

## Specification

Selective solid medium, according to Mossel, for the isolation and identification of *Bacillus cereus* from food samples according to ISO standards.

## Presentation

	Packaging Details	Shelf Life	Storage
20 Prepared plates 90 mm with: 21 ± 2 ml	1 box with 2 packs of 10 plates/pack. Single cellophane.	3 months	2-14 °C

## Composition

Composition (g/l):	
Peptone from casein.....	10.000
Mannitol.....	10.000
Sodium chloride.....	10.000
Meat extract.....	1.000
Phenol red.....	0.025
Agar.....	12.000
Polymixin B Sulphate.....	100,000IU
Egg Yolk .....	100 ml

## Description /Technique

### Description

Mossel's formulation is developed to detect and enumerate *B. cereus* in any food stuff. It is both selective and differential for this microorganism. Polymyxin addition inhibits most of accompanying bacteria, but does not affect the growth of *B. cereus*. This bacterium does not ferment mannitol and thus there is no change in the colour of the indicator around the colonies. The lecithinase activity of *B. cereus* produces a halo or zone of white precipitate around the colonies.

A count of *B. cereus* over 100.000 cells/g of food sample is considered hazardous, since the accumulated phosphoril-choline may cause toxic symptoms in children. For this reason a viable enumeration must be performed to evaluate the real population of cells.

### Technique

For plate inoculation follow the laboratories standard methods or the applicable norms (spiral plating method, econometric methods, streak plating, dilution banks, spread plating with drigalsky rod, among them).

According to the authors, dehydrated or dry samples must be treated in the following way: 20 g of sample is mixed with 90 mL of Tryptone Water for a minimum period of 1 hour, at room temperature. Afterwards, add an additional 90 mL of Tryptone Water and homogenize. If necessary dilute 1:10. Proceed to a 1/10 serial dilution bank using Tryptone water as the diluent if necessary. With a Drigalsky loop, spread aliquots of 0,1 mL over the surface of the agar plates and let the agar medium absorb the aliquots. Incubate the plates at 30°C for 18-24 hours to allow spore germination before giving definite results.

Suspected colonies have the following appearance: irregular borders, pink colour becoming purple in the centre, with a halo of white precipitate (mannitol +). Colonies with yellow halos must be discounted (mannitol -).

Confusion with other colonies of Gram positive bacilli is possible, and hence, confirmation tests must be carried out i.e. glucose fermentation, gelatine degradation and nitrate reduction.

## Quality control

### Physical/Chemical control

Color : Orange

pH: 7.2 ± 0.2 at 25°C

### Microbiological control

Inoculate: Practical range 100 ± 20 CFU. min. 50 CFU (productivity)/ 10<sup>4</sup>-10<sup>6</sup> (selectivity).

Microbiological control according to ISO 11133:2014/A1:2018.

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Aerobiosis. Incubation at 30 ± 1 °C, read after 24 ±3h - 44 ±4h

### Microorganism

*Bacillus cereus* ATCC® 11778, WDCM 00001

*Escherichia coli* ATCC® 25922, WDCM 00013

*Bacillus subtilis* ATCC® 6633, WDCM 00003

### Growth

Good (≥50%)-pink colonies with halo of precipitation

Inhibited

Yellow colonies without halous

### Sterility Control

Incubation 48 h at 30-35 °C and 48 h at 20-25 °C: NO GROWTH.

Check at 7 days after incubation in same conditions.

## Bibliography

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- ISO 7932 Standard (2004) 3rd ed. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of presumptive *Bacillus cereus*. Colony count technique at 30°C.
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## Storage

Storage conditions: 2-14°C

Avoid direct contact with surfaces that can freeze product.