HIMEDIA

Technical Data

Tryptone Yeast Sodium Sulphite Agar Base

M2046I

Intended Use:

Recommended for the enumeration of *Clostridium perfringens* from water samples. The composition and performance criteria of this medium are as per the specifications laid down in ISO 14189:2013.

Composition**

ISO 14189:2013-Tryptose Sulphite Cycloserine Agar (TSC)

Ingredients	g/ L
Enzymatic digest of casein	15.000
Enzymatic digest of soya	5.000
Yeast extract	5.000
Sodium metabisulphite	1.000
Ferric ammonium citrate	1.000
Agar	9.00 -18.00
Final pH (complete medium, at 25°C)	7.6±0.2
Supplement to be added after autoclaving	**g / L**
D-cycloserine	4.000

Tryptone Yeast Sodium Sulphite Agar Base M2046I

Ingredients	g/ L
Tryptone #	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
T.S.C. Supplement - FD014	**mg / vial**
D-Cycloserine	200mg

Key : # - Enzymatic digest of casein, ## Enzymatic digest of soya

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 21.0 grams 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. Aseptically add the rehydrated contents of one vial of T.S.C. Selective Supplement (FD014). Mix well and pour 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). Tryptone Yeast Sodium sulphite Agar Base has been recommended by the ISO Committee for the isolation of *C.perfringens* from water samples using membrane filtration technique (3). Tryptone, Soya peptone and yeast extract provide nitrogenous compounds, carbon, long chain amino acids, sulphur, 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. The water sample to be tested is filtered through 0.45 micron filter membrane and the membrane filter is then placed on Tryptone Yeast Sodium sulphite Agar Base and incubated anaerobically at 43-45°C for 18-24 hours. Sulfite reacts with ferric salt to produce sulfide which results in production of black or grey to yellow brown colonies. Confirmatory test : Smear some growth of 24 hours old culture of *Clostridium perfringens* from Blood Agar / Columbia Agar Base/ Tryptone Soya Agar (incubated anaerobically at 34-38°C) on sterile filter paper. Add 2-3 drops of Acid phosphatase Reagent (R096) on to the colonies of filter paper, observe for appearance of strong purplish colour developed within 3-4 min which is positive reaction for *Clostridium perfringens*.

Type of specimen

Water samples

Specimen Collection and Handling:

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (3,4).

After use, contaminated materials must be sterilized by autoclaving before discarding.

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. For further identification confirmatory test is highly recommended.

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

Colour and Clarity of prepared medium

Amber coloured clear to slightly opalescent gel forms in Petri plates.

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 response was observed after an incubation (anaerobic atmosphere)at 44 ± 1°C for 21 ± 3 hours, with added sterile T.S.C. Selective Supplement (FD014). Recovery rate is considered as 100% for bacteria growth on Reference medium - Soyabean Casein Digest Agar (Tryptone Soya Agar).

Selectivity : Cultural response was observed after an incubation (anaerobic atmosphere) at 44 ± 1°C for 21 ± 3 hours, with added sterile T.S.C. Selective Supplement (FD014).

Organism	Inoculum (CFU)	Growth	Recovery^	Sulphite Reduction	Acid phosphatase test $
Productivity					
Clostridium perfringens ATCC 12916 (00080*)	50-100	luxuriant	>=50%	positive, black coloured colonies	Positive Reaction
Clostridium perfringens ATCC 13124 (00007*)	50-100	luxuriant	>=50%	positive, black coloured colonies	Positive Reaction
Clostridium perfringens ATCC 10543 (00174*)	50-100	luxuriant	>=50%	positive, black coloured colonies	Positive Reaction
Selectivity					
** *Bacillus spizizenii* ATCC 6633 (00003*)	>=10^4	inhibited	0%		

Key : (*) - Corresponding WDCM numbers

(**) - Formerly known as *Bacillus subtilis* subsp. *spizizenii*

($) - Acid phosphatase test - Positive reaction : Development of purplish colour within 3-4 minutes on addition of R096 -(Acid phosphatase reagent)

(^) - Recovery obtained for productivity is >=70% when compared to a previously validated batch of Tryptone Yeast Sodium Sulphite Agar Base

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 in order 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 (5,6.

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. International Organization for Standardization (ISO- 14189:2013) Water quality - Enumeration of Clostridium perfringens Method using membrane filtration
4. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6. 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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