

# MOTILITY NITRATE CP 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	5.0 g
Beef extract	3.0 g
Galactose	5.0 g
Potassium nitrate	1.0 g
Disodium hydrogen phosphate	2.5 g
Agar	3.0 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

Motility Nitrate CP Medium is prepared according to the formulation recommended by ISO 7937<sup>1</sup> and by FDA-BAM<sup>2,3</sup> for the confirmation of *C. perfringens* colonies isolated from foods and other materials, based on motility test and nitrate reduction reaction. Motility Nitrate CP Medium should be used in combination with Lactose Gelatin Medium for confirmatory purposes.<sup>1,3</sup>

Essential growth factors are provided by enzymatic digest of casein and beef extract which are sources of nitrogen, carbon and minerals. Galactose is the fermentable carbohydrate and a source of energy Disodium hydrogen phosphate is used as buffering agent to control the pH in the medium. The agar at a concentration of 0.3% is used to demonstrate the motility of organisms. Potassium nitrate serves as a base for evaluation of nitrate reduction to nitrite; galactose and glycerol improve the consistency of the reaction. *C. perfringens* is nonmotile and reduces nitrates to nitrites.

### 4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 19.5 g in 1000 mL of cold purified water and add 5 g of Glycerol (REF 421015). Heat to boiling with frequent agitation to dissolve completely the powder. Dispense 10 mL portions into test tubes and sterilise by autoclaving at 121°C for 15 minutes. If not used the same day, just before to use, heat in boiling water or steam for 15 minutes, then cool rapidly to the incubation temperature.

### 5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	colourless, limpid
Final pH at 20-25 °C	7.3 ± 0.2

### 6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Motility Nitrate CP Medium	Dehydrated medium	4017262	500 g (25.6 L)

### 7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, microbiological tubes, Glycerol (REF 421015), appropriate apparatus for anaerobic culture, 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* one of the following two techniques may be followed:

- confirmation technique using Nitrate Motility CP Medium (REF 401726) and Lactose Gelatin Medium (REF 401576).<sup>1,3</sup>
- confirmation technique using Lactose Sulfite Medium (REF 401579).<sup>1</sup>

#### Confirmation using Nitrate Motility CP Medium

- Stab-inoculate each well-isolated characteristic colony selected from TSC Agar into the freshly deaerated Nitrate Motility CP Medium.
- Incubate under anaerobic conditions at 37 °C for 24 h.
- Examine the tube of medium for the type of growth along the stab line.
- Test for the presence of nitrite by adding, with a graduated pipette and the rubber bulb, 0.2 mL to 0.5 mL of the nitrite detection reagent<sup>^</sup> to each tube of medium. WARNING: carry out this test under a fume hood.

<sup>^</sup>Nitrite detection reagent

Dissolve 0.1 g of 5-Amino-2-naphthalenesulfonic acid in 100 mL of 15 % acetic acid solution. Filter through a filter paper. Store in a well-stoppered brown bottle (preferably with a bulb type dropper) at 3 °C ± 2 °C.

Sulfanilic acid solution: Dissolve 0.4 g of sulfanilic acid in 100 mL of 15 % acetic acid solution. Filter through a filter paper. Store in a well-stoppered brown bottle (preferably with a bulb type dropper) at 3 °C ± 2 °C.

Preparation of complete reagent: mix equal amounts of the two solutions just before use. Discard unused reagent immediately.

### 10 - READING AND INTERPRETATION

Nonmotile organisms produce growth only in and along stab line, while motile organisms usually produce diffuse growth out into the medium, away from the stab.

The formation of a red colour after the addition of nitrite detection reagent confirms the reduction of nitrate to nitrite. If no red colour is formed within 15 mm, add a small amount of zinc dust and allow to stand for 10 min. If a red colour is formed after the addition of zinc dust, no reduction of nitrate has taken place. Bacteria that produce black colonies in TSC Agar, are non-motile, reduce nitrate to nitrite, produce acid and gas from lactose, and liquefy gelatin in 48 h are considered to be *C. perfringens*. Cultures that show a faint reaction for nitrite (i.e. a pink colour) shall be eliminated, since *C. perfringens* consistently gives an intense and immediate reaction.



### 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
<i>C. perfringens</i> ATCC 13124	37°C / 24-48 H / AN	non-motile, positive to nitrate reduction test
<i>C. bifermentans</i> NCTC 506	37°C / 24-48 H / AN	negative to nitrate reduction test

AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection; NCTC: National Type Cultures Collection.

### 12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of dehydrated Motility Nitrate CP Medium is tested for the specific reactions by comparing the results with a previously approved Reference Batch.

The medium is tested by stabbing the tubes with pure culture of *C. perfringens* ATCC 13124 and *C. bifermentans* NCTC 506, *C. sporogenes* ATCC 19404 and *E. coli* ATCC 8739. After incubation at 37°C for 24-48 hours, *C. perfringens* exhibits a growth not diffusing along the stab line, positive to nitrate reduction test, *C. bifermentans* and *C. sporogenes* are negative to nitrate reduction test, *E. coli* is negative to motility test.

### 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](http://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](http://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 Motility Nitrate CP Medium in tubes can be stored at 3 °C ± 2 °C for 4 weeks but, just prior to use, heat in boiling water or flowing steam for 15 min, then cool rapidly to the incubation temperature.<sup>1</sup>

### 15 - REFERENCES

1. ISO 7937:2004. Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of *Clostridium perfringens* -- Colony-count technique
2. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM). M102: Motility-Nitrate Medium, Buffered (for *C. perfringens*).
3. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM). Chapter 16: *Clostridium perfringens*

### TABLE OF APPLICABLE SYMBOLS

<b>REF</b> or <b>REF</b> Catalogue number	<b>LOT</b> 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.