CLOSTRIDIUM BOTULINUM AGAR BASE
CLOSTRIDIUM BOTULINUM ANTIMICROBIC SUPPLEMENT

Dehydrated culture medium and selective supplement

1 - INTENDED USE

Selective and differential medium for the isolation and presumptive identification of *Clostridium botulinum* from foods and other materials.

2 – COMPOSITION*

CLOSTRIDIUM BOTULINUM AGAR BASE
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Pancreatic digest of casein	40 g
Yeast extract	5 g
Glucose	2 g
Disodium hydrogen phosphate	5 g
Sodium chloride	2 g
Agar	20 g
Magnesium sulphate	10 mg

CLOSTRIDIUM BOTULINUM ANTIMICROBIC SUPPLEMENT
(VIAL CONTENT FOR 500 mL OF MEDIUM)

D-cycloserine	125 mg
Trimethoprim	2 mg
Sulphamethoxazole	38 mg

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Clostridium botulinum is an anaerobic, rod-shaped sporeforming bacterium that produces a protein with characteristic neurotoxicity. Under certain conditions, these organisms may grow in foods, producing toxin(s).[1]
Clostridium Botulinum Agar Base completed with the antimicrobials of Clostridium Botulinum Antimicrobic Supplement corresponds to the formulation devised by Dezfulian et al. for the detection of *C. botulinum* from faeces and called CBI medium.[2]
According to Dezfulian, qualitative tests indicated complete recovery of *C. botulinum* types A, B, F, and G and the isolation of *C. botulinum* types A, B, and F from seeded faecal specimens was easily achieved with CBI medium.[1]
The Biolife medium has been used for the evaluation of a family outbreak of alimentary botulisms by Pavic et. al.[3] The authors reported that this medium is more suitable for the isolation of lecithinolytic *C. botulinum* type A, B and F than Sulphite Agar and Columbia Agar.
Pancreatic digest of casein provides nitrogen, carbon, minerals and amino acids for the microbial growth. Yeast extract is a source of vitamins particularly of the B-group. Glucose is a source of carbon and energy. Sodium chloride maintains the osmotic balance. Disodium phosphate buffers the medium while magnesium sulphate helps for the sporulation of the organisms. Egg yolk is the substrate to detect lecithinase and lipase activities. Lecithinase degrades lecithin producing an insoluble, opaque precipitate in the medium surrounding the colonies. Lipase break down free fats causing an iridescent sheen on the surface of the colonies. The mixture of D-cycloserine, trimethoprim and sulphamethoxazole helps in the selective isolation of *C.botulinum* by inhibiting accompanying flora.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 37 g in 450 mL of cold purified water, heat to boiling with frequent agitation, and sterilise by autoclaving at 121°C for 15 minutes. Cool to approximately 47-50°C and add, under aseptic conditions, the contents of one vial of Clostridium Botulinum Antimicrobic Supplement reconstituted with 2.5 mL of sterile distilled water followed by 2.5 mL of acetone, and 50 mL of Egg Yolk Emulsion (cat. N° 4244160). Mix well and distribute into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Clostridium Botulinum Agar Base

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	yellow, opalescent
Prepared plates appearance	yellow, opaque
Final pH at 20-25 °C	7.4 ± 0.2

Clostridium Botulinum Antimicrobic Supplement

Freeze-dried supplement appearance	high, dense, white pellet
Reconstituted supplement appearance	colourless, slightly opalescent

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Clostridium Botulinum Agar Base	Dehydrated medium	4013062	500 g (6.7 L)
Clostridium Botulinum Antimicrobic Supplement	Freeze-dried supplement	4240066	10 vials, each for 500 mL of medium

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops, swabs and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, sterile Petri dishes, controlled atmosphere generators and jars, 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.

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9 - TEST PROCEDURE

Inoculate the food homogenate and the serial dilutions of the sample onto the surface of the plates. Incubate with anaerobic atmosphere at 35-37° for 48 hours.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
C. botulinum grows with large colonies surrounded by an opaque halo of egg yolk precipitation with an iridescent sheen on the surface of the growth.

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.

12 – LIMITATION OF THE METHOD

- The selectivity of the medium can interfere with the isolation of less resistant type G and nonproteolytic type F strains of *C. botulinum*.[2]
- Although *C. botulinum* type G can also be recovered on the medium, lack of lipase activity makes the differentiation of the colonies of this organism from those of other bacteria more difficult.[2]
- Although this medium seems to be superior to nonselective media, the simultaneous use of both media for the isolation of *C. botulinum* from the primary specimen, and if necessary, from the enriched culture, is recommended, to avoid missing drug-susceptible strains of *C. botulinum* such as some type E strains.[2]

13 - PRECAUTIONS AND WARNINGS

- The medium base and the supplement are for microbiological control and for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplements shall be used in association according to the described directions. Apply Good Manufacturing Practice in the production process of prepared media.
- Dehydrated media must be handled with suitable protection. Clostridium Botulinum Antimicrobic Supplement is classified as dangerous. Before use, consult the Material Safety Data Sheets.
- 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.
- Be careful when opening the metal ring of the supplement vials to avoid injury.
- The supplement is sterilized by membrane filtration.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplement or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplement and the inoculated plates with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplement as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheets 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

Dehydrated medium

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).

Freeze-dried supplement

Upon receipt, store the product in the original package at 2-8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

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/bottles) and the applied storage conditions (temperature and packaging).

15 - REFERENCES

1. FDA-BAM Chapter 17: Clostridium botulinum. Content current as of:10/31/2017
2. Dezfulian M, McCroskey LM, Hatheway CL, Dowell Jr VR. Selective medium for isolation of Clostridium botulinum from human feces. J Clin Microbiol 1981; Mar; 13(3):526-31.
3. Pavic, S et al. About suitability of C.Botulinum Agar for the isolation of B type C.botulinum from dry cured dalmatian ham and stools of patients during family outbreack. Zavod za javno zdravstvo Zupanije splitsko-dalmatinske. 2000.

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	

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.

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