Dehydrated Culture Media

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MYP AGAR (Mannitol Egg Yolk Polymyxin Agar)

CODE: CM0929

A medium for the enumeration of Bacillus cereus in food samples.

Typical Formula*	**gm/litre**
Meat extract	1.0
Peptone	10.0
Mannitol	10.0
Sodium chloride	10.0
Phenol Red	0.025
Agar	12.0

pH 7.2 ± 0.2 @ 25°C

*** Adjusted as required to meet performance standards**

POLYMYXIN B SUPPLEMENT

Code: SR0099

Vial contents (each vial is sufficient for 500ml of medium)	**per vial**	**per litre**
Polymyxin B	50,000IU	100,000IU

Directions
Suspend 21.5g in 450ml distilled water and bring gently to the boil to dissolve. Sterilise by autoclaving at 121°C for 15 minutes.
Cool to approximately 49°C and aseptically add 50ml Egg Yolk Emulsion SR0047 and 1 vial of Polymyxin B Supplement SR0099, reconstituted as directed. Mix well and pour into sterile petri dishes.

Description
Bacillus cereus has been recognised as a causative agent of food poisonings since the 1950s. Many early isolation techniques used blood agar, relying on haemolysis and colony morphology for the detection of suspect *Bacillus cereus*, and then a range of tests for confirmation. The main problem with these media was that they were not selective and were generally only useful in detecting high numbers of *Bacillus cereus*.
Bacillus cereus is a Gram-positive, rod shaped, facultatively aerobic sporeforming organism. The cells are large (3.5mm) and sometimes form short chains or long strings with central to terminal ellipsoidal spores that do not distend the cell. Unstained globules within the cells occur when they are grown on glucose containing media.

Although the presence of *Bacillus cereus* has been determined in foods related to outbreaks of illness since the early years of the 20th century[1], its role as a causative agent in food poisoning was not established until the 1950s.

Two distinct syndromes, emetic and diarrhoeal, may occur in *Bacillus cereus* associated foodborne illnesses, related to two different metabolites. The diarrhoeal type of illness is caused by a heat labile, high molecular weight protein, while the vomiting (emetic) type is caused by a heat stable, low molecular weight peptide[2].

A wide range of foods have been associated with the diarrhoeal syndrome, including meat based dishes, soups, vegetables, puddings and sauces[2]. The emetic syndrome seems to be associated to a

more limited range of foods with approximately 95% of all cases related to fried or cooked rice[3]. Foods implicated in *Bacillus cereus* induced illnesses usually contain at least 10^5cfu/g, although about 10 % of outbreaks have been associated with food containing less than this[2]. In almost all cases the implicated food has been held for too long at unsatisfactory storage temperatures.

MYP Agar is a selective and differential medium developed by Mossel *et al.*[4]. The diagnostic features of the medium rely upon the failure of *Bacillus cereus* to utilise mannitol and the ability of most strains to produce phospholipase C. The medium is made selective by the addition of Polymyxin B which will inhibit Gram-negative bacteria. MYP Agar has proved to be very effective for detecting *B. cereus* even for ratios as challenging as one cell of *Bacillus cereus* to 10^6 cells of other organisms[4].

Technique
Dry the surface of the agar medium. Prepare the food sample by making appropriate dilutions in Peptone Water CM0009.
Spread 0.1ml of these dilutions over the surface of the agar plate using a sterile glass spreader. Incubate at 30° C for 18-40 hours.

Count the number of typical colonies and calculate the viable count.

Typical colonies of *Bacillus cereus* are rough and dry with a bright pink background surrounded by an egg yolk precipitate.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.
Polymyxin B Supplement SR0099 should be stored at 2-8° C.
Store the prepared plates at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder
Prepared medium: Red coloured gel

Quality control

Positive control:
Expected results
Bacillus cereus ATCC® 11778*
Good growth; bright pink colonies; zone of egg yolk precipitation
Negative control:

Escherichia coli ATCC® 25922*
No growth

*** This organism is available as a Culti-Loop®**

References
1. Lübenau, C. (1906). *Zbl. Bakt., I.* **40**: 433-437
2. Kramer, J. M. and R. J. Gilbert (1989) *Bacillus cereus* and other *Bacillus* species. In: Foodborne Bacterial Pathogens. pp.21-70. Doyle, M. P. (Ed.). Marcel Dekker, New York.
3. Jenson, I. and C. J. Moir (1997) *Bacillus cereus* and other *Bacillus* species. In: Foodborne Microorganisms of Public Health Significance. 5th Edition. pp.379-406. A. D. Hocking (Ed.). AIFST (NSW Branch) Food Microbiology Group, Australia.
4. Mossel, D.A.A.; Koopman, M.J. and Jongerius, E. (1967) *Appl. Microbiol*. **15**, 650-653.