# COLUMBIA AGAR EP

## Dehydrated culture medium

### 1 - INTENDED USE

General-purpose medium for the detection of clostridia in non-sterile pharmaceutical products.

### 2- COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

| | |
|---|---|
| Pancreatic digest of casein | 10 g |
| Peptic digest of meat | 5 g |
| Pancreatic digest of heart | 3 g |
| Yeast extract | 5 g |
| Maize starch | 1 g |
| Sodium Chloride | 5 g |
| Agar | 14 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

Columbia Blood Agar was first described in 1966 by Ellner, Stoessel, Drakeford and Vasi[1] of the Columbia University, who combined meat and casein peptones and defibrinated sheep blood into one medium. After 2 years trial, this medium showed remarkably improved growth promoting properties and was found to be superior to blood agar previously used for differentiating β and α haemolytic organisms.[1]
Columbia Agar EP complies with the formulation given by harmonised microbiological methods of European Pharmacopoeia.[2] The medium is recommended for the procedure of clostridia detection, for the subculture from the Reinforced Medium for Clostridia.
Peptones provide carbon, nitrogen and trace elements for bacterial growth, yeast extract is a source of vitamins, particularly of the B-group B. Sodium chloride maintains the osmotic balance, maize starch is included to absorb toxic by-products contained in the specimen and is an energy source for bacterial growth.

### 4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 43 g in 1000 mL of cold purified water; heat to boiling with frequent agitation. Sterilize by autoclaving at 121°C for 15 minutes. Allow to cool to 47-50°C and add, where necessary, gentamicin sulphate corresponding to 20 mg of gentamicin base and pour into Petri dishes.

### 5 - PHYSICAL CHARACTERISTICS

| | |
|---|---|
| Dehydrated medium appearance | pale yellow, fine, homogeneous, free-flowing powder |
| Solution appearance | pale yellow, slightly opalescent |
| Final pH at 20-25 °C | 7.3 ± 0.2 |

### 6 - MATERIALS PROVIDED - PACKAGING

| Product | Type | REF | Pack |
|---|---|---|---|
| Columbia Agar EP | Dehydrated culture medium | 4011342 | 500 g (11.6 L) |

### 7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and swabs, incubator and laboratory equipment as required, controlled atmosphere generators and jars, ancillary culture media and reagents.

### 8 - SPECIMENS

Non sterile pharmaceutical products. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.[2]

### 9- TEST PROCEDURE

1. Prepare a sample using a 1 in 10 dilution (with a minimum total volume of 20 mL) of not less than 2 g or 2 mL of the product to be examined.
2. Divide the sample to 2 portions of at least 10 mL. Heat 1 portion at 80 °C for 10 minutes and cool rapidly. Do not heat the other portion.
3. Use 10 mL or the quantity corresponding to 1 g or 1 mL of the product to be examined of both portions to inoculate suitable amounts of Clostridium Broth (REF 401304).
4. Incubate under anaerobic conditions at 30-35°C for 48 h.
5. After incubation, make subcultures from each container on Columbia Agar EP plates and incubate under anaerobic conditions at 30-35°C for 48-72 h.

### 10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological, chromatic characteristics of the colonies.
The occurrence of anaerobic growth of rods (with or without endospores) giving a negative catalase reaction indicates the presence of clostridia. This is confirmed by identification tests.

### 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 is listed the test strains useful for the quality control of Columbia Agar EP.

| CONTROL STRAINS | | | INCUBATION T°/ T / ATM | EXPECTED RESULTS |
|---|---|---|---|---|
| *C. sporogenes* | ATCC | 19404 | 30-35°C / 48 h / AN | growth |

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 Columbia Agar EP is tested for productivity by comparing the results with a previously approved Reference Batch.
Productivity is tested by a quantitative test with *C.sporogenes* ATCC 19404: the plates are inoculated with decimal dilutions in saline of colony suspensions and incubated at 30-35°C for 48 hours in anaerobic atmosphere. The colonies are enumerated on both batches and the productivity ratio (*Pr*) is calculated. If *Pr* is ≥ 0.7 the results are considered acceptable and conform to the specifications. Furthermore the productivity characteristics are tested by semi-quantitative ecometric technique with the following strains: *C.perfringens* ATCC 13124 and *C.sporogenes* ATCC 19404. After incubation the amount of growth is evaluated and recorded. All strains show a good growth.

## 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 the 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 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.
- 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 for the validation of the period of validity of the finished products, according to the type (plates/tubes/bottles) and the storage method applied (temperature and packaging).

## 15 - REFERENCES

1. Ellner PD, Stoessel CJ, Drakeford E, Vasi, F. A new culture medium for medical bacteriology. Am. J. Clin. Path 1966; 45: 502-504.
2. European Pharmacopoeia 11th Edition, 2022, Vol. 1; 2.6.13 Microbiological Examination of non-sterile products: test for specified micro-organisms: 01/2021:20631.

**TABLE OF APPLICABLE SYMBOLS**

| | | | | |
|---|---|---|---|---|
| REF or REF<br>Catalogue number | LOT Batch code | IVD *In vitro* Diagnostic Medical Device | Manufacturer | Use by |
| Temperature limitation | Contents sufficient for <n> tests | Consult Instructions for Use | Keep away from direct light | Store in a dry place |

**REVISION HISTORY**

| Version | Description of changes | Date |
|---|---|---|
| Revision 2 | Updated layout and content | 2022/07 |

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

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