GB

NATIONAL STANDARD OF

THE PEOPLE'S REPUBLIC OF CHINA

GB 4789.40-2016

National Food Safety Standard –

Food Microbiological Examination –

Examination of Cronobacter (Enterobacter Sakazakii)

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Issued by: National Health and Family Planning Commission of the People 's Republic of China;

China Food and Drug Administration.

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Foreword

This Standard replaces GB 4789.40-2010 *National food safety standard Food microbiological examination: Enterobacter sakazakii*, SN/T 1632.1-2013 *Detection of enterobacter sakazakii from dehydrated powdered milk for export - Part 1: Isolation and enumeration*.

Compared with GB 4789.40-2010, the main changes in this Standard are as follows:

- modified the standard's name to "National Food Safety Standard - Food Microbiological Examination - Examination of Cronobacter (Enterobacter Sakazakii)";
- modified the number of suspicious colonies picked.

National Food Safety Standard – Food Microbiological Examination – Examination of Cronobacter (Enterobacter Sakazakii)

1 Scope

This Standard specifies the examination method for Cronobacter in food.

This Standard applies to the examination of Cronobacter in infant formula, milk and dairy products and their raw materials.

2 Equipment and materials

In addition to microbial laboratory routine sterilization and training equipment, other equipment and materials are as follows:

2.1 Thermostat incubator: 25°C ± 1°C, 36°C ± 1°C, 44°C ± 0.5°C

2.2 Refrigerator: 2°C ~ 5°C

2.3 Constant temperature water bath: 44°C ± 0.5°C

2.4 Balance: resolution of 0.1 g

2.5 Homogenizer

2.6 Oscillator

2.7 Sterile pipettes: 1 mL (with 0.01 mL scale), 10 mL (with 0.1 mL scale) or micro pipette and suction head

2.8 Sterile conical flask: capacity of 100 mL, 200 mL, 2000 mL

2.9 Sterile Petri dish: 90 m in diameter

2.10 pH meter or pH colorimetric tube or precision pH test paper

2.11 Automatic microbial biochemical identification system

3 Medium and reagent

3.1 Buffer peptone water: see A.1

3.2 Modified lauryl sulfate tryptose broth-vancomycin medium, mLST-Vm): see A.2

3.3 Enterobacter sakazakii chromogenic medium

3.4 Trypticase soy agar, TSA: see A.3

3.5 Biochemical identification kit

3.6 Oxidase reagent: see A.4

3.7 L-lysine decarboxylase medium: see A.5

3.8 L-ornithine decarboxylase medium: see A.6

3.9 L-arginine bishydrolase medium: see A.7

3.10 Carbohydrate fermentation medium: see A.8

3.11 Citrus citrate medium: see A.9

Method One Qualitative test of Cronobacter

4 Examination procedures

See Figure a for the examination procedures of Cronobacter.

7 Operation steps

7.1 Sample dilution

7.1.1 Solid and semi-solid samples: sterilely weigh 100 g, 10 g, 1g of samples, respectively add into 900 mL, 90 mL, 9 mL of BPW that have been preheated to 44°C. Gently shake to fully dissolved, make 1:10 sample homogenizing solution, culture at 36°C ± 1°C for 18h ± 2h. Respectively transfer 1 mL to inoculate in 10 mL LmST-Vm broth, culture at 44°C ± 0.5°C for 24h ± 2h.

7.1.2 Liquid sample: use a sterile pipette to take 100 mL, 10 mL, 1 mL of samples, respectively add into 900 mL, 90 mL, 9 mL of BPW that have been preheated to 44°C. Gently shake to fully dissolved, make 1:10 sample homogenizing solution, culture at 36°C ± 1°C for 18h ± 2h. Respectively transfer 1 mL to inoculate in 10 mL LmST-Vm broth, culture at 44°C ± 0.5°C for 24h ± 2h.

7.2 Separation, identification

Same with 5.2 and 5.3.

8 Result and report

Combining the colony morphology, biochemical characteristics and according to the confirmed number of positive tubes of Cronobacter, check MPN search table and report the MPN value of Cronobacter per 100 g (mL) of sample (see Table B.1).

sterilization. The vancomycin solution can be stored at 0°C ~ 5°C for 15 d.

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

Add 0.1 mL of vancomycin solution into per 10 mL of mLST. The final concentration of vancomycin in the mixture is 10 μg/mL.

NOTE: mLST-Vm must be used within 24h.

A.3 Tryptone soy agar (T S A)

A.3.1 Composition

Tryptone	15.0 g
Plant peptone	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Distilled water	1000 mL

A.3.2 Preparation method

Heating and stirring to dissolved. Boiling for 1 min. Adjust pH to 7.3 ± 0.2. Autoclave at 121°C for 15 min.

A.4 Oxidase reagent

A.4.1 Composition

N, N, N ', N' - tetramethylphenylene diamine hydrochloride	1.0 g
Distilled water	1000 mL

A.4.2 Preparation method

Make a small amount of fresh preparation. Store in the refrigerator from light. Use within 7d.

A.4.3 Test method

Use a glass rod or a disposable inoculation needle to pick up a single characteristic colony and coat it on a filter paper plate moistened with an oxidase reagent. If the filter paper does not become magenta, purple or dark blue within 10s, the oxidase test is negative, otherwise the oxidase test is positive.

NOTE: Do not use nickel/chromium material in the experiment.

A.5 L-lysine decarboxylase medium

A.5.1 Composition

A.7.2 Preparation method

Heat and dissolve the components, and if necessary, adjust pH to 6.8 ± 0.2. Sub-packaging 5 mL for each tube. Autoclave at 121°C for 15 min.

A.7.3 Experimental method

Inoculate the culture into the L-arginine decarboxylase medium, just under the liquid level of the liquid medium. Culture at 30°C ± 1°C for 24h ± 2h. Observe the results. L-arginine decarboxylase test is positive; the medium is purple; the negative is yellow.

A.8 Carbohydrate fermentation medium

A.8.1 Basal medium

A.8.1.1 Composition

Casein (enzyme digestion)	10.0 g
Sodium chloride	5.0 g
Phenol red	0.02 g
Distilled water	1000 mL

A.8.1.2 Preparation method

Heat and dissolve the components, and if necessary, adjust pH to 6.8 ± 0.2. Sub-packaging 5 mL for each tube. Autoclave at 121°C for 15 min.

A.8.2 Sugar solution (D-sorbitol, L-rhamnose, D-sucrose, D-melibiose, amygdalin)

A.8.2.1 Composition

Sugar	8.0 g
Distilled water	100 mL

A.8.2.2 Preparation method

Respectively weigh 8 g of D-sorbitol, L-rhamnose, D-sucrose, D-honey disaccharide, amygdalin and dissolve into 100 mL of distilled water. Filter for sterilization, make 80 mg/mL sugar solution.

A.8.3 Complete medium

A.8.3.1 Composition

Basal medium	875 mL
Sugar solution	125 mL

A.8.3.2 Preparation method

Aseptically add each saccharide solution to the basal medium and mix well.