MANNITOL EGG YOLK POLYMYXIN AGAR

INTENDED USE
Remel Mannitol Egg Yolk Polymyxin (MEP) Agar is a solid medium recommended for use in qualitative procedures for the isolation of *Bacillus* spp. from foods.

SUMMARY AND EXPLANATION
Food poisoning by *Bacillus cereus* may occur when foods have been prepared and held without adequate refrigeration before being served. *B. cereus* is an aerobic sporeformer which is commonly found in vegetables, soil, and many raw and processed foods. MEP Agar was formulated for cultivation and enumeration of *B. cereus* in foods. It is recommended by the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA).[1-3]

PRINCIPLE
Beef extract and peptone supply essential amino acids, peptides, and nitrogenous substances necessary for bacterial growth. Mannitol is the carbon energy source and sodium chloride maintains osmotic equilibrium. Acid produced in the fermentation of mannitol, by *Bacillus* spp. other than *B. cereus*, causes the phenol red indicator to change the colony color from pink to yellow. *B. cereus* does not ferment mannitol and produces pink colonies on MEP Agar. Egg yolk supplies lecithin and serves to reduce the toxic effect of organic peroxides which may accumulate in the agar. Lecithinase, an enzyme produced by *B. cereus,* metabolizes lecithin and produces an insoluble, opaque precipitate in the medium surrounding growth. Polymyxin B is a selective agent which inhibits many gram-negative bacilli and gram-positive cocci.

REAGENTS (CLASSICAL FORMULA)*

Mannitol	10.0 g	Phenol Red	25.0 mg
Peptone	10.0 g	Polymyxin B	10.0 mg
Sodium Chloride	10.0 g	Egg Yolk Suspension	100.0 ml
Beef Extract	1.0 g	Agar	15.0 g
		Demineralized Water	900.0 ml

pH 7.2 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PRECAUTIONS
This product is for Laboratory Use only. It is not intended for use in the diagnosis of disease or other conditions.

PROCEDURE
1. Prepare serial dilutions of the material for testing in a suitable broth medium (e.g., tryptic soy-polymyxin broth) and incubate at 30 ± 2°C for 24-48 hours.[2,3]
2. Subculture positive broth tubes to MEP Agar following the plate count method, the most probable number (MPN) method, or established laboratory procedures.
3. Incubate MEP Agar plates for 24-48 hours at 30 ± 2°C.
4. Examine plates for characteristic colony morphology. *B. cereus* colonies do not ferment mannitol and form pink colonies; other *Bacillus* spp. usually form yellow colonies. *B. cereus* colonies produce lecithinase which is characterized by a wide zone of precipitation (turbidity) surrounding the individual colonies in the medium.
5. Confirmative identification of colonies which are characteristic of *B. cereus* requires additional biochemical testing following established laboratory procedures. Consult appropriate references for further instructions.[2,3]

INTERPRETATION OF THE TEST
Mannitol Fermentation:
Positive Test - Yellow colonies indicating mannitol fermentation
Negative Test - Pink to red colonies (becoming more intense over time) indicating mannitol has not been fermented

Lecithin Production:
Positive Test - An opaque precipitate in the medium surrounding the colonies indicating lecithinase has been produced
Negative Test - No opaque precipitate in the medium

QUALITY CONTROL
Each lot number of Mannitol Egg Yolk Polymyxin Agar has been manufactured, packaged, and processed in accordance with current Good Manufacturing Practice regulations. All lot numbers have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures.

CONTROL	INCUBATION	RESULTS
Bacillus cereus ATCC® 11778	Aerobic, up to 48 h @ 33-37°C	Pink colonies w/ opaque zone
Escherichia coli ATCC® 25922	Aerobic, up to 48 h @ 33-37°C	Inhibition (complete)
Staphylococcus epidermidis ATCC® 12228	Aerobic, up to 48 h @ 33-37°C	Inhibition (partial to complete)

(Continued on back)

LIMITATIONS

1. MEP Agar is not 100% selective. Organisms other than *B. cereus*, including other *Bacillus* spp., may grow.[3]
2. Organisms which grow on MEP Agar and ferment mannitol may produce acid which diffuses throughout the agar making it difficult to distinguish mannitol-fermenting from nonfermenting organisms.[3]

BIBLIOGRAPHY

1. Horowitz, W. 2002. Official Methods of Analysis of AOAC International. 17th ed., 1st rev. AOAC International, Washington, D.C.
2. Food and Drug Administration. 2001. Bacteriological Analytical Manual Online. Chapter 14, Updated May 2009. Authors: E.J. Rhodehamel and S.M. Harmon. Contacts: N. Belay, D.B. Shah, and R.W. Bennett. AOAC International, Gaithersburg, MD.
3. Downes, F.P. and K. Ito. 2001. Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA, Washington, D.C.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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