



# CLOSTRIDIUM AGAR (Reinforced Clostridial agar)

## Dehydrated culture medium

### 1 - INTENDED USE

For the cultivation and enumeration of clostridia and other anaerobes.

### 2 - COMPOSITION - TYPICAL FORMULA\*

(AFTER RECONSTITUTION WITH 1 L OF WATER)

|                 |        |
|-----------------|--------|
| Yeast extract   | 3.0 g  |
| Beef extract    | 10.0 g |
| Peptone         | 10.0 g |
| Glucose         | 5.0 g  |
| Soluble starch  | 1.0 g  |
| Sodium chloride | 5.0 g  |
| Sodium acetate  | 3.0 g  |
| L-cysteine HCl  | 0.5 g  |
| Agar            | 15.0 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

Reinforced Clostridial Medium has been formulated by Hirsch and Grinstead in 1954<sup>1</sup>, while the solid version of the medium (Clostridium Agar or Reinforced Clostridial Agar) corresponds to the formulation described by Barnes and Ingram<sup>2</sup>.

Clostridium Agar is very rich and is used for the cultivation and enumeration of clostridia, lactobacilli and other anaerobes and facultative anaerobes, in foodstuffs and other materials.<sup>3</sup>

Peptone and beef extract provide nitrogen, carbon, minerals and amino acids for the microbial growth. Yeast extract is a source of vitamins, particularly of the B-group and glucose is a source of carbon and energy. Sodium chloride maintains the osmotic balance while sodium acetate buffers the medium. L-cysteine, a reducing agent, favours the growth of anaerobes. Soluble starch helps to detoxify metabolic by-products. Agar is the solidifying agent. According to Hirsch and Grinstead,<sup>1</sup> polymyxin B 0.02 g/L can be added to inhibit Gram-negative bacteria. According to APHA<sup>4</sup>, for enumeration of lactic streptococci the medium must be supplemented with an additional 5 g/L glucose to bring to 10 g/L glucose per litre and prussian blue may be incorporated into basal medium to 0.3 g/L as needed.

### 4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 52.5 g in 1000 ml of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 115°C for 20 minutes. If required, cool to 45-50 °C and add 0.02 g/L of Polymyxin B in form of a filter-sterilized aqueous solution.

### 5 - PHYSICAL CHARACTERISTICS

|  |   |
|--|---|
| Dehydrated medium appearance           | pale yellow, fine, homogeneous, free-flowing powder |
| Solution and prepared tubes appearance | pale yellow, slightly opalescent                    |
| Final pH at 20-25 °C                   | 6.8 ± 0.2   |

### 6 - MATERIALS PROVIDED - PACKAGING

| Product          | Type              | REF     | Pack          |
|------------------|-------------------|---------|---------------|
| Clostridium Agar | Dehydrated medium | 4013032 | 500 g (9.5 L) |

### 7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

### 8 - SPECIMENS

Water, food and pharmaceutical samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards and regulations.

### 9 - TEST PROCEDURE,

Prepare the decimal dilutions of the sample and inoculate tubes or dishes by poured plate technique.

From each dilution, take an aliquot and add it to plates or tubes. Pour the melted medium at 45-50°C onto the sample and allow to solidify. Incubate 2-5 days at an optimal temperature (e.g., 35 °C). An anaerobic environment can be achieved in tubes by covering with oil immediately after the medium is solidified. If plates are used, they must be incubated in an anaerobic atmosphere.

### 10-READING AND INTERPRETATION

Colonies of the bacteria growing on Clostridium Agar exhibit an opaque, yellowish colouration. The presence of rods with or without endospores that are negative in the catalase test indicates the presence of clostridia. The culture result must be confirmed by biochemical identification.

### 11 - USER QUALITY CONTROL

All manufactured lots of the product 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 | 30 or 35°C / 48h / AN   | growth           |
| <i>C. sporogenes</i> ATCC 19404  | 30 or 35°C / 48h / AN   | growth           |

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 Clostridium Agar, is tested for productivity by comparing the results with a previously approved Reference Batch.

Productivity is tested by dilution to extinction method, by inoculating decimal dilutions of organisms in the tubed medium and incubating at 30°C for 48 hours in anaerobic atmosphere and recording the highest dilution showing growth in Reference Batch ( $G_{r_{RB}}$ ) and in Test Batch ( $G_{r_{TB}}$ ). Productivity is tested with the following strains: *C. perfringens* ATCC 13124, *C. sporogenes* ATCC 19404, *C. bifementans* NCTC 506. The productivity index  $G_{r_{RB}}-G_{r_{TB}}$  for each test strain shall be  $\leq 1$ .

### 13 - LIMITATIONS OF THE METHOD

- The medium is not selective: other sporeforming anaerobes as well *C. butyricum*, lactobacilli and streptococci exhibit good growth.<sup>3</sup>
- Biochemical, immunological, molecular, or mass spectrometry testing should be performed on isolates, from pure culture, for complete identification.

### 14 - PRECAUTIONS AND WARNINGS

- This product is for Laboratory use 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 plates 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.

### 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 for the validation of the shelf life of the finished products, according to the type (tubes/bottles) and the storage method (temperature and packaging). According to MacFaddin the prepared medium may be stored at +2°C /+8°C for up to 2 weeks.<sup>3</sup>

### 16 - REFERENCES

- Hirsch A, Grinsted, E. Methods for the growth and enumeration of anaerobic sporeformers from cheese, with observations on the effect of nisin. J Dairy Res 1954; 21: 101-110.
- Barnes EM, Ingram M. The effect of redox potential on the grown Clostridium welchii strain isolated from horse muscle. J Appl Bact 1956; 19: 177-185.
- MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
- APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015.

### TABLE OF APPLICABLE SYMBOLS

|                                |                                   |                              |                             |        |
|--------------------------------|-----------------------------------|------------------------------|-----------------------------|--------|
| REF or REF<br>Catalogue number | LOT<br>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 | 2022/07 |

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

