



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

GB 4789.34-2016

**National food safety standard - Food Microbiological
Examination - Examination of Bifidobacterium**

食品安全国家标准 食品微生物学检验 双歧杆菌检验

(English Translation)

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Foreword

This Standard replaces GB 4789.34-2012 National Food Safety Standard - Food Microbiological Examination - Identification of Bifidobacterium.

Compared with GB 4789.34-2012, the main changes in this Standard are as follows.

- added the counting method for Bifidobacterium;
- added MRS medium;
- modified the application scope of this Standard;
- modified Annex B as optional.

National Food Safety Standard - Food Microbiological Examination - Examination of Bifidobacterium

1 Scope

This Standard specifies the identification and counting method for Bifidobacterium.

This Standard applies to the identification and counting of pure bacteria strain of Bifidobacterium. This Standard applies to the identification of bacteria when the food only contains single Bifidobacterium. This Standard applies to the counting when the food only contains Bifidobacterium, i.e., the food may

contain one or more different Bifidobacterium species.

2 Equipment and materials

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

2.5 Sterile pipettes. 1mL (with 0.01mL scale), 10mL (with 0.1mL scale) or micro-pipettes (200μL ~ 1000μL) and matching tips.

4 Inspection procedures

Bifidobacterium inspection procedures are shown in Figure 1.

5.1 Sterile requirements

All operating procedures shall follow the sterile procedures.

5.2.2.1 Sample processing. take 25.0g (mL) of sample into a sterile conical flask or homogeneous bag containing 225.0mL of physiological saline, homogenizing at 8000r/min ~ 10000r/min for 1min ~ 2min or using slapping homogenizer to beat 1min ~ 2min to make 1.10 sample homogenizing solution.

5.2.3.1 Smear microscopy. pick up Bifidobacterium single colony growing in Bifidobacterium plate or MRS plate for dyeing. Bifidobacterium is Gram-positive, short rod-shaped, slender rod-shaped or spherical, can form a variety of branches or bifurcation and other forms, non-acid, no spores, no power.

5.3.1.1 Preparation of solid and semi - solid samples. weigh aseptically 2.0g of sample; place into a sterile homogeneous cup containing 198.0mL of diluent, homogenizing at 8000r/min ~ 10000r/min for 1min ~ 2min, OR place in a sterile homogeneous bag containing 198.0mL of diluent;

5.3.4.1 It can be observed with the naked eye, if necessary, with a magnifying glass or colony counter. Record the dilution factor and the corresponding number of colonies. The colony counting is expressed in colony-forming units (CFU).

5.3.5.1 If the number of colonies on a dilution plate is within the appropriate counting range, calculate the average of the two plate colonies and multiply the average by the corresponding dilution factor as the total number of colonies per gram or milliliter.

5.3.5.2 If there are two consecutive dilutions of the number of plate colonies in the appropriate counting range, it shall calculate according to equation (1).

5.4 Results and report

According to the results of 5.2.3.1, 5.2.3.2, 5.2.3.3, the species name of Bifidobacterium shall be reported. Issue the report according to the results of

5.3.6 colony counting, in CFU/g (mL).

Annex A

Medium and reagent

A.1 Bifidobacterium agar medium

A.1.1 Ingredients

A.1.2 Preparation

A.2 PYG liquid medium

A.2.1 Composition

Peptone 10.0 g

Glucose 2.5 g

Yeast 5.0 g

Cysteine-HCl 0.25 g

Salt solution 20.0 mL

Vitamin K1 solution 0.5 mL

Hemoglobin solution 5 mg/mL 2.5 mL

Adding distilled water to 500.0 mL

A.2.2 Preparation

A.3 MRS medium

A.3.1 Composition

A.3.2 Preparation

Add all components of A.3.1 to distilled water, dissolve by heating, calibrate to pH 6.2 ± 0.1 , autoclave at 121°C for 15min ~ 20min after sub-packaging.

Annex B

Detection of organic acid metabolites of Bifidobacterium

B.1 Preparation of Bifidobacterium culture medium

Inoculate the Bifidobacterium cultured on Bifidobacterium agar plate or MRS agar plate into PYG liquid medium. Then use non-inoculated PYG liquid medium for blank control, anaerobic, culture at $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 48h.

B.2 Preparation of standard solution

B.3 Method

B.3.1 Treatment of acetic acid

Take 2.0ml ~ 3.0mL of Bifidobacterium culture medium into 10mL centrifuge tube, add 0.2mL of 50% sulfuric acid solution (volume fraction), mix, add 2.0mL of acetone, mix and add excess sodium chloride, shake for 1min, then add 2.0mL of ether, shake for 1min, centrifuge at 3000 r/min for 5min.

B.3.2 Treatment of lactic acid

Take 2.0mL ~ 3.0mL of Bifidobacterium culture medium into 10mL colorimetric tube, place in a 100°C water bath for 10min, add 0.2mL of 50% (volume fraction) sulfuric acid solution, mix, add 1.0mL of methanol, place in a 58°C water bath for 30min. Add 1.0 mL of water, add 1.0 mL of chloroform, shake 3min, centrifuge at 3000 r/min for 5min, take chloroform layer for analysis. Use same operation steps for lactic acid standard and blank culture