T.S.C. Agar Base (Tryptose Sulfite Cycloserine) ISO

Cat. No. MM-M-029

For the detection and enumeration of Clostridium perfringens from foods.

Principles and uses:

T.S.C. Agar Base (Tryptose Sulfite Cycloserine) was originally formulated by Harmon for presumptive identification and enumeration of Clostridium perfringens from food. This medium has been documented as one of the most useful media for the quantitative recovery of C. perfringens, while suppressing growth of other facultative anaerobes. This media is recommended by ISO Normative Committee. Depending on the formula, supplements are added to increase the selectivity of the medium. Egg Yolk Emulsion (Cat. MMS-M-E152) supplement is added for the demonstration of lecithinase activity, and after incubation, lecithinase-producers produce an opaque area in the colony surroundings.
The superior nutrient base provides optimal conditions for the development of Clostridia. Tryptose and soy peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group, essential for bacterial growth. Ferric ammonium citrate and disodium disulfite are H2S indicators. Bacteriological agar is the solidifying agent. Cycloserine inhibits the accompanying bacterial flora and may cause the colonies which develop to remain smaller.
Colonies producing hydrogen sulfide are characterized by a blackening due to the reaction with the ferric salt. The degradation of the egg yolk lecithin produces insoluble products which accumulate around the colonies, forming a white precipitate. After 24 hours incubation, all black colonies, lecithinase positive as well as the lecithinase negative ones, have to be considered as positive presumptive C. perfringens and the corresponding confirmation tests have to be made.

Formula per Litre:

Enzymatic digest of casein	15g	Bacteriological agar	15g
Disodium disulfite (Anhydrous)	1.0g	Ferric ammonium citrate	1.0g
Yeast extract	5.0g	Enzymatic digest of soya	5.0g

Preparation:

Suspend 42 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 ºC for 15 minutes. Cool the medium according to the applicable normative and aseptically add two vials of Clostridium perfringens Selective Supplement (Cat. MMS-M-X020) reconstituted (each one) in 5 ml of warm sterile distilled water. If desired, 25 ml of Egg Yolk Emulsion Supplement (Cat. MMS-M-E152) can be added (Not indicated in ISO Normative). Homogenize gently and dispense into Petri dishes.

Instructions for use:

Enumeration of Clostridium perfringens in food according to ISO 7937:

- Transfer 1 ml of the initial suspension to the empty Petri dishes.
- Pour 10 to 15 ml of the TSC Agar (44-47ºC) into the dish and mix with the inoculum by gently rotating each dish. When the medium has solidified, add an overlayer of 10 ml of the TSC agar.
- Incubate under anaerobic conditions at 37ºC for 20±2 hours.
- After 24 hours incubation, all black colonies, lecithinase positive as well as the lecithinase negative ones, have to be considered as positive presumptive C. perfringens and the corresponding confirmation tests have to be made.

Enumeration of Clostridium perfringens in water samples according to ISO 14189:

- Filter a measured volume of water, to yield between 10-80 colonies on membrane.
- Place the membrane on a TSC Agar plate.
- Incubate the plates with the filters, under anaerobic conditions at 44±1ºC for 21±3 hour, inverted.
- After 24 hours incubation, all black colonies, lecithinase positive as well as the lecithinase negative ones, have to be considered as positive presumptive C. perfringens and the corresponding

confirmation tests have to be made.

Quality control:

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Amber, slightly opalescent	7.6 ± 0.2

Microbiological test:

According to ISO 11133, Food microbiology: Clostridium perfringens and Escherichia coli. Incubation conditions: (37±1ºC, anaerobic atmosphere / 20±2 h).
Inoculation conditions: Productivity quantitative (100±20. Min. 50 CFU) / Selectivity (10^4-10^6 CFU). Reference media: TSA
According to ISO 11133, Water microbiology: Clostridium perfringens and Bacillus subtilis. Incubation conditions: (44±1ºC, anaerobic atmosphere / 21±3h).
Inoculation conditions: Productivity quantitative (100±20. Min. 50 CFU) / Selectivity (10^4-10^6 CFU).

Reference media: TSA

Microorganisms	Specification	Characteristic reaction
Clostridium perfringens ATCC 13124	Good growth > 50%	Black colonies
Escherichia coli ATCC 25922	Total inhibition	
Bacillus subtilis ATCC 6633	Total inhibition	

Storage:

Temperature: 2ºC - 25ºC

Bibliography:

Sahidi S.A. and Ferguson A.R. (1971) Appl. Microbiol, 21 500-506. Harmon S.M., Kauttar D.A. and Peeler J.T. (1971) Appl. Microbiol. 21 922-927. Hauschild A.H.W. and Hilsheimar R. (1973) Appl. Microbiol. 27. 78-82.
International standard ISO 7937 Microbiology of food and animal feeding stuffs-Horizontal method for enumeration of Clostridium perfringens –colony count technique
International standard ISO 14189 Water quality — Enumeration of Clostridium perfringens — Method using membrane filtration