



# CERTIFIED REFERENCE MATERIAL

## BCR<sup>®</sup> – 528

### CERTIFICATE OF ANALYSIS

<b><i>Bacillus cereus</i> in milk powder</b>			
Colony forming particles of <i>Bacillus cereus</i> according to the procedure <sup>3)</sup>	Number of colony forming particles (cfp)		Number of accepted sets of data p
	Certified value <sup>1)</sup> [cfp/capsule]	Uncertainty interval <sup>2)</sup> [cfp/capsule]	
MEYP (ISO 7932) after 24 h incubation	53.4	51.7 – 55.2	11 <sup>5)</sup>
MEYP (ISO 7932) after 48 h incubation	53.7	52.1 – 55.4	11 <sup>5)</sup>
PEMBA (L 00.00 - 25) <sup>4)</sup> after 24 h incubation	55.0	52.8 – 57.4	11 <sup>6)</sup>
PEMBA (L 00.00 - 25) <sup>4)</sup> after 48 h incubation	55.8	53.6 – 58.0	11 <sup>6)</sup>
<p>1) This certified value is the geometric mean of 11 accepted sets of data, independently obtained from 11 laboratories. The certified value is the number of colony forming particles (cfp) of the test strain in one analytical portion. Analytical portion: A volume of <math>(0.10 \pm 0.02)</math> mL from 10 mL peptone saline solution (see overleaf) in which one capsule has been reconstituted. The certified values are traceable to the respective (ISO) procedure used.</p> <p>2) These values are the 95% confidence interval, associated with the certified value of the specified procedure.</p> <p>3) MEYP: Mannitol Egg Yolk Polymyxin agar PEMBA: Pyruvate Egg yolk Mannitol Bromothymol blue Agar</p> <p>4) German Federal Food Law method number L 00.00 – 25 (1992)</p> <p>5) Based on the results of 154 capsules.</p> <p>6) Based on the results of 153 capsules.</p>			

This certificate is valid for one year after purchase.

Sales date:

The minimum amount of sample to be used is the entire capsule.

#### NOTE

This material has been certified by BCR (Community Bureau of Reference, the former reference materials programme of the European Commission). The certificate has been revised under the responsibility of IRMM.

Brussels, December 1994  
Latest revision: September 2013

Signed: \_\_\_\_\_

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Indicative Values		
Colony forming particles of <i>Bacillus cereus</i> according to the procedure	Number of colony forming particles (cfp)	
	Indicative value <sup>1)</sup> [cfp]	Uncertainty interval <sup>2)</sup> [cfp]
SBA <sup>3)</sup> (Analysis no 67) <sup>4)</sup> after 24 h incubation	50.1	48.4 – 51.7
<p>1) This value is the geometric mean based on the results of 4 laboratories. The value is the number of colony forming particles (cfp) of the test strain in one analytical portion. Analytical portion: A volume of (0.10 ± 0.02) mL from 10 mL peptone saline solution (see overleaf) in which one capsule has been reconstituted. The indicative value is traceable to the respective procedure.</p> <p>2) These values are the 95% confidence interval, associated with the indicative value.</p> <p>3) Sheep Blood Agar.</p> <p>4) Nordic Committee on Food Analysis method number 67, second edition (1982).</p>		

## DESCRIPTION OF THE SAMPLE

The CRM consist of 0.317 g artificially contaminated with spray dried milk contained in an ochre/white gelatine capsule. The strain used for the contamination is *Bacillus cereus* (ATCC 9139). The capsules are packed in a plastic container with a silica gel desiccant bag. The entire capsule should be reconstituted according to the instruction for use. Additional information on the material is given in the report.

## ANALYTICAL METHOD USED FOR CERTIFICATION

- Spread plate technique according to ISO 7932 (1987) using the Mannitol Egg Yolk Polymyxin agar (MEYP).
- Spread plate technique according to the German Federal Food Law method no L 00.00 – 25 (1992) using the Pyruvate Egg yolk Mannitol Bromothymol blue Agar (PEMBA).
- Spread plate technique according to the Nordic Committee on Food Analysis method no 67, second edition (1982) using the Sheep Blood Agar (SBA).

## PARTICIPANTS

- Laboratory for Water and Food Microbiology, National Institute of Public Health and Environmental Protection, Bilthoven (NL)
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## SAFETY INFORMATION

The usual laboratory safety precautions for Biosafety level II pathogens apply.

This CRM is intended for in vitro analysis only. **DO NOT TOUCH THE CAPSULES BY HAND, USE STERILE FORCEPS OR WEAR STERILE GLOVES. THE CAPSULES SHOULD NOT BE OPENED.**

## INSTRUCTIONS FOR USE

To make it ready for use, the material has to be reconstituted according to the following procedure:

### 1. Material

- Peptone saline solution (PS), ingredients (g/L): Peptone 1, NaCl 8.5. Sterilized at  $(121 \pm 1)^\circ\text{C}$  for  $(15 \pm 1)$  min
- Water bath  $(38.5 \pm 0.5)^\circ\text{C}$
- Whirl mixer
- Sterile test tubes, e.g. 25 mm diameter x 190 mm length, with caps

### 2. Reconstitution procedure

Fill one or a number of test tubes with  $(10.0 \pm 0.2)$  mL PS of room temperature. Place the tubes in the water bath for at least 30 min. Add one capsule to each tube. After 10 min place the tube on the whirl mixer and mix well for 5-10 sec (mixing speed about 1000 rpm). Replace the tube in the water bath. Repeat for the other tubes. Mix again after 20 and 30 min as described above. After the third mixing, place the tube directly in melting ice. Use the dissolved capsule solution within 2 h. Note: In order to assure good dispersion of the gelatine capsule it is critically important that the tubes are kept out of the water bath for the minimum length of time.

Further information on the reconstitution procedure of this CRM can be found in Annex E of the report.

This CRM is intended to be used for:

1) Checking the spread plate technique according to ISO 7932 (1987) using the Mannitol Egg Yolk Polymyxin agar (MEYP).

For this purpose pipette  $(0.100 \pm 0.002)$  mL of the cooled capsule solution onto the surface of a MEYP plate and spread the inoculum as quickly as possible over the surface by means of a sterile spreader trying not to touch the sides of the plate. Incubate at  $(30 \pm 1)^\circ\text{C}$  for  $(48 \pm 4)$  h. Count the number of colonies after  $(24 \pm 2)$  h and/or after  $(48 \pm 4)$  h.

2) Checking the spread plate technique according to the German Federal Food Law method no L00.00 – 25 (1992) using the Pyruvate Egg yolk Mannitol Bromothymol blue Agar (PEMBA).

For this purpose pipette  $(0.100 \pm 0.002)$  mL of the cooled capsule solution onto the surface of a PEMBA plate and spread the inoculum as quickly as possible over the surface by means of a sterile spreader trying not to touch the sides of the plate. Incubate at  $(37 \pm 1)^\circ\text{C}$  for  $(48 \pm 4)$  h. Count the number of colonies after  $(24 \pm 2)$  h and/or after  $(48 \pm 4)$  h.

3) Checking the spread plate technique according to the Nordic Committee on Food Analysis method no 67, second edition (1982) using the Sheep Blood Agar (SBA).

For this purpose pipette  $(0.100 \pm 0.002)$  mL of the cooled capsule solution onto the surface of a SBA plate and spread the inoculum as quickly as possible over the surface by means of a sterile spreader trying not to touch the sides of the plate. Incubate at  $(30 \pm 1)^\circ\text{C}$  or at  $(37 \pm 1)^\circ\text{C}$  for  $(24 \pm 2)$  h.

Further information on the use of this CRM and on the confidence limits (geometric mean) for the most frequently used combinations of capsules and replicates for MEYP and PEMBA can be found in Chapter 9 of the report.

Dispose in accordance with good laboratory practice.

## STORAGE

Upon receipt the material shall be stored at  $(-20 \pm 5)^\circ\text{C}$  until reconstitution.

However, the European Commission cannot be held responsible for changes that happen during storage of the material at the customer's premises, especially of opened samples.

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## NOTE

A technical report on the production of BCR-528 is available on the internet (<http://www.irmm.jrc.be>).

A paper copy can be obtained from IRMM on request.