

Clostridium Perfringens Supplement (TSC)

Cat. 6020

Selective supplement for the enumeration of Clostridium perfringens.

Practical information

Applications	Categories
Selective enumeration	Clostridium perfringens
Detection	Clostridium perfringens

Industry: Water / Food

Regulations: ISO 14189 / ISO 7937

Principles and uses

T.S.C. AGAR BASE was originally formulated by Harmon for presumptive identification and enumeration of Clostridium perfringens from water and 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. T.S.C Agar Base is recommended by ISO Normatives 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. After incubation, lecithinase-producers generate an opaque area in the colony surroundings.

The 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 generates 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 vial

D-Cycloserine (mg)	200
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Preparation

Aseptically reconstitute 1 vial of Clostridium perfringens Supplement (Cat. 6020) with 5 ml of warm sterile distilled water. Mix gently until complete dissolution and aseptically add to 500 ml of T.S.C. Agar Base (Cat. 1029), autoclaved and cooled to 44-47 °C. If desired, add 25 ml of Egg Yolk Emulsion (Cat. 5152) (not indicated in ISO Normative). Mix well and distribute into sterile containers.

Instructions for use

Colony count technique, Food microbiology 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.

Method using membrane filtration, Water microbiology 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	Lyophilized tablet	N/A	Transparent	

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

Microrganisms	Specification	Characteristic reaction
Clostridium perfringens ATCC 12916	Good growth >50%	Black colonies
Clostridium perfringens ATCC 13124	Good growth > 50%	Black colonies
Escherichia coli ATCC 25922	Total inhibition	
Bacillus subtilis ATCC 6633	Total inhibition	

Storage

Temp. Min.:2 °C

Temp. Max.:8 °C

Bibliography

Hauschild, A.H.W., Hilsheimer, R., and Griffith, D.W. 1974. Enumeration of faecal Clostridium perfringens spores in egg yolk - free Tryptose – Sulfite – Cycloserine Agar. Appl. Microb., 27:527-530.

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.

NF T 90-415: October 1985. Water testing. Detection and enumeration of the spores of sulfite-reducing anaerobies and of sulfite-reducing Clostridia. General method by the standing tube technique.