NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 4789.40-2024

National food safety standard - Food Microbiological Testing - Cronobacter Testing

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Foreword

This Standard serves as a replacement of GB 4789.40-2016 National Food Safety Standard -

Food Microbiological Examination - Examination of Cronobacter.

In comparison with GB 4789.40-2016, the main changes are as follows.

---The title of the Standard is modified;

---The scope of application of this Standard is expanded to supplementary foods for infants and young children;

---PCR identification method is added as optional content;

---The yellow pigment-producing and amygdalin items in picking yellow colonies and biochemical identification are deleted;

---The pH adjustment method for culture media and reagents is modified;

---The preheating, selective enrichment temperature and TSA plate incubation temperature and time are modified;

---The culture temperature of biochemical reactions and the preparation of amino acid culture medium are modified.

National Food Safety Standard - Food Microbiological

Examination - Examination of Cronobacter

1 Scope

This Standard specifies the examination method for Cronobacter spp. in food.

This Standard is applicable to the examination of cronobacter in formula foods and supplementary foods for infants and young children, milk and dairy products and their raw materials.

2 Equipment and Materials

In addition to the routine sterilization and culture equipment of the microbiology laboratory, other equipment and materials are as follows.

2.6 Sterile pipette. 1 mL (with a scale of 0.01 mL), 10 mL (with a scale of 0.1 mL) or

micropipette and tip.

2.15 PCR reaction tube.

2.16 1.5 mL centrifuge tube.

2.17 10 ?L inoculation loop.

3.9 L-arginine dihydrolase medium. see A.7.

3.13 5 U/?L thermostable DNA polymerase.

3.14 2.5 mmol/dNTPs. dATP, dTTP, dCTP, dGTP.

3.15 25 mmol/L MgCl2.

3.17 Cronobacter quality control strain. ATCC 29544 or equivalent strain provided by an

organization with strain preservation qualifications.

3.18 Escherichia coli quality control strain. ATCC 25922 or equivalent strain provided by an

organization with strain preservation qualifications.

4 Examination Procedures

The examination procedures of cronobacter are shown in Figure 1.

5.2.1 Gently mix the mLST-Vm broth culture, use 10 ?L inoculation loop to respectively take

PCR Identification (optional)

5.3 PCR Identification (optional)

The PCR test environmental conditions and process control shall be implemented with reference to the stipulations of GB/T 27403.

5.3.1 DNA template preparation

The thermal lysis method can be adopted to prepare the template. Pick 2 ~ 3 suspicious cronobacter colonies from each TSA plate into 500 ?L of sterilized deionized water.

5.3.2.1 Primer

The primer information for PCR identification is shown in Table 1.

5.3.2.2 PCR reaction system

The composition of the PCR reaction system is shown in Table 2.

5.3.3 Control settings

In each PCR identification, use the cronobacter standard strain DNA template as a positive control, and the Escherichia coli standard strain DNA template as a negative control. During the extraction process, set the sterilized deionized water as a DNA extraction blank control.

The PCR reaction requires additional sterilized deionized water as a blank control for the PCR

reaction system.

5.4 Confirmatory Test

When 5.3 is selected, pick colonies from TSA plates with positive PCR result for biochemical

identification. TSA plates with negative PCR result will no longer be subjected to the biochemical identification.

6 Result and Report

In accordance with the colony characteristics, confirmatory test (biochemical identification) and / or PCR identification results, report whether or not cronobacter was detected in the 100

g (mL) of sample.

8 Result and Report

Based on the colony characteristics, confirmation test (biochemical identification) or PCR identification results, in accordance with the number of positive tubes, in which, cronobacter was detected, check the MPN retrieval table, and report the MPN value of cronobacter in 100

g (mL) of sample (see Table C.1 in Appendix C).

Appendix A

Culture Media and Reagents

A.1 Buffered Peptone Water, BPW

A.1.1 Ingredients

Peptone 10.0 g

Sodium chloride 5.0 g

Disodium hydrogen phosphate 9.0 g

Potassium dihydrogen phosphate 1.5 g

Distilled water 1,000 mL

A.2.2 Vancomycin solution

A.2.2.1 Ingredients

Vancomycin 10.0 mg

Distilled water 10.0 mL

A.2.3 Modified lauryl sulfate tryptose broth-vancomycin medium, mLST-Vm

For each 10 mL of mLST, add 0.1 mL of vancomycin solution. The final concentration of