TSC without Cycloserin/Agar
(Tryptone-Sulfite-Cycloserin)

355-4419
356-9644

DEFINITION
Base agar medium used for the detection and enumeration of sulfite-reducing anaerobic bacteria in food products and in water.

STANDARDS
FOOD MICROBIOLOGY
- **NF ISO 15213 (September 2003)**: Food Microbiology. Horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions.
- **NF EN ISO 7937 (February 2005)**: Food Microbiology. Horizontal method for the enumeration of *Clostridium perfringens* - Colony count technique.
- **Obligatory microbiological criteria for foodstuffs of animal origin - General methods of bacteriological analysis** (Decree of 21 December 1979 in JO dated 19 January 1980, amended by Decrees of: 7 September 1984 in JO dated 29 September 1984; 5 March 1985 in JO dated 23 March 1985; 2 June 1988 in JO dated 8 July 1988; 13 March 1989 in JO dated 20 April 1989).

WATER
- **ISO/DIS 6461-2 (June 2005):** Water quality - Detection and enumeration of *Clostridium perfringens*. - Part 2: Membrane filtration method (ISO 6461-2: 1986 review).
- **NF EN 26461-2 (July 1993):** Water quality - Detection and enumeration of spores of sulfite-reducing anaerobic micro-organisms *(Clostridia)*. Part 2: Membrane filtration method.
- **NF T90-415 (October 1985):** Water test - Detection and enumeration of spores of sulfite-reducing anaerobic bacteria and of *Clostridium* - General method by incorporation of agar in deep tubes.
- **Official Journal of the European Communities - Council Directives 98/83/EC of Commission of 3 November 1998** on the quality of water intended for human consumption - Annex III: Specifications for the analysis of parameters.
- **NF T90-461/A2 (May 2007):** Water quality - Microbiology - Quality control for culture media.

PRINCIPLE
The principle of the medium relies on the ability of *Clostridium* to reduce sulfites to sulphide which, in the presence of ferric citrate, forms a black precipitate of iron sulfide around the colonies. Due to the presence of D-cycloserin, the medium inhibits other bacteria.
Incubation at 46°C enhances the selectivity of the medium with regard to *Clostridium perfringens*.

PRESENTATION
Base medium
- **Ready-to-use**
  100 ml x 6 bottles **code 355-4419**
- **Dehydrated**
  500 g **code 356-9644**

STORAGE
- Dehydrated: + 15°C to 25°C, in carefully-sealed bottles in a cool, dry place.
- Ready-to-use: + 2°C to 8°C
- Expiration date and batch number are shown on the package.

THEORETICAL FORMULA

Peptone	15 g
Soy peptone	5 g
Yeast extract	5 g
Na_2SO_5	1 g
Ferric ammonium citrate III	1 g
Agar	15 g
Distilled water	1,000 ml

Final pH $_{(25°C)}$ = 7.6 ± 0.2

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)
- Distilled water
- 4% solution of D-cycloserin sterilized by filtration (ISO/DIS 6461-2 and ISO 7937)

EQUIPMENT REQUIRED (NOT SUPPLIED) (non-exhaustive)
- Scales
- Sterile weighing bags
- Grinder
- Hotplate
- Mixer-homogenizer

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- Test tubes (16 x 160 mm) with autoclave proof stoppers
- 150 ml Pyrex bottles with autoclave-proof stoppers
- Sterile Petri dishes (ø = 90 mm)
- Sterile pipettes (1 ml, etc)
- Equipment for anaerobiotic culture (cloche, catalyser, etc)
- Water-bath precise to ± 1°C
- Thermostatically-controlled incubator or incubation room, precise to ± 1°C
- Autoclave
- All usual laboratory equipment.

PREPARATION OF DEHYDRATED MEDIUM

Base medium

Always shake well before use.

Dissolve 42 g of powder in 1 liter of distilled water. Wait for 5 minutes, then mix thoroughly until a homogenous suspension is obtained.
Heat gently, swirling frequently, then bring to boiling point until completely dissolved.
Dispense in tubes or bottles (100 ml) and sterilize in autoclave at 121°C (± 1°C) for 15 minutes.
Reconstitution ratio: 42 g/l
500 g of powder makes 11.9 liters of base medium.

Base medium + cycloserin

At the moment of use, add 100 ml of base medium, melted and cooled to 44°C - 49°C according to the standards, 1 ml of a 4% solution of D-cycloserin sterilized by filtration.

Base medium + cycloserin + egg yolk

Egg yolk can be added to the aforementioned formula 8 ml of a 50% solution of egg yolk in physiological water for 100 ml of the aforementioned preparation.
In these cases, decimal dilutions of the product are spread at 0.1 ml per plate.

PROTOCOL

• Preparation of samples

According to the standards applicable to the product concerned.

• Inoculation and incubation

Food standards

Depth inoculation: (NF V 08-019)
Place 1 ml of stock solution or each decimal dilution in 2 Petri dishes and pour 12 - 15 ml of melted agar cooled to 44° - 49°C, depending on the standards. Homogenize and leave to solidify. Pour another layer of agar (about 5 ml). Leave to dry.
Incubate in anaerobiosis at 37°C ± 1°C for 20 hours.

Water standards

• Inoculation in tubes: (NF T90-415)
Mix a tube of test specimen with a tube of melted medium (20 ml) cooled to 44°C - 49°C, depending on the standards. Incubate at 37°C ± 1°C for 24 and/or 48 hours.

• Membrane-filtration technique: (NF EN 26461-2)
Filter the solution and recover the membrane. Place it in a Petri dish (top side uppermost), avoiding the inclusion of air bubbles under the filter.
Pour about 18 ml of TSC without D-cycloserine medium at 44°C - 49°C, depending on the standards. Allow to cool on a fresh and horizontal surface.
Incubate in anaerobiosis at 37°C ± 1°C for 24 and 48 hours.

• Membrane-filtration method: (ISO/DIS 6461-2 *)
Filter a measured volume of water avoiding the formation of bubbles between the membrane and the filtration apparatus.
Recover the membrane and place it grid side up on a Petri dish containing TSC with D-cycloserin, avoiding the inclusion of air bubbles under the filter.
Incubate in anaerobiosis at 44°C ± 1°C for 21 h ± 3 hours.

N.B.: ISO/DIS 6461-2 standard is a working draft. It has to be published and approved at the national levels to come into effect.*

READING AND INTERPRETATION

The colonies of sulfite-reducing anaerobes are black with a black halo.
Only dishes presenting colonies that are clearly distinct from one another should be used for enumeration.
For the specific enumeration of *C. perfringens*, further tests should be carried out on a representative number of typical colonies.

N.B.: When egg yolk is present in the medium, colonies of *C. perfringens* are black and usually surrounded by an opaque halo caused by the lecithinase of this bacterium.

PRECAUTIONS

- D-cycloserin should not be added to a base medium at a temperature exceeding 49°C.
- To avoid re-oxygenation of the medium, it should not be stirred violently.
- Comply with Good Laboratory Practice.

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PERFORMANCES / QUALITY CONTROL OF THE TEST

The growth performances of the media are verified with the following strains:

STRAINS	Results after 20h culture at 37°C (anaerobic atmosphere)	
Productivity		
Clostridium perfringens ATCC 13124	**Appearance**	Black colonies
	PR	≥ 0.7
Clostridium perfringens ATCC 12916	**Appearance**	Black colonies
	PR	≥ 0.7
Selectivity		
Escherichia coli ATCC 25922	**Growth**	No growth

*** PR = Total colony count obtained on 2 plates of TSC/total colony count on 2 plates of TCS agar.**

STRAINS	Results after 48h culture at 37°C (anaerobic atmosphere)	
Clostridium perfringens ATCC 13124	**Appearance**	Black colonies
Escherichia coli RIVM WR1 **	**Growth**	No growth

** RIVM WR1 is equivalent to NCTC 13167

QUALITY CONTROL OF MANUFACTURER

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

KEY WORDS

TCS / Sulfite-reducing anaerobes / *Clostridium perfringens* / Food products / Water / Detection / Enumeration / Filtration / MPN / Medium.

BIBLIOGRAPHY

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