition of zinc dust, no reduction of nitrate has taken place.

Inoculation and reading of Lactose Gelatin Medium, Modified (M987I):

Inoculate each selected colony into the freshly deaerated lactose-gelatin medium (5.8). Incubate under anaerobic conditions at 37 °C for 24 h.

Examine the tubes of the lactose-gelatin medium for the presence of gas and a yellow colour (due to acid formation) indicating fermentation of lactose. Chill the tubes for 1 h at 5°C and check for gelatin liquefaction. If the medium has solidified, re-incubate for an additional 24 h and again check for gelatin liquefaction.

Interpretation:

Bacteria that produce black colonies in T.S.C. media (M837I), are non-motile, usually reduce nitrate to nitrite, produce acid and gas from lactose, and liquefy gelatin in 48 h are considered to be *C. perfringens.* Cultures that show a faint reaction for nitrite (i.e. a pink colour) shall be eliminated, since *C.perfringens* consistently gives an intense and immediate reaction.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. Pre-enrichment may be required.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to brownish yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Reaction
Reaction of 4.2% w/v aqueous solution at 25°C. pH : 7.6±0.2
pH
7.40-7.80
Cultural Response
Productivity: Cultural characteristics observed in anaerobic atmosphere with added T.S.C. Selective Supplement (FD014), after an incubation at 37±1°C for 20 ± 4 to 44 ± 4 hours. Recovery rate is considered as 100% for bacteria growth on Reference Medium - Soyabean Casein Digest Agar.
Selectivity: Cultural characteristics observed in anaerobic atmosphere with added T.S.C. Selective Supplement (FD014), after an incubation at 37±1°C for 20 ± 4 to 44 ± 4 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Characteristic reaction
Productivity				
*Clostridium perfringens ATCC 1*3124 (00007)	50-100	luxuriant	>=50%	Black colonies
Clostridium perfringens ATCC 12916 (00080)	50-100	luxuriant	>=50%	Black colonies
Selectivity				
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited		
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited		

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Reference

1.Harmon S. M., Kauttar D.A. and Peiler J. T., 1971, Appl. Microbiol., 22:688.
2.Harmon S. M. and Kautter D.A., 1987, J. Asso. Off. Anal. Chem., 70: 994.
3.Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
4.Microbiology of food,animal feeding stuffs and water- Preparation, production,storage and performance culture media, EN ISO 11133:2014 /Amd. 1:2018 (E).
5.International Organization for Standardization (ISO- 7937:2004) : Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of Clostridium perfringens- Colony count technique
6.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
7.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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