BACILLUS CEREUS AGAR BASE (MYP)
BACILLUS CEREUS SELECTIVE AGAR (MYP)
Dehydrated and ready-to-use culture medium

MYP: typical Bacillus *cereus* colonies

1 - INTENDED USE
For the detection and enumeration of *Bacillus cereus* group in foodtuffs and other samples.

2 – COMPOSITION*
DEHYDRATED AND READY-TO-USE IN FLASKS BACILLUS CEREUS AGAR BASE (MYP) - TYPICAL FORMULA PER LITRE

Beef extract	1.00 g
Peptone	10.00 g
D-mannitol	10.00 g
Sodium chloride	10.00 g
Phenol red	0.025 g
Agar	12.00 g

BACILLUS CEREUS SELECTIVE AGAR (MYP) - READY TO USE PLATES

Bacillus Cereus Agar Base	43 g
Egg Yolk Emulsion 20%	100 mL
Polymyxin B sulphate	100,000 IU
Purified water	900 mL

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Bacillus cereus is a group of ubiquitous facultative anaerobic sporeforming Gram-positive rods commonly found in soil. *Bacillus cereus* Group comprises six species: *B. cereus, B. thuringiensis, B. weihenstephanensis, B. mycoides, B. pseudo-mycoides,* and *B. anthracis*. *Bacillus cereus* is a foodborne pathogen that can produce two toxins, one heat stable and emetic and the other thermolabile causing diarrhea.[1] The infection is caused by ingestion of food such as meat, rice and vegetables contaminated with *B. cereus*, and left at room temperature after cooking.
For the enumeration of vegetative cells and spores of *Bacillus cereus* in food, a mannitol-egg yolk-phenol red (MYP) agar has been developed by Mossel in 1967[2] exploiting the failure of *B. cereus* to dissimilate mannitol, and the ability of most strains to produce phospholipase C. MYP agar is recommended by ISO 7932,[3] ISO 21871[4], FDA-BAM[5] and APHA[6] for detection and enumeration of *B. cereus* in food.
Bacillus Cereus Selective Agar MYP contains beef extract and peptone that provide carbon, nitrogen, and minerals for microbial growth. This medium relies on the selective inhibitory component polymyxin B and two indicator systems: mannitol/phenol red and egg yolk.
The growth of many unwanted organisms is suppressed by polymyxin B, while target-organisms will not attack mannitol but dissimilate egg yolk and consequently give rise to typical bacilliform colonies with purple-red zones and halos.[7] Non-target organisms that ferment mannitol produce acid products and form yellow colonies.

4A - DIRECTIONS FOR DEHYDRATED MEDIUM
Suspend 21.5 g of Bacillus Cereus Agar Base MYP in 450 mL of cold distilled water. Heat to boiling with frequent agitation, sterilize by autoclaving at 121°C for 15 minutes and cool to 44-47 °C. Reconstitute under aseptic conditions the contents of one vial of Bacillus Cereus Antimicrobic Supplement (Ref. 4240001) with 5 mL of sterile purified water.
ISO 7932, ISO 21871: add 5 mL of Bacillus Cereus Antimicrobic Supplement (REF 4240001) and 50 mL of Egg Yolk Emulsion 20%, (REF 42111205). Mix well and distribute into sterile Petri dishes.
FDA-BAM: Dispense 225 mL of boiled medium base into 500 mL Erlenmeyer flasks. Autoclave 15 min at 121°C and cool to 44-47 °C. To 225 ml medium base add 2.25 mL of Bacillus Cereus Antimicrobic Supplement (REF 4240001) and 12.5 mL of Egg Yolk Emulsion 50% (REF 42111601). Mix well and distribute into sterile Petri dishes.

4A - DIRECTIONS FOR READY-TO-USE MEDIUM IN FLASKS
Liquify the contents of the flask in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottle may be placed into a jar containing water, placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to 44-47°C. Reconstitute under aseptic conditions the contents of one vial of Bacillus Cereus Antimicrobic Supplement (REF 4240001) with 5 mL of sterile purified water, add 1 mL to the base medium and mix.
ISO 7932, ISO 21871: add 10 mL of Egg Yolk Emulsion 20% (REF 42111205), mix well and distribute into sterile Petri dishes.
FDA-BAM: add 5 mL of Egg Yolk Emulsion 50%, (REF 42111601), mix well and distribute into sterile Petri dishes

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	red-orange, fine, homogeneous, free-flowing powder
Solution and prepared medium in flask appearance	red, slightly opalescent
Prepared plates appearance	pink, opaque
Final pH at 20-25 °C	7.2 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Bacillus Cereus Agar Base (MYP)	Dehydrated medium	4011112	500 g (11.6 L)
Bacillus Cereus Agar Base (MYP)	Ready-to-use flask	5111112	6 x 90 mL
Bacillus Cereus Selective Agar (MYP)	Ready-to use plates	541112M	2 x 10 plates ø 90 mm

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, sterile Petri dishes, Egg Yolk Emulsion (REF 42111205 or 42111601), Bacillus Cereus Antimicrobic Supplement (REF 4240001), ancillary culture media and reagents.

8 – SPECIMENS

Products intended for human consumption and the feeding of animals, and environmental samples in the area of food production and food handling. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.[3-6]

9 - TEST PROCEDURE

For the isolation and enumeration of presumptive *B. cereus* in foodtuffs, according ISO 7932, the following method is recommended:

1. Prepare the test sample in accordance with the specific International Standard appropriate to the product concerned.
2. Distribute 0.1 mL of test sample if the product is liquid, or of the initial suspension if solid, on the surface of two agar plates (90mm). Repeat the procedure using further decimal dilutions.
3. If low number of *B. cereus* is expected, distribute 1 mL of test sample if the product is liquid or 1 mL of the initial suspension if solid on each of two agar plates (140mm) or over the surface of three 90mm plates.
4. Incubate at 30°C in aerobic conditions for 18-24 hours. If colonies are not visible incubate the plates for further 24 hours before counting.

10 - READING AND INTERPRETATION

Count the *B. cereus* colonies in plates with less than 150 colonies having the following characteristics: large, pink (indicating that mannitol fermentation has not occurred) and generally surrounded by a zone of precipitation (indicating the production of lecithinase).
If the plates have a high content of background flora which ferments mannitol, the characteristic coloration of colonies and background may be reduced or no longer visible. In addition, some presumptive *Bacillus cereus* strains have only a slight or absent egg yolk reaction. In such cases and in any other doubtful cases, these colonies should also be submitted to the confirmation.
Typical and atypical colonies on MYP Agar shall be confirmed by means of the haemolysis test on sheep blood agar.
Select five presumptive colonies from each plate and streak the selected colonies onto the surface of sheep blood agar order to allow a good interpretation of the haemolysis reaction. Incubate at 30 °C for 24 h ± 2 h and observe the haemolysis reaction.
Results confirming presumptive *Bacillus cereus* strains:

- Formation of pink colonies surrounded by precipitate on MYP agar.
- Positive haemolysis reaction.

Optional tests intended for complementary investigations (i.e., epidemiological) on isolated *Bacillus cereus* group strains[3]: 1) detection of *cytK-1* or cttK2-gene variants of the gene encoding Cytotoxin K; 2) Detection of *Bacillus cereus* group strains able to produce cereulide; 3) Motility test for *B. antracis* screening; 4) Microscopic examination of the parasporal crystal from B*acillus thuringiensis*
Other tests useful for differentiating typical strains of *B. cereus* from other members of the *B. cereus* group:[5] 1) Microscopic observation (large Gram-positive rods in short-to-long chains; spores are ellipsoidal, central to sub-terminal, and do not swell the sporangium); 2 Glucose fermentation (+); 3) Voges Proskauer Reaction (+); 4) Nitrate reduction (+); 5) Motility test (+); 6) Decomposition of tyrosine (+); 7) Growth in presence of 0.001% lysozyme.

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
B. cereus ATCC 11778	18-24h /30°C/A	growth, pink colonies with opaque halo
E. coli ATCC 11775	18-24h /30°C/A	no growth
B. subtilis ATCC 6633	18-24h /30°C/A	growth, yellow colonies without opaque halo

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 – PERFORMANCE CHARACTERISTICS

Prior to release for sale, representative samples of all lots of dehydrated Bacillus Cereus Agar Base (MYP), supplemented with Egg Yolk Emulsion and Bacillus Cereus Antimicrobic Supplement and ready-to-use media (Test Batch:TB) are tested for productivity, specificity and selectivity by comparing the results with Tryptic Soy Agar.
The productivity is tested by a quantitative method with the target strain *B. cereus* ATCC 11778: the plates are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 30°C for 18-24 hours. The colonies are enumerated on both media and the productivity ratio (Pr: CFU_{TB}/CFU_{TSA}) is calculated. If Pr is ≥ 0.5 and if the colonies morphology and colour are typical (pink colonies with opaque halo) the results are considered acceptable and conform to the specifications.
Moreover, the productivity characteristics are tested by semi-quantitative ecometric technique with the following target strains: *B. cereus* ATCC 14579 and *B. thuringiensis* ATCC 10792. After incubation, the amount of growth and the colony characteristics are evaluated: the target strains exhibit good growth, with pink colonies with opaque halo.
The specificity is assessed by semi-quantitative ecometric technique with *B. subtilis* ATCC 6633. After incubation, the amount of growth and the colony characteristics are evaluated: *B. subtilis* exhibits good growth with yellow colonies without opaque halo.
The selectivity is assessed with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of *E. coli* ATCC 25922. The growth of *E. coli* is totally inhibited.

13-LIMITATIONS OF THE METHODS

- It appears that the spores of many, if not most, strains of *B. cereus* germinate readily on the surface of culture media used for enumeration. In most cases does not seem to be needed a heat shock treatment to induce germination. Sometimes a heat shock procedure is desirable, for example for spore counts or to inhibit growth of vegetative bacterial cells. In such cases, a treatment for 15 min at 70 °C is recommended.[3]
- The confirmatory tests may in some instances be inadequate for distinguishing *B. cereus* from culturally similar organisms that could occasionally be encountered in food. These organisms include 1) the insect pathogen *B. thuringiensis*, which produces protein toxin crystals; 2) *B. mycoides*, which characteristically produces rhizoid colonies on agar media; and 3) *B. anthracis*, which exhibits marked animal pathogenicity and is non-motile. With the exception of *B. thuringiensis*, which is currently being used for insect control on food and forage crops, these organisms are seldom encountered in the routine examination of food.[5]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

14 - PRECAUTIONS AND WARNINGS

- The medium base, the supplements and the ready-to-use media 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 and antibiotics containing supplements must be handled with suitable protection. 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 screw cap flasks to prevent injury due to breakage of glass.
- When using a hot plate and/or a water bath, boil sufficiently long to dissolve the whole medium.
- Wear heat-protective gloves during medium liquefaction. Do not place the hot flasks into an ice bath or in cold water to accelerate cooling as this might cause cracks in the glass.
- The time required for complete liquefaction of the medium may vary considerably and depends on the actual temperature of the heating device, its wattage, the size and volume of the bottle.
- Once the bottled medium is liquefied, it cannot be solidified and dissolved a second time.
- Ready-to-use flasks are subject to terminal sterilization by autoclaving.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium and supplements or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplements and the inoculated plates with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplements 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.

15 - STORAGE CONDITIONS AND SHELF LIFE

Ready to use plates

Upon receipt, store plates in their original pack at +2°C /+8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g., microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

Ready-to-use medium in flasks

Upon receipt, store flasks in their original pack at +2°C /+8°C away from direct light. If properly stored, the flasks may be used up to the expiration date. Do not use the flasks beyond this date. Flasks from opened secondary packages can be used up to the expiration date. Opened flasks must be used immediately. Before use, check the closing and the integrity of the screw cap. Do not use flasks with signs of deterioration (e.g., microbial contamination, abnormal turbidity, precipitate, atypical colour).

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). 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). According to ISO 7932, the plates may be stored prior to drying at between +2 °C and +8 °C for up to 4 days.[3]

16- REFERENCES

1. Turenne C, Alexander DC Bacillus and other Aerobic Endospore-Forming Bacteria. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.

2. Mossel DAA, Koopman MJ, Jongerius E. Enumeration of Bacillus cereus in food. Appl Microbiol 1967;15(3):650-3.
3. ISO 7932:2004/AMD 1:2020 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of presumptive Bacillus cereus — Colony-count technique at 30 degrees C — Amendment 1: Inclusion of optional tests
4. ISO 21871:2006 - Microbiology of food and animal feeding stuffs -- Horizontal method for the determination of low numbers of presumptive Bacillus cereus -- Most probable number technique and detection method
5. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 14: Bacillus cereus. Content current as of: 06/29/2021
6. APHA Compendium of Methods for the Microbiological Examination of Food, 5th Ed., American Public Health Association, Washington D.C., 2015
7. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 233-234.

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	For single use only

REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2022/05
Revision 3	Modifications to chapters 2, 5, 6, 7, 12, 14, 15	2022/12
Revision 4	Modifications of chapters 4A and 4B and other minor changes	2024/12

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