

LACTOSE SULFITE MEDIUM

Dehydrated culture medium

1 - INTENDED USE

For the confirmation of *Clostridium perfringens* colonies isolated from foods and other materials.

2 – COMPOSITION*

TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Enzymatic digest of casein	5.000 g
Yeast extract	2.500 g
Sodium chloride	2.500 g
Lactose	10.000 g
L-cysteine hydrochloride	0.300 g
Disodium disulfite anhydrous	0.750 g
Ammonium iron (III) citrate	0.625 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

Lactose Sulfite Medium is prepared according to the formulation recommended by ISO 7937¹ for the confirmation test of *C. perfringens* based on lactose fermentation and sulfite reduction. The reaction obtained in Lactose Sulfite Medium when incubated at 46 °C is very specific for *C. perfringens* and some colonies of *Clostridium paraperfringens* and *Clostridium absonum*.¹

Essential growth factors are provided by enzymatic digest of casein which is a source of nitrogen, carbon and minerals. The yeast extract is a source of vitamins, particularly of the B-group. Sodium chloride is a source of electrolytes and maintains the osmotic equilibrium. Lactose is the fermentable carbohydrate. Ferric ammonium citrate and disodium disulfite are indicators of sulphite reduction by *C. perfringens* which produces blackening of the medium.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 21.7 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Dispense 9 mL portions into test tubes with inverted Durham tubes and sterilise by autoclaving at 121°C for 15 minutes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale yellow, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	yellow, limpid
Final pH at 20-25 °C	7.1 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Lactose Sulfite Medium	Dehydrated medium	4015792	500 g (23 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, microbiological tubes, Durham tubes, ancillary culture media and reagents.

8 – SPECIMENS

Colonies isolated on plating media such as TSC Agar.

9 - TEST PROCEDURE

For the confirmation of *C. perfringens* in foods, ISO 7937¹ recommends to choose one of the following two techniques:

- confirmation technique using Lactose Sulfite Medium.
- confirmation technique using Nitrate Motility Medium (REF 401726) and Lactose Gelatin Medium (REF 4015762)

Confirmation using Lactose Sulfite Medium

After incubation of the enumeration plates (TSC Agar REF 402158), inoculate each selected typical colony into fluid Thioglycolate Medium (REF 402137) and incubate under anaerobic conditions at 37 °C for 18 h to 24 h.

After incubation, transfer with no delay 5 drops of the thioglycolate culture to the Lactose Sulfite Medium by means of a sterile pipette. Incubate aerobically at 46 °C for 18 h to 24 h in the water bath.

10 - READING AND INTERPRETATION

Examine the tubes of Lactose Sulfite Medium for the production of gas and the presence of a black colour (iron sulphide precipitate). Durham tubes more than one-quarter full of gas and tubes having a black precipitate are considered positive.

In case of doubt, when the Durham tube in a blackened medium is less than one-quarter full of gas, transfer with no delay, using a sterile pipette, 5 drops of the previous growth in Lactose Sulfite Medium to another tube of Lactose Sulfite Medium. Incubate in the water bath at 46 °C for 18 h to 24 h. Examine this tube as described above.

Bacteria which form characteristic colonies in the TSC Medium and which give a positive confirmation with the Lactose Sulfite Medium are considered as being *C. perfringens*. In all other cases, the tubes should be considered as negative.

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
C. perfringens ATCC 13124

 INCUBATION T° / T / ATM
 46°C / 18-24 H / A

 EXPECTED RESULTS
 growth with gas production into Durham tubes, presence of a black precipitate

A: aerobic 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 Lactose Sulfite Medium is tested for the specific reactions by comparing the results with a previously approved Reference Batch.

The medium is tested by inoculation the tubes with pure culture of *C. perfringens* ATCC 13124 and *C. perfringens* NCTC 13170. After incubation at 46°C for 24 hours the strains exhibit a good gas production and a black precipitate.

Moreover, the medium is tested with *C. sporogenes* ATCC 19404. After incubation, the strain grows with gas production and without blackening of broth

13 - 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.

14 - 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 ISO 7937 the complete medium Lactose Sulfite Medium in tubes should be used the same day of the preparation.¹

15 - REFERENCES

1. 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	 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 3	Updated layout and content	2022/08

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

