TECHNICAL DATA SHEET

BACILLUS CEREUS AGAR (ACC. TO MOSSEL)

ENUMERATION OF *BACILLUS CEREUS*

1 INTENDED USE

Bacillus cereus Agar is used for the detection and enumeration of spores and vegetative cells of *Bacillus cereus* in food products. It is used in the enumeration at 30°C of presumptive *Bacillus cereus* according to the standard (NF EN ISO 7932). It is also recommended in the detection or for small numbers method NF EN ISO 21871.

The typical composition corresponds to the MYP agar (mannitol, egg yolk, polymyxin agar) described in the standards NF EN ISO 7932 and NF EN ISO 21871.

2 HISTORY

In 1967, Mossel et al. recommended the use of a mannitol-phenol red-egg yolk medium, whose principles were based on two factors : the lack of mannitol fermentation by *Bacillus cereus* and the presence of a lecithinase in majority of tested strains.
The authors showed that satisfactory selectivity was obtained with polymyxin B at 10 mg / liter.

3 PRINCIPLES

Tryptone and meat extract favor the growth of *Bacillus cereus*.

Sterile egg yolk emulsion is used to detect the presence of lecithinase present in most *Bacillus cereus* strains. Insoluble breakdown products of egg yolk lecithin accumulate around the colonies, forming a whitish precipitate.

Mannitol is used to differentiate contaminating microorganisms which ferment it, causing phenol red to turn yellow.

Polymyxin is used to inhibit accompanying microflora when the tested sample is heavily contaminated.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of complete media :

- Tryptone ..... 10,0 g
- Meat extract..... 1,0 g
- D-mannitol..... 10,0 g
- Sodium chloride ..... 10,0 g
- Phenol red..... 25,0 mg
- Polymyxin B..... $1x10^5$ IU
- Sterile Egg Yolk emulsion ..... 100 mL
- Bacteriological agar..... 13,5 g

pH of the ready-to-use media at 25 °C : 7,2 ± 0,2.

For 44,5 g of dehydrated base media BK116

- Tryptone ..... 10,0 g
- Meat extract..... 1,0 g
- D-mannitol ..... 10,0 g
- Sodium chloride..... 10,0 g
- Phenol red ..... 25,0 mg
- Bacteriological agar ..... 13,5 g

For one vial of supplement BS066 (50 mL)

- Sterile Egg Yolk emulsion .....50,0 mL

For one vial of supplement BS055 (50 mL)

- Polymyxin B (sulfate)..... $5x10^4$ IU
- Sterile egg yolk emulsion..... 50,0 mL

Per vial of supplement Polymyxin B BS007 Qsp 500 mL

- Polymyxin B ..... $5x10^4$ IU)

5 PREPARATION

- Dissolve 44,5 g of dehydrated media (BK116) in 0,9 liters of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense 90 mL into vials or flasks.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool and maintain in a molten state at 44-47 °C.

✓ **Reconstitution : 44,5 g for 900 mL**

✓ **Sterilization : 15 min at 121 °C**

- Into each 90 mL vial of base media, aseptically add 10 mL of sterile egg yolk emulsion with Polymyxin B (BS055).
- Mix well.
- Pour into sterile Petri plates and let solidify on a cold, flat surface.

NOTE :
In the place of 10 mL of sterile egg yolk emulsion with Polymyxin B, it is possible to add 10 mL of sterile egg yolk emulsion (BS066) and 1 mL of rehydrated Polymyxin B supplement (supplement BS007 reconstituted with 5 mL sterile distilled water).

6 INSTRUCTIONS FOR USE

Enumeration of *Bacillus cereus* at 30 °C (NF EN ISO 7932)

- Dry the plates in an incubator, covers partially removed..
- Transfer 0.1 mL of the sample to analyze and its serial dilutions to the plates prepared as above or onto complete ready-to-use plates (BM038, BM199).
- Spread the inoculum on the surface of the agar with a sterile triangle.
- Incubate at 30 °C for 18 to 24 hours. If the colonies are not readily visible, prolong the incubation an additional 24 hours.

✓ **Inoculation : 0,1 mL on surface**

✓ **Incubation : 18 h to 48 h at 30 °C**

Detection or low bacterial numbers of *Bacillus cereus* (NF EN ISO 21871)

- On the surface of the media prepared as above or by using pre-poured plates (BM038, BM199), re-inoculate a loop of each enrichment broth.
- Incubate at 30 °C for 18 to 24 hours. If the colonies are not readily visible, prolong the incubation an additional 24 hours.

✓ **Inoculation : A loop on the surface**

✓ **Incubation : 18 h to 48 h at 30 °C**

7 RESULTS

Presumed colonies of *Bacillus cereus* are pink (mannitol-negative) and almost always surrounded by a halo of precipitate, indicating the production of lecithinase. In general they measure 2 to 5 mm and present irregular, stringy borders.

NOTE :
If the plates have a high level of contaminating flora that ferment mannitol, the pink color of the agar and the *Bacillus cereus* colonies may cause visibility problems in identification.

See ANNEX 1 : PHOTO SUPPORT.

8 QUALITY CONTROL

Dehydrated base media : pinkish powder, free-flowing and homogeneous.
Supplement Polymyxin B : white, giving rise to a colorless and limpid solution upon reconstitution.
Egg yolk supplements : yellowish emulsion, opaque, presenting a re-suspendable precipitate.
Prepared (complete) media : pink to orange agar, opaque.

Typical culture response after 24 (1) or 48 hours of incubation at 30 °C (NF EN ISO 11133) :

Microorganisms		Growth	Characteristics
(1) *Bacillus cereus*	WDCM 00001	*PR ≥ 50 %*	Pinkish colonies with a halo
Bacillus subtilis ssp. spizizenii	WDCM 00003	Limited, score 1-2	Yellow colonies without a halo
Escherichia coli	WDCM 00013	Inhibited, score 0	-

9 STORAGE / SHELF LIFE

Dehydrated base media : 2-30 °C.
Pre-poured media in Petri plates : 2-8 °C.
Sterile egg yolk emulsion : 2-8 °C
Sterile egg yolk emulsion with Polymyxin B : 2-8 °C
Polymyxin B Selective Supplement : 2-8 °C.
The expiration dates are indicated on the labels.

Prepared base media in vials (*) : 180 days at 2-8 °C.
Prepared complete media in plates (*) : 30 days at 2-8 °C.
Rehydrated Polymyxin B Supplement (*) : 30 days at 2-8 °C.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated base media (without egg yolk nor Polymyxin B) :
500 g bottle BK116HA
Sterile egg yolk emulsion with Polymyxin B :
10 x 50 mL vials BS05508
Sterile egg yolk emulsion :
10 x 50 mL vials BS06608
Polymyxin B Selective Supplement:
10 vials BS00708
Pre-poured media in Petri plates (Ø 90 mm) :
20 plates BM03808
120 plates BM19908

11 BIBLIOGRAPHY

Mossel, D.A.A., Koopman, M.J., and Jongerius, E. 1967. Enumeration of *Bacillus cereus* in Foods. App. Microb., 15, (3): 650-653.

NF EN ISO 7932. Juillet 2005. Microbiologie des aliments. Méthode horizontale pour le dénombrement de *Bacillus cereus* présomptifs. Technique par comptage des colonies à 30 °C.

NF EN ISO 21871. Juillet 2006. Microbiologie des aliments. Méthode horizontale pour le dénombrement de *Bacillus cereus* présumés en petit nombre. Technique du nombre le plus probable et méthode de recherche.

NF EN ISO 11133. Juillet 2014. Microbiologie des aliments, des aliments pour animaux et de l'eau. Préparation, production, stockage et essais de performance des milieux de culture.

12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

Document code : BACILLUS MOSSEL_ENv14
Creation date : 02-2003
Updated : 10-2022
Origin of revision : Minor correction

ANNEX 1 : PHOTO SUPPORT

MYP agar (for *Bacillus cereus* acc. to Mossel)

Detection and enumeration of *Bacillus cereus.*

Results :

Growth obtained after 24 hours of incubation at 30 °C.