TSC AGAR BASE
D-CYCLOSERINE ANTIMICROBIC SUPPLEMENT
D-CYCLOSERINE 4-MUP SUPPLEMENT
TSC AGAR – TSC AGAR MUP

Dehydrated and ready to use culture medium and selective supplements

1 - INTENDED USE

For the isolation and enumeration of *Clostridium perfringens* in foods, waters and other materials.

2 – COMPOSITION*

TSC AGAR BASE – DEHYDRATED AND READY TO USE MEDIUM IN FLASKS
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Enzymatic digest of casein	15 g
Soy Peptone	5 g
Yeast extract	5 g
Sodium metabisulphite anhydrous	1 g
Ferric ammonium citrate	1 g
Agar	15 g

D-CYCLOSERINE ANTIMICROBIC SUPPLEMENT
(VIAL CONTENT FOR 500 ML OF MEDIUM)

D-Cycloserine	200 mg

D-CYCLOSERINE 4-MUP ANTIMICROBIC SUPPLEMENT
(VIAL CONTENT FOR 500 ML OF MEDIUM)

D-cycloserine	200 mg
4-methylumbelliferyl phosphate	50 mg

TSC AGAR - READY TO USE PLATES

TSC Agar Base	1000 mL
D-cycloserine	400 mg

TSC MUP AGAR - READY TO USE PLATES

TSC Agar Base	1000 mL
D-cycloserine	400 mg
4-methylumbelliferyl phosphate	100 mg

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Food poisoning caused by *Clostridium perfringens* may occur when foods such as raw meats, poultry, dehydrated soups and sauces, raw vegetables, and spices are cooked and held without maintaining adequate heating or refrigeration before serving.[1] The enumeration of *C. perfringens* in food samples plays a key role in the epidemiological investigation of food-borne disease outbreaks and for this purpose various culture media have been proposed since the 1950s.
Tryptose Sulfite Cycloserine (TSC) Agar was developed by Harmon[2] using the same basal medium as Shahidi-Ferguson-Perfringens (SFP) agar, but with 400 mg/L of D-cycloserine substituted for the kanamycin and polymyxin. The TSC agar method was improved by eliminating the egg yolk and using pour plates by Hauschild.[4] TSC Agar, with and without the addition of egg yolk, is probably the best medium of those currently available for the purpose of enumeration of *C. perfringens*.[5]
The complete medium TSC Agar with D-cycloserine and without egg yolk emulsion, meets the requirements given by ISO 7937[6] (will be replaced by ISO 15213-2 under development)[7] for the samples of the food chain and ISO 14189[8] for water. With and without egg yolk emulsion, TSC Agar meets the requirements of FDA-BAM[1].
TSC Agar Base supplemented with D-cycloserine and 4-methylumbelliferyl phosphate simplifies the enumeration of *C. perfringens* mainly when high number of small colonies are present.[9]
For the detection of sulphite reducing *Clostridium* spp. in food samples with Iron Sulfite Agar, consult ISO 15213-1.[10]
The enzymatic digest of casein and soy peptone provide nitrogen, carbon, minerals and amino acids for the microbial growth. The yeast extract is a source of vitamins particularly of the B-group. Ferric ammonium citrate and sodium metabisulfite are indicators of sulphite reduction by *C. perfringens* which produces black colonies. D-cycloserine is an antibiotic inhibiting cell-wall biosynthesis in bacteria which helps in the selective isolation of *C. perfringens* by inhibiting accompanying flora.
The detection of acid phosphatase has been shown to be a useful diagnostic tool for identifying *C. perfringens*.
C. perfringens can metabolize 4-methylumbelliferyl phosphate (MUP) using the enzyme acid phosphatase to produce 4-methylumbelliferone, which fluoresces when placed under long-wavelength (365-nm) ultraviolet light.[9]

4A- DIRECTIONS MEDIUM PREPARATION (DEHYDRATED MEDIUM)

TSC Agar. Suspend 21 g of TSC Agar Base in 500 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add the content of one vial of D-Cycloserine Antimicrobic Supplement (REF 4240002), reconstituted with 5 mL of sterile purified water. If required, 25 mL of Egg Yolk Emulsion (REF 42111601) may be added to the precooled medium base. Mix well and pour into sterile Petri dishes.
TSC MUP Agar. Suspend 21 g of TSC Agar Base in 500 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add the contents of one vial of D-Cycloserine 4-MUP Supplement (REF 4240049), reconstituted with 5 mL of sterile distilled water. Mix well and pour into sterile Petri dishes.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

4B- Directions for medium preparation (medium in flasks and in tubes)

Liquefy the contents of the flask or the tube in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottles and the tubes may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to 47-50°C. Reconstitute the contents of one vial of D-Cycloserine Antimicrobic Supplement (REF 4240002) or D-Cycloserine 4-MUP Supplement (REF 4240049) with 5 mL of sterile purified water and add to TSC Agar Base in the following quantities:
100 mL flasks: 1 mL/flask
200 mL flask: 2 mL/flask
15 mL tube: 0.15 mL/tube
Mix well and distribute into sterile Petri dishes.

5 - Physical characteristics

TSC Agar Base

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates/flasks/tubes appearance	yellow, limpid
Final pH at 20-25 °C	7.6 ± 0.2
D-Cycloserine Antimicrobic Supplement	
Freeze-dried supplement appearance	short, dense, white pellet
Reconstituted supplement appearance	colourless limpid
D-Cycloserine 4-MUP Supplement	
Freeze-dried supplement appearance	short, dense, white pellet
Reconstituted supplement appearance	colourless limpid

6 - Materials provided - packaging

Product	Type	REF	Pack
TSC Agar Base	Dehydrated medium	4021582	500 g (11.9 L)
D-Cycloserine Antimicrobic Supplement	Freeze-dried supplement	4240002	10 vials, each for 500 mL of medium
D-Cycloserine 4-MUP Supplement	Freeze-dried supplement	4240049	10 vials, each for 500 mL of medium
TSC Agar	Ready-to-use plates	542158	2 x 10 plates ø 90 mm
TSC Agar	Ready-to-use plates	492158	3 x 10 plates ø 55 mm
TSC Agar MUP	Ready-to-use plates	492158X	3 x 10 plates ø 55 mm
TSC Agar Base	Ready-to-use tubes	552158B	20 x 15 mL
TSC Agar Base	Ready-to-use flasks	5121582	6 x 100 mL
TSC Agar Base	Ready-to-use flasks	5121584	6 x 200 mL

7 - Materials required but not provided

Autoclave, water-bath, sterile loops, swabs and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, sterile Petri dishes, controlled atmosphere generators and jars, membrane filters, ancillary culture media and reagents.

8 - Specimens

Waters, foods and animal feeding stuffs. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.[6-8]

9 - Test procedure

Enumeration of *C. perfringens* in foods with TSC Agar (ISO/DIS 15213-2)[7]

1. Prepare the test sample, the initial suspension and the dilutions, in accordance with the specific International Standard dealing with the product concerning.
2. If it is the intention to count only spores, heat the decimal dilution series to 80 °C in a water bath for 10 min ± 1 min.
3. Transfer by means of sterile pipettes 1 mL of the test sample (if liquid) or 1 mL of the initial suspension and 1mL of each decimal dilution, in duplicate, to the centres of empty Petri dishes.
4. Pour 12-15 mL of Egg Yolk free TSC Agar into each 90 mm dish and mix well with the inoculum. Pour 45 ml to 50 ml for 140 mm Petri dishes.
5. Carefully mix the inoculum with the medium by rotating the Petri dishes.
6. After complete solidification, pour about 5 ml of medium for 90 mm Petri dishes or 10 ml for 140 mm Petri dishes as overlay, to prevent the development of spreading colonies on the surface of the medium.
7. Allow to solidify and incubate in anaerobic jars or other suitable containers and incubate at 37 °C for 20 ± 2 hours. Longer incubation may result in excess blackening of the plates
8. Count the typical colonies in the plates containing less than 150 suspect colonies (90 mm Petri dishes) or less than 360 colonies (140 mm Petri dishes).
9. To confirm the presence of *C. perfringens,* choose one of the following two techniques:

Acid phosphatase test (REF192010) or SIM agar test (REF 402036)

Enumeration of *C. perfringens* in foods with TSC MUP Agar[11]

1. Transfer by means of sterile pipettes 0.1 mL of the test sample (if liquid) or 0.1 mL of the initial suspension and 0.1mL of each decimal dilution, in duplicate, to the surface of the TSC 4 MUP Agar plates.
2. Incubate in anaerobic jars or other suitable containers and incubate at 44°C for 22 ± 2 hours.
3. Count the fluorescent colonies observed under Wood's lamp (360 nm) on the plates containing between 15 and 150 characteristic colonies.
4. Confirm the suspected colonies with the catalase test (-) and with inverted CAMP Test (+).

Enumeration of *C. perfringens* in water with TSC Agar (ISO 14189)[8]

1. Filter a measured volume of sample, or a dilution of it, through a membrane with a pore size of 0.45 µm sufficient to retain spores of clostridia.
2. Using aseptic technique, roll the membrane filter used to collect the water sample onto the surface of the agar, so as to avoid the formation of air bubbles between the filter and the agar surface.

3. Incubate TSC Agar anaerobically at 44 ± 1 for 21 ± 3 hours.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
On TSC Agar, *C. perfringens* usually produce black or grey to yellow brown colonies as a result of the reduction of sulphite to sulphide.
On TSC MUP Agar, *C. perfringens* usually produce black or grey to yellow brown colonies, fluorescent when observed under Wood's lamp.
For a complete explanation of the identification criteria and methods, refer to the quoted references.[1,6-8,11]

11 - USER QUALITY CONTROL

All manufactured lots of the products are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control:

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. perfringens ATCC 13124	37°C/ 18-24 H / AN	growth, black colonies (TSC MUP Agar: fluorescent under Wood's Lamp)
B. subtilis ATCC 6633	37°C/ 18-24 H / AN	totally inhibited

AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, representative samples of all lots of dehydrated TSC Agar Base with and without D-Cycloserine Antimicrobic Supplement, D-Cycloserine 4-MUP Supplement and ready to use plates of TSC Agar and TSC MUP Agar are tested for productivity and selectivity with target and non-target strains.
Productivity is tested by quantitative pour plate and membrane filtration techniques with the target strain *C. perfringens* ATCC 13124 with incubation at 37°C and 44°C for 20 hours.
Selectivity is tested with modified Miles-Misra method with the following non-target strains: *B. subtilis* ATCC 6633, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853, *P. mirabilis* ATCC 10005. In addition, *C. sporogenes* ATCC 19404 is testes by dilution to extinction technique.
TSC Agar without D-cycloserine is tested by quantitative pour plate method with *C. perfringens* ATCC 13124 and by ecometry with *E. coli* ATCC 25922.
In TSC Agar Base supplemented with D-cyscloserine or D-cycloserine and MUP, with all the inoculation and incubation methods, *C. perfringens* grows with black colonies (fluorescent under Wood Lamp for TSC MUP Agar) and the measured productivity index is always higher than 0.7. *B. subtilis* is totally inhibited while *E. coli, P. mirabilis* and *P. aeruginosa* are partially inhibited.
In TSC Agar without D-cyscloserine, *C. perfringens* grows with black colonies and *E. coli* grows with colourless colonies.

13 - LIMITATIONS OF THE METHOD

- Black colonies must be confirmed as *C. perfringens* by appropriate tests.

14 - PRECAUTIONS AND WARNINGS

- The products are for microbiological control and for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplements shall be used in association according to the described directions. Apply Good Manufacturing Practice in the production process of prepared media.
- Dehydrated media and antibiotics containing supplements must be handled with suitable protection. Before use, consult the Material Safety Data Sheets.
- This culture medium contains raw materials of animal origin. The ante and post mortem controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that the product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Be careful when opening screw cap flasks and tubes to prevent injury due to breakage of glass. Be careful when opening the metal ring to avoid injury.
- When using a hot plate and/or a water bath, boil sufficiently long to dissolve the whole medium.
- Wear heat-protective gloves during medium liquefaction. Do not place the hot flasks into an ice bath or in cold water to accelerate cooling as this might cause cracks in the glass.
- The time required for complete liquefaction of the medium may vary considerably and depends on the actual temperature of the heating device, its wattage, the size and volume of the bottle.
- Once the bottled or tubed medium is liquefied, it cannot be solidified and dissolved a second time.
- Ready-to-use flasks and tubes are subject to terminal sterilization by autoclaving.
- The supplements are sterilized by membrane filtration.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplement or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplement and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplements as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheets are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products

intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Ready to use plates

Upon receipt, store plates in their original pack at +2°C /+8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g., microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

Ready-to-use medium in flasks and tubes

Upon receipt, store flasks and tubes in their original pack at +2°C /+8°C away from direct light. If properly stored, the flasks and tubes may be used up to the expiration date. Do not use the flasks or tubes beyond this date. Flasks and tubes from opened secondary packages can be used up to the expiration date. Opened flasks and tubes must be used immediately. Before use, check the closing and the integrity of the screw cap. Do not use flasks or tubes with signs of deterioration (e.g., microbial contamination, abnormal turbidity, precipitate, atypical colour).

Dehydrated medium

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

Freeze-dried supplements

Upon receipt, store the product in the original package at +2°C /+8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type and the applied storage conditions (temperature and packaging). According to ISO 14189[8] the TSC Agar plates with D-cycloserine prepared by the user, may be stored at +2°C /+8°C for up to 7 days; the basal medium (without D-cycloserine) may be stored at +2°C /+8°C for up to 4 weeks.

16 - REFERENCES

1. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM). Chapter 16: Clostridium perfringens.
2. Harmon SM, Kautter DA, Peeler JT. Improved medium for enumeration of Clostridium perfringens Appl Microbiol 1971 Oct;22(4):688-92.
3. Shahidi SA, Ferguson AR. New quantitative, qualitative, and confirmatory media for rapid analysis of food for Clostridium perfringens. Appl. Microbiol. 1971; 21:500-506.
4. Hauschild AH, Hilsheimer R. Evaluation and modifications of media for enumeration of Clostridium perfringens. Appl Microbiol 1974 Jan;27(1):78-82.
5. Mead GC. Selective and differential media for Clostridium perfringens. Int J Food Microbiol 1985; 2:89-98
6. ISO 7937:2004. Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of Clostridium perfringens -- Colony-count technique
7. ISO/DIS 15213-2 6b Microbiology of the food chain — Horizontal method for the detection and enumeration of Clostridium spp. — Part 2: Enumeration of Clostridium perfringens by colony-count technique
8. ISO 14189:2013 Water quality — Enumeration of Clostridium perfringens — Method using membrane filtration
9. Adcock PW, Saint CP. Rapid Confirmation of Clostridium perfringens by Using Chromogenic and Fluorogenic Substrates. Appl Environ Microbiol. 2001 Sep; 67(9): 4382–4384.
10. ISO 15213-1:2023. Microbiology of the food chain - Horizontal method for the detection and enumeration of Clostridium spp. Part 1: Enumeration of sulfite-reducing Clostridium spp. by colony-count technique.
11. Manuel Suisse des Denrées Alimentaires (MSDA). Chapitre 56, Microbiologie. Juillet 2000

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	This side up	Store in a dry place	Fragile
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	For single use only

REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2023/03

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it