

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

Cat. 1029

For the detection and enumeration of *Clostridium perfringens* from foods.

Practical information

Applications	Categories
Selective enumeration	<i>Clostridium perfringens</i>
Detection	<i>Clostridium perfringens</i>

Industry: Water / Food

Regulations: ISO 11133 / ISO 14189 / ISO 7937

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. 5152) 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 in g/L

Enzymatic digest of casein	15	Bacteriological agar	15
Disodium disulfite (Anhydrous)	1	Ferric ammonium citrate	1
Yeast extract	5	Enzymatic digest of soya	5

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 °C for 15 minutes. Cool the medium according to the applicable normative and aseptically add two vials of *Clostridium perfringens* Selective Supplement (Cat. 6020) reconstituted (each one) in 5 ml of warm sterile distilled water. If desired, 25 ml of Egg Yolk Emulsion Supplement (Cat. 5152) 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 (44-47 °C) 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 37 °C 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±1 °C 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 (25°C)
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±1 °C, anaerobic atmosphere / 20±2 h).

Inoculation conditions: Productivity quantitative (100±20. Min. 50 CFU) / Selectivity (10⁴-10⁶ CFU).

Reference media: TSA

According to ISO 11133, Water microbiology: *Clostridium perfringens* and *Bacillus subtilis*.

Incubation conditions: (44±1 °C, anaerobic atmosphere / 21±3h).

Inoculation conditions: Productivity quantitative (100±20. Min. 50 CFU) / Selectivity (10⁴-10⁶ CFU).

Reference media: TSA

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

Storage

Temp. Min.: 2 °C

Temp. Max.: 25 °C

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 Hilsheimer 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