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NATIONAL STANDARD OF
THE PEOPLE'S REPUBLIC OF CHINA

GB 5413.19-2010

National food safety standard
Determination of free biotin in foods for infants and
young children, milk and milk products

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Table of Contents

Foreword.....	3
1 Scope	4
2 Normative references	4
3 Principle.....	4
4 Reagents and materials	4
5 Apparatus.....	5
6 Analysis procedures	6
7 Result representation	9
8 Precision.....	10
9 Other	10
Annex A	11

Foreword

This Standard replaces GB/T 5413.19-1997 "Determination of Free Biotin Content in Formulated Foods for Infants and Young Children and Milk Powder".

The Appendix A of this Standard is normative.

This Standard replaces the following previous standards:

- GB 5413-1985, GB/T 5413.19-1997.

National food safety standard

Determination of free biotin in foods for infants and young children, milk and milk products

1 Scope

This Standard specifies the determination method of free biotin in foods for infants and young children, milk and milk products.

This standard applies to determination of free biotin content in formulated food for infants and young children and milk powder.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

3 Principle

It uses the specificity and sensitivity of *Lactobacillus plantarum* to free biotin to quantitatively determine the content of the test substance in the specimen. In the culture medium which contains all nutrients, except the test substance, the microbial growth and the content of test substance is linear. According to the comparison between the transmittance and the standard curve, the content of test substance in the specimen can be calculated.

4 Reagents and materials

Unless otherwise specified, the reagents used in the method are analytically pure; the water used is Grade 3 water regulated in GB/T 6682.

4.1 Strain: (*Lactobacillus plantarum*), ATCC 8014.

4.2 d-Biotin or Vitamin H ($C_{10}H_{16}N_2O_3S$), purity $\geq 99\%$.

4.3 Culture medium.

- 5.1 Balance: resolution is 0.1 mg.
- 5.2 pH meter: accuracy ≤ 0.02 .
- 5.3 Spectrophotometer.
- 5.4 Vortex mixer.
- 5.5 Centrifuge: speed ≥ 2000 rpm.
- 5.6 Constant temperature incubator: $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$.
- 5.7 Refrigerator: $2^{\circ}\text{C} \sim 5^{\circ}\text{C}$.
- 5.8 Sterile pipette: 10 mL (with 0.1 mL calibration) or micropipettor or sucker.
- 5.9 Bottle top dispenser: 0 mL $\sim$ 10 mL.
- 5.10 Erlenmeyer flask: 200 mL.
- 5.11 Measuring flask (category A): 100 mL, 250 mL, 500 mL.
- 5.12 Single calibration pipette (category A): volume is 5 mL.
- 5.13 Funnel: diameter is 90 mm.
- 5.14 Quantitative filter papers: diameter 90 mm.
- 5.15 Test tube: 18 mm $\times$ 180 mm.

NOTE: Before using glass apparatus, use active agents (sodium lauryl or household detergents are added into washing water) to clean the hard glass measuring tube and other necessary glassware. Dry them for 2 h at 200°C after cleaning.

6 Analysis procedures

6.1 Preparation of inoculum suspension

6.1.1 After activating strain (4.1), transfer it into Lactobacillus agar culture medium (4.3.1); incubate it overnight at $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$. Transfer 2~3 generations to enhance vitality. Transfer it from solid culture to Lactobacillus broth culture medium (4.3.2) to cultivate.

6.1.2 Centrifugalize the culture solution in Lactobacillus broth at 2000 rpm for 2 min $\sim$ 3 min to decant the supernatant liquid. Add 10 mL of sodium chloride solution (4.7). Well mix it. Centrifugalize for 2 min $\sim$ 3 min. Clean so for 3 $\sim$ 4 times. Pipette right amount of this bacterial suspension in 10 mL of sodium chloride solution (4.7) for test.

6.8.2 Take inoculated blank tube S2 as blank. Adjust the absorbance as 0. Sequentially read out the absorbance A of other each test tube. Read the absorbance A of specimen blank together.

6.8.3 Use vortex mixer to completely mix each test tube (or add a drop of foam suppressor). Immediately transfer the culture solution into cuvette for determination. The wavelength is 550 nm. Till the reading is stable for 30 s, read out the absorbance A. The stability time of each test tube must be same. Take the content of biotin standard product as X-coordinate, the absorbance A as Y-axis to draw standard curve.

6.8.4 In the culture test tube of test solution, the absorbance of specimen blank tube shall be less than 0.05. If the absorbance of specimen blank tube is greater than 0.02, the absorbance value of the test tube which is added with 1 mL of test solution must minus 1/5 of the absorbance value of its specimen blank tube; the absorbance value of the test tube which is added with 2 mL of test solution must minus 2/5 of the absorbance value of its specimen blank tube, et cetera. Take it as the reference for calculation result. According to the absorbance A of test solution, check the concentration of biotin in this test solution from standard curve. Calculate the concentration of biotin in specimen according to dilution factor and sampling weight. Discard the specimen tubes of which the absorbance value exceeds S3~S10 range of standard curve tube.

6.8.5 For each number of test solution tube, use the absorbance value of each test tube to calculate the content of biotin in this number of test solution. Calculate the average value of biotin content in this number of test solution. The concentration measured of each test tube must not exceed $\pm 15\%$ of this average value. If the qualified test number is less than 2/3 of total tube number of test solution of all these four numbers, it shall require re-inspection. If the qualified tube number is greater than or equal to 2/3 of the original tube number, re-calculate the average value of biotin content in per milliliter of determination solution in each number of effective test tube. Based on this average value, calculate the total average value C_x of all numbers of test tubes for the calculation of biotin content in specimen.

7 Result representation

The biotin content in specimen shall be calculated according to equation (1):

$$X = \frac{C_x}{m} \times \frac{f}{1000} \times 100 \dots\dots\dots (1)$$

Where,

X - biotin content in specimen, in micrograms per hundred grams ($\mu\text{g}/100\text{g}$);

Annex A

(Normative)

Culture medium and reagents

A.1 Lactobacillus agar culture medium

A.1.1 Components

Potolytic peptone, 15g; yeast extract, 5g; glucose, 10g; tomato juice, 100 mL; potassium dihydrogen phosphate, 2.0 g; polyethylene sorbitan monooleate, 1.0 g. Add distilled water to 1000 mL, adjust pH to 6.8 ± 0.2 ($25^{\circ}\text{C} \pm 5^{\circ}\text{C}$).

A.1.2 Method

Add 10.0 g of agar into A.1.1. Heat it to boiling to make agar melt. Mix evenly. Fractionally pack it into tubes, 10 mL for each. Sterilize it at high temperature of 121°C for 15 min for use.

A.2 Lactobacillus broth culture medium

A.2.1 Component

Potolytic peptone, 15g; yeast extract, 5g; glucose, 10g; tomato juice, 100 mL; potassium dihydrogen phosphate, 2.0 g; polyethylene sorbitan monooleate, 1.0 g. Add distilled water to 1000 mL, adjust pH to 6.8 ± 0.2 ($25^{\circ}\text{C} \pm 5^{\circ}\text{C}$).

A.2.2 Method

Heat the components in A.2.1 to boiling. Mix evenly. Fractionally pack it into tubes, 10 mL for each. Sterilize it at high temperature of 121°C for 15 min for use.

A.3 Culture medium of broth determination

A.3.1 Components

Casamino acids for vitamin determination, 12.0 g; glucose, 40.0 g; sodium acetate, 20.0 g; L-cystine, 0.2 g; DL- tryptophan, 0.2 g; adenine sulfate, 20.0 mg; hydrochloric acid guanine, 20.0 g; uracil, 20.0 g; thiamine hydrochloride, 2.0 mg; riboflavin, 2.0 mg; niacin, 2.0 mg; calcium pantothenate, 2.0 mg; pyridoxine hydrochloride, 4.0 g; p-amino benzoic acid, 200.0 μg ; dipotassium phosphate, 1.0 g; potassium dihydrogen phosphate, 1.0 g; magnesium sulfate, 0.4 g; sodium chloride 20.0 g; ferrous sulfate, 20.0 mg; manganese sulfate, 20.0

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