SULFITE POLYMYXIN SULFADIAZINE (SPS) AGAR

Dehydrated and ready to use culture medium

1 - INTENDED USE

For the isolation and enumeration of *Clostridium perfringens* and other clostridia.

2 – COMPOSITION*

TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptic digest of casein	15.00
Yeast extract	10.00
Sodium sulfite	0.50
Ferric citrate	0.50
Polymyxin B sulphate	0.01
Sulphadiazine	0.12
Agar	13.50

*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] The enumeration of *C.perfringens* in food samples plays a key role in the epidemiological investigation of food-borne disease outbreaks.
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.* Mossel *et al.*[2] and Mossel,[3] reported on an iron-sulphite agar medium containing 0.05 % sodium sulphite and 10 ppm polymyxin B sulphate, which yielded quantitative recovery of pure cultures of several species of clostridia. Angelotti et al.[4] modified Mossel's formulation by adding sulphadiazine for suppressing the growth of *Enterobaceriaceae* and proposed Sulfite Polymyxin Sulfadiazine (SPS) Agar. The low sulphite content in the medium permits adequate blackening of colonies and at the same times allows sulphite-sensitive clostridia to grow.[5] According to MacFaddin[5] the medium can be used for the isolation and enumeration of *C. perfringens* and *Clostridium botulinum* from foodstuffs.
The medium is moderately selective: polymyxin B and sulfadiazine are inhibitors to most organisms other than *Clostridium* spp. The selectivity may be increased by the addition of neomycin 20 mg/L for the inhibition of *Clostridium bifermentans*.[4] Essential growth factors are provided by casein peptone 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* and other clostridia which produces black colonies.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 39.6 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely the medium and sterilise by autoclaving at 118°C for 15 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 118°C for 15 minutes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	beige-grey, limpid
Final pH at 20-25 °C	7.0 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Sulfite Polymyxin Sulfadiazine (SPS) Agar	Dehydrated medium	4020942	500 g (12.6 L)
Sulfite Polymyxin Sulfadiazine (SPS) Agar	Ready-to-use plates	492094	3 x 10 plates ø 55 mm
Sulfite Polymyxin Sulfadiazine (SPS) Agar	Ready-to-use flasks	5120942	6 x 100 mL
Sulfite Polymyxin Sulfadiazine (SPS) Agar	Ready-to-use flasks	5120943	6 x 100 mL

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.

1. Pipette 1 mL of decimal dilutions of the homogenised foodstuff into sterile plates and add 15-20 mL of SPS Agar.
2. Tilt the plate to mix the inoculum with the agar and allow to solidify.
3. Alternatively inoculate by stabbing deep tubes with the initial suspension of the specimen and its dilutions.
4. Incubate the plates or the tubes at 35-37ºC for 24 hours 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.
Most clostridia including *C. perfringens* and *C. botulinum* reduce sulphite to sulphide and causes colonies to turn black.

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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/ 48 H / AN	growth with black colonies
E. coli ATCC 25922	37°C/ 48 H / AN	inhibited

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

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of dehydrated SPS Agar (Test Batch:TC) is assessed for productivity and selectivity by comparing the results with a previously approved Reference Batch (RB).
Productivity is tested by a poured plate quantitative technique with the target strains *C. perfringens* ATCC 13124, *C. sporogenes* ATCC 19404 and *C. bifermentans* NCTC 506: the plates are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 35-37°C for 48 hours in anaerobic atmosphere. The colonies are enumerated on both batches and the productivity ratio ($Pr:CFU_{TB}/CFU_{RB}$) is calculated. If Pr is ≥ 0.7 and if the colonies morphology and colour are typical (black colonies) the results are considered acceptable and conform to the specifications.
The selectivity is assessed with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the following non-target strains: *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *E. faecalis* ATCC 19433. The growth of *E. coli* is markedly inhibited while the growth of *S. aureus* and *E. faecalis* is fair to good with white colonies.

13 – LIMITATION OF THE METHOD

- SPS Agar is not sufficient for identification. Organisms other than clostridia may grow and all black colonies should be checked for presence of spores.[5]
- Black colonies should be confirmed as *C. perfringens* by appropriate tests: motility (-), nitrate reduction (+), acid and gas from lactose (+), gelatin liquefaction (+).[6]

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.
- Be careful when opening screw cap flasks to prevent injury due to breakage of glass.
- 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 medium is liquefied, it cannot be solidified and dissolved a second time.
- Ready-to-use flasks are subject to terminal sterilization by autoclaving
- 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 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

Dehydrated medium

Upon receipt, store at +2°C /+8°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 (plates/tubes) and the applied storage conditions (temperature and packaging). According to MacFaddin, the tubed medium prepared by the user can be stored at +2°C /+8°C for 6 months.[5]

Ready to use plates

Upon receipt, store plates in their original pack at 2-8°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, pronounced pink colour, excess of moisture).

Ready-to-use medium in flasks

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

16 - REFERENCES

1. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM). Chapter 16: Clostridium perfringens.
2. Mossel DAA, Debruin S, Van Diepen HMJ, Vendring CMA, Zoutewelle G. The enumeration of anaerobic bacteria, and of Clostridium species in particular, in foods. J Appl Bacteriol 1956; 19:142-154.
3. Mossel DAA. Enumeration of sulphite reducing clostridia occurring in foods. J Sci Food Agr 1959; 10:662-669
4. Angelotti R, Hall HE, Foster MJ, Lewis KH. Quantitation of Clostridium perfringens in foods. Appl Microbiol 1962 May;10(3):193-9.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
6. ISO 7937:2004. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of Clostridium perfringens - Colony-count technique

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	2022/08

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