



**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB 4789.14-2014

**National food safety standard - Food Contact Materials and
Articles - Food Microbiological Examination - Bacillus Cereus**

食品安全国家标准 食品微生物学检验 蜡样芽胞杆菌检验

(English Translation)

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Table of Contents

Foreword...	3
1 Scope...	4
2 Equipment and materials...	4
3 Culture media and reagents...	4
4 Plate count method of bacillus cereus (first method)...	5
5 MPN count method of bacillus cereus (second method)...	12
Annex A Culture media and reagents...	15
Annex B Retrieval table of bacillus cereus most probable numbers...	23

Foreword

This Standard replaces GB/T 4789.14-2003, Microbiological examination of food hygiene - Examination of bacillus cereus.

Compared with GB/T 4789.14-2003, the major changes of this Standard are as follows.

- it changes the Chinese name of the standard;
- it adds the second method, the MPN counting method of bacillus cereus;
- it changes the culture conditions for selective isolation medium (MYP), from culturing for 12 h ~ 20 h at 37°C to culturing for 24 h ~ 48 h at 30°C ± 1°C;
- it adds the lysozyme examination;
- it changes the operating procedures;
- it adds Annex A and Annex B.

National Food Safety Standard -

Food Microbiological Examination - Bacillus Cereus

1 Scope

This Standard specifies the examination method of bacillus cereus in food.

The first method of this Standard applies to the count of bacillus cereus in food which has a high content of bacillus cereus; and the second method applies to the count of bacillus cereus in food which has a low content of bacillus cereus.

2 Equipment and materials

In addition to conventional sterilization and culture equipment for microbiology laboratories, the other equipment and materials are as follows.

- a) refrigerators. $2^{\circ}\text{C} \sim 5^{\circ}\text{C}$;
- b) thermostatic incubators. $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$, $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$;
- c) homogenizers;
- d) electronic balances. sensitivity 0.1 g;
- e) sterile conical flasks. 100 mL, 500 mL;
- f) sterile pipettes. 1 mL (having 0.01 mL scale), 10 mL (having 0.1 mL scale) or micropipettes and tips;
- g) sterile plates. diameter 90 mm;
- h) sterile examination tubes. 18 mm x180 mm;
- i) microscopes. 10x ~ 100x (oil immersion lens);
- j) L spreader.

4.1 Examination procedures

The examination procedures of the plate count method of bacillus cereus are as shown in Figure 1.

4.2.1 Sample treatment

Frozen samples shall be unfrozen not exceeding 15 min below 45°C and not exceeding

18 h at $2^{\circ}\text{C} \sim 5^{\circ}\text{C}$. If they can't be examined promptly, they shall be stored at $-10^{\circ}\text{C} \sim$

-20°C . Non-frozen, perishable samples shall be examined as soon as possible; and if they can't be examined promptly, they shall be stored in a refrigerator at $2^{\circ}\text{C} \sim 5^{\circ}\text{C}$ and examined within 24 h.

4.2.3 Sample dilution

Absorb 1 mL of the 1.10 sample homogeneous solution of 4.2.2 to add to a dilution tube containing 9 mL of PBS or normal saline; fully mix up to make 1.100 sample

homogeneous solution. In accordance with the estimation of sample contamination degree, operate as above and make into ten-fold incremental serial dilution sample homogeneous solution in succession. After each dilution, replace one 1 mL sterile pipette or tip.

4.2.5.1 Isolation

Under normal conditions, allow plates to stand for 10 min after applying. If sample solution is difficult to absorb, it can be placed in an incubator to culture for 1 h at $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$; turn over plates after sample homogeneous solution is evenly absorbed; place them upside down in the incubator; culture for $24 \text{ h} \pm 2 \text{ h}$ at $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$. If the colonies are not typical, continue culturing for $24 \text{ h} \pm 2 \text{ h}$ before observing. On MYP agar plates, typical colonies are of a faint pink colour (indicating unfermented mannitol) and there are white to pale orchid pink precipitation ring (indicating the production of lecithinase).

4.3.1 Dyeing microscopic examination

Pick single colonies of pure culture for Gram's microscopic examination. *Bacillus cereus* is Gram positive bacillus of size ($1 \mu\text{m} \sim 1.3 \mu\text{m}$) $\times$ ($3 \mu\text{m} \sim 5 \mu\text{m}$); the spore is oval which is located at the centre or one end of thallus, not expanding on thallus; both ends of thallus are flat, normally arranged in the shape of short chains or long chains.

4.3.2.4 Root growth test

Item *Bacillus cereus* *Bacillus thuringiensis* *Bacillus mycoides* *Bacillus anthracis* *Bacillus megaterium*

4.4.1.1 Select plates having typical bacillus cereus colonies and plates of a total

colony count between 20 CFU $\sim$ 200 CFU on three plates of the same dilution degree; count typical colonies. In case of the phenomena of a) $\sim$ f), calculate in accordance with Equation (1) of 4.4.2.1. In case of the phenomenon of g), calculate in accordance with Equation (2) of 4.4.2.2.

5.1 Examination procedures

The examination procedures of the MPN count method of *Bacillus cereus* is shown in Figure 2.

5.2.1 Sample treatment

As in 4.2.1.

5.2.2 Sample preparation

As in 4.2.2.

5.2.3 Sample dilution

As in 4.2.3.

5.2.5 Culture

Use an inoculating loop to transfer 1 loop from each tube; inoculate on MYP agar plates by streaking; culture for $24\text{ h} \pm 2\text{ h}$ at $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$. If colonies are not typical, continue to culture for $24\text{ h} \pm 2\text{ h}$ before observation.

5.2.6 Confirmatory appraisal

Select 5 typical colonies from each plate (select all if less than 5); inoculate them on nutrient agar plates for pure culture by streaking; culture for $24\text{ h} \pm 2\text{ h}$ at $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$; carry out confirmatory test. See 4.3.

Annex A

Culture media and reagents

A.1 Phosphate buffer solution (PBS)

A.1.1 Composition

Potassium dihydrogen phosphate 34.0 g

Distilled water 500.0 mL

A.3 Trypticase soy polymyxin broth

A.4 Nutrient agar

A.6 Motility medium