Instructions for Use

REF - KM0186 ***Bacillus cereus* Agar (PEMBA)**

Dehydrated Culture Media

1. Intended Use

For *in vitro* diagnostic use. KM0186 *Bacillus cereus* Agar (PEMBA) is a differential selective medium for the isolation and enumeration of *Bacillus cereus* in clinical specimens and food samples.

2. Composition*

Ingredient	g/L
Peptone	1.0
Mannitol	10.0
Magnesium sulphate	0.1
Sodium chloride	2.0
Disodium hydrogen phosphate	2.5
Potassium dihydrogen phosphate	0.25
Bromothymol blue	0.12
Sodium pyruvate	10.0
Agar	15.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Bacillus cereus Selective Agar (PEMBA) was developed by Holbrook and Anderson [1] for the isolation and enumeration of *B. cereus* in foods. It meets the requirements for a medium that is sufficiently selective to be able to detect small numbers of *B. cereus* cells and spores in the presence of large numbers of other food contaminants. The medium is also sufficiently diagnostic that colonies of *B. cereus* are readily identified and confirmed by microscopic examination.

KM0186, when prepared as a final medium, is recommended by the International Organization for Standardization [2] and the UK Standards for Microbiology Investigations [3 4] and is tested in accordance with ISO 11133:2014 [5].

4. Principle

The medium is made selective by the inclusion of polymyxin B sulphate (E&O LS1051), sodium pyruvate is also present which is said to improve egg yolk precipitation and enhance sporulation. As *B. cereus* is mannitol-negative, the colonies are bluish in colour due to the presence of the bromothymol blue indicator, with a surrounding precipitate of the same colour due to lecithinase production from the egg yolk.

5. Preparation Instructions

Suspend 41.0g/L of dehydrated culture media in 950ml of deionised/purified water and allow to soak whilst mixing for 10 minutes.

Heat with frequent agitation and boil for one minute to completely dissolve powder.

Autoclave to sterilise at 121°C for 15 minutes before cooling in a water bath to 45-50°C. Aseptically add 50ml Egg Yolk Emulsion (BM0140) and the appropriate number of vials of *Bacillus cereus* (PEMBA) Supplement (LS1051).

Gently homogenize the final medium before aseptically dispensing the specified volume into appropriate sterile containers and allowing to cool.

Prepared media may be kept at between 2 and 8°C for up to 14 days away from direct sunlight.

6. Physical Characteristics

	Dehydrated Medium	Prepared Medium
Appearance and Colour	Straw fine powder	Yellow/green opaque firm gel
pH	N/A	7.2 ± 0.2

7. Materials Provided

KM0186 can be provided in the formats detailed below. Each tub is labelled with product name, product code, lot number, expiry date, instructions, and appropriate warnings.

Product Code	Product Format
KM0186-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0186-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0186-10KG-10000	1 x 10kg Dehydrated Culture Media Tub

8. Materials Needed but not Provided

Standard microbiological laboratory materials *e.g.,* autoclave, sterile loops or swabs, collection containers, incubators, and quality control organisms.

Ingredients for preparation of media supplied by E&O Laboratories e.g., supplements E&O BM0140 Egg Yolk Emulsion and E&O LS1051 *Bacillus cereus* (PEMBA) Supplement.

9. Specimens

KM0186 *Bacillus cereus* Agar (PEMBA) is suitable for the testing of the following specimens:

- Food Specimens: products for human consumption, animal feed and environmental samples
- Clinical Specimens: rectal swabs and stool specimens.

Sampling and transport equipment must be used in accordance with the end user's suppliers' recommendations for the conservation of *Bacillus* species. Refer to appropriate standard method or local guidance on sample collection and subsequent processing.

10. Test Procedures and Interpretation of results

Allow the plates to come to room temperature.

Based on sample type and information provided, check to see if the specimen needs to be pre-enriched prior to inoculation to the prepared medium.
Inoculate plated media directly with the sample, or subculture onto plated media after incubation in enrichment broth where required.

Food and Environmental Specimens:

For solid samples, prepare a homogenised suspension using a ratio of 1:9 of sample to Maximum Recovery Diluent (E&O BM0760). With homogenised and liquid specimens, media should be inoculated with 1ml of the specimen and incubate at 30 ± 1°C aerobically for 24 hours.

Clinical Specimens [3 4]

- Process the clinical specimen as required by local guidelines.
- Prepare a homogenised suspensions using both a ratio of 1:9 of sample to Phosphate Buffered Saline (E&O BM1360) and 1:99 of sample to Phosphate Buffered Saline (E&O BM1360).
- Inoculate specimens onto the prepared KM0186 medium.
- Incubate the plates at 36 ± 1°C aerobically for 16-24 hours. Further incubation at room temperature up to 48 hours may be required.

After incubation, examine agar for colonies (typical colony appearance outlined in Quality Control table below). If no initial colonies were seen at 24 hours, re-incubate the for a further 24 hours at 30 ± 1°C. Perform further biochemical or mass spectroscopy testing to confirm identity of presumptive positive isolates. Refer to relevant local guidelines.

11. Quality Control

Organism	Incubation	Result (Specificity)
B. cereus (NCTC 10320)	37 ± 1°C aerobically for 24-48 hours	Growth: Turquoise/blue colonies with zone of precipitate
B. subtilis (NCTC 10400)	37 ± 1°C aerobically for 24-48 hours	Growth: White/Yellow colonies with zone clearing
E. coli (NCTC 12241)	37 ± 1°C aerobically for 24-48 hours	Inhibited

It is the responsibility of the user to perform Quality Control testing taking into consideration the intended use of the medium, and in agreement with any local relevant guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

12. Performance

To fully verify KM0186 Bacillus cereus Agar (PEMBA) performance, dehydrated culture media samples were used to prepare PEMBA Agar and tested to assess colony morphology and recovery level (where an acceptable range is ≥70% and ≤120%) compared to a non-selective reference medium. Prepared samples were inoculated with 30-150cfu and 10^4-10^5 cfu for the non-target organisms and incubated at 37 ± 1°C aerobically for 24-48 hours. All samples of prepared media produced grew and showed good recovery and the correct morphology of the required test organisms: *Bacillus cereus* (NCTC 10320) and *Bacillus subtilis* (NCTC 10400) and no recovery of the non-target test organism: *Escherichia coli* (NCTC 12241). Therefore, it can be concluded that KM0186 Bacillus cereus Agar (PEMBA), meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

13. Limitations of the Media

- It should be noted that some *Proteus* spp. and Gram-positive cocci may grow on this medium.
- Due to natural variation, some strains may grow poorly on this medium.

14. Precautions and Warnings

This product is considered hazardous under CLP regulations. Refer to KM0186 Material Safety Data Sheet. Wear such PPE as recommended by laboratory COSHH assessment. During and after use, always handle all materials in a manner conforming to Good Laboratory Practices and consider that material under test should be regarded as a potential biohazard if mishandled.

15. Storage conditions and Shelf life

Store product in the original container with the lid tightly closed at between 10 and 30°C in low humidity conditions away from direct sunlight. Kept under these conditions, the product may be used up to the date of expiry shown on the product label.

Do not use if the product is not free-flowing or displays any sign of colour change, formation of large lumps or hardening of the powder. Additionally, do not use medium if it has been stored inappropriately, the packaging has been damaged or has passed the expiry date.

Dehydrated culture media does not need to be used all at once; replace the cap and ensure that the container is tightly closed and stored according to labelled instructions.

Dispose of in accordance with local and national authority requirements.

16. References

1. Holbrook, R. and Anderson, J. (1980) An improved selective and diagnostic medium for the isolation of Bacillus cereus in foods. Can. J. Microbiol. 26(7), pp.753-759.
2. International Organization for Standardization (2006) 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. Geneva: ISO.
3. Public Health England (2018) Identification of *Bacillus* species. UK Standards for Microbiology Investigations. Bacteriology – Identification, ID 9(3.1). London: Standards Unit, Microbiology Services.
4. Public Health England (2020) Gastroenteritis. UK Standards for Microbiology Investigations. Syndromic, S 7(2). London: Standards Unit, National Infection Service.
5. International Organization for Standardization (2014) 11133:2014 Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media. Geneva, ISO.

Version History*

001	29/09/23	New Document Created

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

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TABLE OF APPLICABLE SYMBOLS

REF o REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Σ Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place