TRYPTONE SULFITE NEOMYCIN (TSN) AGAR

Dehydrated culture medium

1 - INTENDED USE

For the isolation and enumeration of *Clostridium perfringens* in foodstuffs and other materials.

2 – COMPOSITION*

TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	15.00 g
Yeast extract	10.00 g
Sodium sulfite	1.00 g
Ferric citrate	0.50 g
Neomycin sulfate	0.05 g
Polymyxin B sulfate	0.02 g
Agar	13.50 g

*The formula 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]
In the 1950s and 1960s, several studies were carried out to develop a suitable culture medium that would allow the isolation and counting of *C.perfringens*.
Tryptone Sulfite Neomycin (TSN) Agar is prepared according to the formulation proposed by Marshall et al.[2] who modified Mossel's medium[3] for the enumeration of sulphite-reducing clostridia in foods. TSN Agar is used for the isolation and enumeration of *C.perfringens* in foodstuffs and other materials with the incubation at 46°C. The medium may be used by stabbing the tubes as well by streaking the plates.
Neomycin and polymyxin B inhibit the accompanying Gram-negative bacteria and are partially inhibitory for *Clostridium bifermentas*. The relatively high incubation temperature helps in the more specific detection of *C. perfringens*. Essential growth factors are provided by tryptone while the yeast extract is a source of vitamins, particularly of the B-group. Ferric citrate and sodium sulphite are indicators of sulphite reduction by *C. perfringens* which grows with black colonies.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 40 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely the medium and sterilise by autoclaving at 115°C for 20 minutes. Cool to 47°C-50°C mix well and pour into sterile Petri dishes. If required, dispense before sterilisation 20 mL in 20x200 mm tubes and autoclave at 115°C for 20 minutes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellowish, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	yellow, limpid
Final pH at 20-25 °C	7.2 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Tryptone Sulfite Neomycin (TSN) Agar	Dehydrated medium	4021592	500 g (12.5 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, microbiological tubes, sterile Petri dishes, appropriate apparatus for anaerobic culture, ancillary culture media and reagents.

8 – SPECIMENS

Foods and animal feeding stuffs. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

9 - TEST PROCEDURE

Prepare the test sample, the initial suspension and the dilutions, in accordance with the specific International Standard dealing with the product concerning.
Inoculate tubes or plates of the medium by stabbing deep tubes or streaking plates with the initial suspension of the specimen and its dilutions. Incubate for 18-24 hours at 46 ± 0.1°C in an anaerobic atmosphere.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
On TSN Agar, *C. perfringens* usually produce black or grey colonies as a result of the reduction of sulphite to sulphide.

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	46°C/ 18-24 H / AN	growth with black colonies
E. coli ATCC 25922	46°C/ 18-24 H / AN	inhibited

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

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12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of dehydrated TSN Agar is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.
Productivity is tested by dilution to extinction method, by stabbing the tubed medium with appropriate decimal dilutions of target organisms, incubating at 46°C for 18-24 hours in anaerobic atmosphere and recording the highest dilution showing growth and blackening in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}). Productivity is tested with the following target strains: *C. perfringens* ATCC 13124, *C. perfringens* ATCC 12916. The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1. The growths exhibit the typical blackening of the medium.
Selectivity tested by dilution to extinction method, by stabbing the tubed medium with appropriate decimal dilutions of non-target organisms, incubating at 46°C for 18-24 hours in anaerobic atmosphere and recording the highest dilution showing in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}). Selectivity is tested with the following non-target strains: *C. bifermentans* NCTC 506, *E. coli* ATCC 25922, S. Enteritidis ATCC 13076 and *P. aeruginosa* ATCC 27853. Non-target strains are partially or totally inhibited and the selectivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≥-1.

13 – LIMITATION OF THE METHOD

- Black colonies must be confirmed as *C. perfringens* by appropriate tests: motility (-), nitrate reduction (+), acid and gas from lactose (+), gelatin liquefaction (+).

14 - PRECAUTIONS AND WARNINGS

- This product is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- 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 this 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.
- Apply Good Manufacturing Practice in the production process of prepared media.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption
- The Certificates of Analysis and the Safety Data Sheet of the product 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

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).
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 Marshall *et al.* TSN Agar must be used the same day of the preparation.[1]

16 - REFERENCES

1. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM). Chapter 16: Clostridium perfringens.
2. Marshall RS, Steenbergen JF, McClung LS, Mossel DAA. Rapid technique for the enumeration of Clostridium perfringens. Appl Microbiol 1965 Jul;13(4):559-63.
3. Mossel DAA. Enumeration of sulphite reducing clostridia occurring in foods. J Sci Food Agr 1959; 10:662-669.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	Store in a dry place	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/08

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

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