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GB 4789.14-2014

National Food Safety Standard Food Microbiological Examination: Examination of 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

National Health and Family Planning Commission

Release Date: Dec. 1, 2014
Implementation Date : May 1, 2015

Translated by ChemLinked

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National Standard of the People's Republic of China

GB 4789.14-2014

In Replacement of GB/T 4789.14-2003

National Food Safety Standard

Food Microbiological Examination: Examination of *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

Date of Publication: 2014-12-01 Date of Implementation: 2015-05-01

Issued by:

National Health and Family Planning Commission of People's Republic of China

Foreword

This standard replaces GB/T 4789.14-2003 “Microbiological Examination of Food Hygiene-Examination of *Bacillus Cereus*”.

Compared with GB 4789.14-2003, major changes of this standard are as follows:

—— the Chinese title of this standard has been revised;

—— the “Method II MPN enumeration of *Bacillus cereus*” has been added;

——the incubation condition for selective isolation medium (MYP) has been revised from “incubate at 37 ℃ for 12 h~20 h” to “incubate at 30 ℃±1 ℃ for 24 h~48 h”;

——the “Lysozyme test” has been added;

——the operation procedures have been revised;

——the Annex A and Annex B have been added.

National Food Safety Standard

Food Microbiological Examination: Examination of *Bacillus Cereus*

1 Scope

This standard specifies the method for examination of *Bacillus cereus* in foods.

Method I of this standard applies to the enumeration of *Bacillus cereus* in foods with high content of *Bacillus cereus*; Method II applies to the enumeration of *Bacillus cereus* in foods with low content of *Bacillus cereus*.

2 Apparatus and Materials

In addition to the conventional apparatus for sterilization and incubation in microbiological laboratory, other apparatus and materials are as follows:

a) Refrigerator: 2 ℃~5 ℃;

b) Constant temperature incubator: 30 ℃±1 ℃, 36 ℃±1 ℃;

c) Homogenizer;

d) Electronic balance: with sensitivity of 0.1 g;

e) Sterile conical flask: with nominal capacities of 100 mL and 500 mL;

f) Sterile pipette: with nominal capacities of 1 mL (graduated in 0.01 mL division) and 10 mL (graduated in 0.1 mL division) or micropipette and pipette tips;

g) Sterile culture dish: with diameter of 90 mm;

h) Sterile tube: 18 mm×180 mm;

i) Microscope: 10×~100× (oil immersion lens);

j) L-shaped spreading rod.

3 Culture Mediums and Reagents

3.1 Phosphate buffer solution (PBS): see section A.1 in Annex A.

3.2 Mannitol yolk polymyxin (MYP) agar: see section A.2 in Annex A.

3.3 Tryptic soy polymyxin broth: see section A.3 in Annex A.

3.4 Nutrient agar: see section A.4 in Annex A.

3.5 Hydrogen peroxide solution: see section A.5 in Annex A.

3.6 Motility medium: see section A.6 in Annex A.

3.7 Nitrate broth: see section A.7 in Annex A.

3.8 Casein agar: see section A.8 in Annex A.

3.9 Manganese sulfate nutrient agar: see section A.9 in Annex A.

3.10 0.5% basic fuchsin: see section A.10 in Annex A.

3.11 Motility medium: see section A.11 in Annex A.

3.12 Sugar fermentation tube: see section A.12 in Annex A.

3.13 V-P medium: see section A.13 in Annex A.

3.14 Tryptic soy sheep blood (TSSB) agar: see section A.14 in Annex A.

3.15 Lysozyme nutrient broth: see section A.15 in Annex A.

3.16 Simmons citrate medium: see section A.16 in Annex A.

3.17 Gelatin medium: see section A.17 in Annex A.

4 Plate Count of *Bacillus Cereus* (Method I)

4.1 Examination procedures

Refer to Figure 1 for the examination procedures for plate count of *Bacillus cereus.*

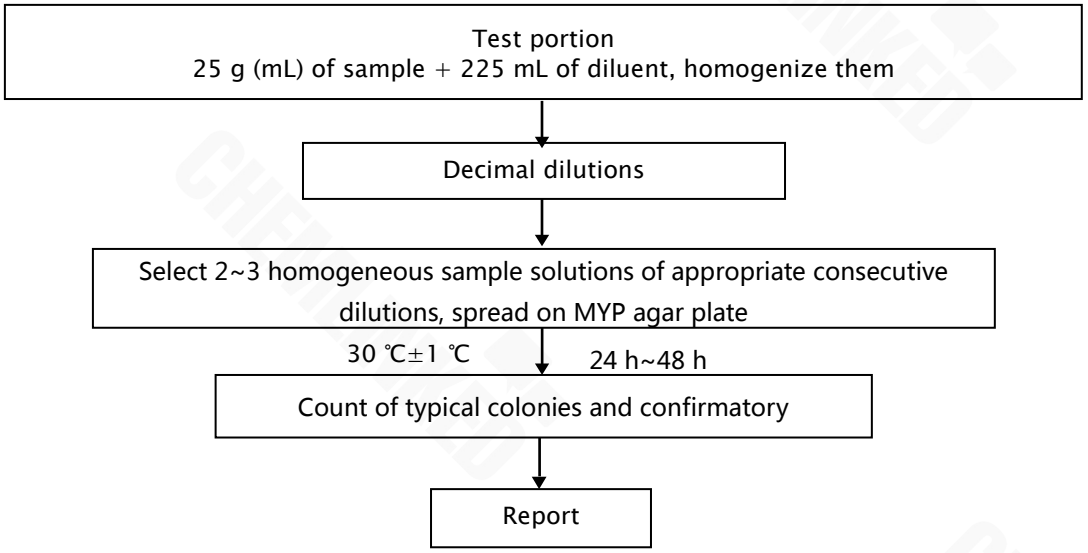

Figure 1 Examination procedures for plate count of *Bacillus cereus*

4.2 Operation procedures

4.2.1 Sample treatment

Frozen sample shall be thawed at 45 ℃ for not more than 15 min or at 2 ℃~5 ℃ for not more than 18 h; if it cannot be examined timely, store it at -10 ℃~-20 ℃; Non-frozen perishable sample shall be examined timely as far as possible; if it cannot be examined

timely, store it in a refrigerator at 2 ℃~5 ℃, and examine it within 24 h.

4.2.2 Sample preparation

Weigh 25g of sample, place in a sterile homogenization cup containing 225 mL of PBS or normal saline, homogenize it at 8000 r/min~10000 r/min for 1 min~2 min with a rotary blade homogenizer, or place in a sterile homogenization bag containing 225 mL of PBS or normal saline, and slap it for 1 min~2 min with a slapping homogenizer. If the sample is liquid, pipette 25 mL of sample, place in a sterile conical flask (appropriate amount of sterile glass beads may be pre-placed in the flask) containing 225 mL of PBS or normal saline, and then shake well to get a 1:10 homogeneous sample solution .

4.2.3 Sample dilution

Pipette 1 mL of 1:10 homogeneous sample solution prepared in Section 4.2.2, place in a dilution tube containing 9 mL of PBS or normal saline and then shake well to get a 1:100 homogeneous sample solution. Based on the estimate of sample contamination status and according to the above operation procedures, prepare homogeneous sample solutions of decimal ascending dilutions. Use a new 1 mL sterile pipette or pipette tip for each dilution.

4.2.4 Sample inoculation

Based on the estimate of sample contamination status, select 2~3 homogeneous sample solutions of appropriate dilutions (for liquid sample, the initial sample can be included), inoculate on 3 MYP agar plates with inoculation amount of 0.3 mL, 0.3 mL and 0.4 mL respectively, and then spread the whole plate with sterile L-shaped spreading rod, and avoid touching the edges of the plate. Before use, if there are drops of water on surface of MYP agar plate, dry it in an incubator at 25 ℃~50 ℃ until the drops of water disappear.

4.2.5 Isolation and incubation

4.2.5.1 Isolation

Generally let the plates stand for 10 min after spreading. If the sample solution is difficult to be absorbed, incubate the plates in an incubator at 30 ℃±1 ℃ for 1 h, invert the plates in the incubator when the homogeneous sample solution is completely absorbed, and incubate at 30 ℃±1 ℃ for 24 h±2 h. If the colonies are not typical, continue incubating for 24 h±2 h, and then observe them. On the MYP agar plate, typical colonies are slightly pink (which indicates non-fermentation of mannitol), surrounded by white to slightly pink precipitation ring (which indicates production of lecithinase).

4.2.5.2 Pure incubation

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