

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

MV837

Intended Use:

Perfringens Agar Base with the addition of selective supplement and enrichment, it is used for the presumptive identification and enumeration of *Clostridium perfringens*.

Composition**

Ingredients	g / L
HiVeg™ hydrolysate No. 1	15.000
HiVeg™ extract	5.000
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

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 23.5 grams in 475 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°) for 15 minutes. Cool to 45-50°C. Add 25 ml of Egg Yolk Emulsion (FD045) and rehydrated contents of 1 vial of S.F.P. Selective Supplement (FD013) / T.S.C. Selective Supplement (FD014). Alternatively if fluorogenic detection is desired add rehydrated contents of CMF Selective Supplement (FD243) instead of FD013/FD014. 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). TSC Agar Base (with FD014) or SFP Agar Base (with FD013) is comparable in performance for isolation of *C. perfringens* (3,4) Perfringens Agar Base is also recommended by APHA (5). Perfringens Agar Base can be made selective either by addition of D-cycloserine (FD014) (1, 2) or Kanamycin and Polymyxin B (FD013) (6). Perfringens HiVeg™ Agar Base is prepared by using vegetable peptones in place of animal based peptones which make the media free of BSE/TSE risks.

HiVeg™ hydrolysate No. 1, Soya peptone, yeast extract, HiVeg™ extract provide nitrogenous compounds, carbon, 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), Kanamycin and Polymyxin B (FD013) help in the selective isolation of *C. perfringens* by inhibiting accompanying flora. Egg yolk emulsion serves as a source of lecithin utilized by *C. perfringens* (MV837).

Type of specimen

Food samples

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5). 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. Further biochemical and serological tests must be carried out for further identification.
2. Some organism may show poor growth due to nutritional variation.

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

Basal medium :Amber coloured clear to slightly opalescent gel. After Addition of Egg Yolk Emulsion (FD045) : Yellow coloured opaque gel forms in Petri plates

Reaction

Reaction of 4.7% w/v aqueous solution at 25°C. pH : 7.6±0.2

pH

7.40-7.80

Cultural Response

Cultural characteristics observed under anaerobic condition with added T.S.C. Selective Supplement (FD014)/S.F.P. Selective Supplement (FD013)/CMF Selective Supplement (FD243) and Egg Yolk Emulsion (FD045), after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Sulphite Reduction	Lecithinase/ Haloes	Fluorescence
<i>Clostridium perfringens</i> ATCC 12924	50-100	luxuriant	≥50%	positive, blackening of medium	Positive reaction, opaque zone around the colony	Positive Reaction
<i>Clostridium sordellii</i> ATCC 9714	≥10 ⁴	inhibited	0%			

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 (4,5).

Reference

1. Harmon S. M., Kauttar D.A. and Peiler J. T., 1971, Appl. Microbiol., 22:688.
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3. Horwitz, (Ed.), Official Methods of Analysis of AOAC International, 17th Ed., AOAC International, Gaithersburg, Md.
4. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
5. 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.

6. Shahidi S. A. and Ferguson A R., 1971, Appl. Microbiol., 21,500.
7. Isenberg (Ed.), 1992, Clinical Microbiology Procedures Handbook, American Society for Microbiology, Washington, D.C.
8. 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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