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

GB 5413.16-2010

Replacing GB/T 5413.16-1997

**National food safety standard -- Determination of folic acid
(folate activity) in foods for infants and young children, milk and
milk products**

(English Translation)

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Table of Contents

Foreword	
1 Scope	
2 Normative references	
4 Reagents and materials	
4.3 Phosphate buffer	
4.9 Medium	
5 Apparatus	
5.5 Incubator. 36 °C ± 1 °C	
6 Step, Analysis	
6.1 Preparation of test broth	
6.2 Preparation of the sample	
6.3 Standard curve tube production	
6.4 Sample tube production	
6.5 Sterilization	
6.6 Inoculation	
6.7 Training	
6.8 Determination	
6.10 Calculation of folic acid in the sample tube	
7 Expression analysis	
8 Precision	
9 Other	

Foreword

This standard replaces GB/T 5413.16-1997 "infant formula milk powder and folic acid (folate activity) determination." this standard compared with GB/T 5413.16-1997, main changes are as follows.

- Phosphate buffer solution was adjusted;
- Increases the processing method of rice;
- Increasing the optical density determination procedure.

This standard replaces the standards previously issued as follows.

--GB 5413-1985, GB/T 5413.16-1997.

National Food Safety Standard Infant foods and dairy products folic acid (folate activity) Measurement

1 Scope

This standard specifies the infant food and dairy products folic acid (folate activity) measurement method.

This standard applies to dairy foods and folic acid (folate activity) measured infants.

2 Normative references

The standard file referenced in the application of this standard is essential. For dated references, only the edition date of the note Apply to this standard. For undated references, the latest edition (including any amendments) applies to this standard.

Principle 3

Use of *Lactobacillus casei* (*Lactobacillus casei*) ATCC 7469 specifically folic acid, folic acid contained in the sample grown produce the acidity and the formation of the optical density to determine the content of folic acid.

4 Reagents and materials

Unless otherwise specified, the reagents used in this method are analytically pure water GB/T 6682 provisions of secondary water.

Chicken 4.1 Pancreas. Weigh 100 mg of dried chicken pancreas, add 20 mL of distilled water, stirred for 15 min, centrifuged 10 min (3000 rev/min), the supernatant was prepared before use.

4.2 0.9% saline solution. Weigh 9.0 g of sodium chloride was dissolved in 1000 mL of water, packed in a stoppered test tube, each tube 10 mL, 121 °C Sterilized 15 min. Ready to once a week.

4.3 Phosphate buffer

4.3.1

phosphate buffer I (0.05 mol/L). Weigh 5.85 g of potassium dihydrogen phosphate, 1.22 g potassium phosphate dibasic, with 1000 mL aqueous solution. Before use by 0.5 g/100 mL ascorbic acid.

4.3.2

II phosphate buffer (for the front cereals and their products processing). Weigh 14.2 g of disodium hydrogen phosphate, dissolved with 1000 mL of water.

Prior to use by 1.0 g/100 mL was added ascorbic acid (4.16) A pH adjustment with sodium hydroxide solution to 7.8 ± 0.1 .

4.3.3

phosphate buffer III (cereals and their products for testing). Weigh 14.2 g disodium hydrogen phosphate, dissolved in 1000 mL of water.

Prior to use by 1.0 g/100 mL ascorbic acid, adjusted with sodium hydroxide solution A (4.16) pH to 6.8 ± 0.1 .

4.3.4

phosphate buffer IV (0.1 mol/L) (for the preparation of cereals and cereal products standard solution). dissolve 13.61 g of potassium dihydrogen phosphate Diluted to 1000 mL in water. (4.10) with potassium hydroxide solution adjusted to $\text{pH } 7.0 \pm 0.1$.

4.4 folic acid standard.

4.5 Ammonia (10.8%).

4.6 toluene (C_7H_8).

4.7 ascorbic acid ($\text{C}_6\text{H}_8\text{O}_6$).

4.8 strains. *Lactobacillus casei* (*Lactobacillus casei*) ATCC 7469.

4.9 Medium

4.9.1

Lactobacillus agar. peptone of milk 15 g, yeast extract 5 g, glucose 10 g, tomato juice, 100 mL, potassium dihydrogen phosphate 2 g, polyethylene sorbitan monooleate 1 g, agar 10 g, add distilled water to 1000 mL, adjust pH to 6.8 ± 0.2 ($20^\circ\text{C} \sim 25^\circ\text{C}$). 121°C Autoclave 15min, standby.

4.9.2

Lactobacillus broth medium. peptone of milk 15 g, yeast extract 5 g, glucose 10 g, tomato juice, 100 mL, potassium dihydrogen phosphate 2 g, polyethylene sorbitan monooleate 1 g, add distilled water to 1000 mL, adjust pH to 6.8 ± 0.2 ($20^\circ\text{C} \sim 25^\circ\text{C}$). 121°C autoclaving Bacteria 15min, standby.

4.9.3

Folate measurement medium. Casein peptone 10 g, glucose 40 g, sodium acetate 40 g, dipotassium phosphate 1 g, potassium dihydrogen phosphate 1 g, DL- tryptophan 0.2 g, L-

aspartic acid 0.6 g, L- cysteine hydrochloride 0.5 g, adenine sulfate, 10 mg, 10 guanine hydrochloride mg, uracil, 10 mg, xanthine 20 mg, polysorbate 0.1 g, glutathione 5 mg, magnesium sulfate 0.4 g, sodium chloride 20 mg, sulfur Ferrous sulfate 20 mg, manganese sulfate 15 mg, riboflavin 1 mg, p- amino benzoic acid 2 mg, vitamin B6 4mg, thiamine hydrochloride 400 µg, Calcium pantothenate 800 µg, niacin 800 µg, biotin 20 µg, add distilled water to 1000 mL, adjust pH to 6.7 ± 0.1 ($20^{\circ}\text{C} \sim 25^{\circ}\text{C}$).

Note: Commercially available commercial synthetic medium effect is more stable.

4.10 potassium hydroxide solution (4 mol/L). Weigh 224 g of potassium hydroxide in 1000 mL beaker with 400 mL of water to dissolve, cooled to Room temperature, transferred to a 1000 mL volumetric flask, and water volume.

4.11 papain solution. 1 g of protease (dynamic ≥ 6000 U/mg, pH 6.0 ± 0.1 , 40°C) was dissolved in 100 mL of phosphate buffer Liquid I (4.3.1) in. Pro preparation before use.

4.12 alpha- amylase solution. 1 g alpha- amylase (1.5 U/mg) was dissolved in 100 mL phosphate buffer I (4.3.1) in. Pro preparation before use.

4.13 0.22 µm filter sterilization.

Preparation of standard solution of 4.14 4.14.1 folic acid standard stock solution (500 µg/mL). Weigh 55 mg ~ 56 mg (accurate to 0.1 mg) of folic acid standard (4.4), with 50 mL of distilled water into 100 mL volumetric flask, add 2 mL of ammonia (4.5). After preparation of the solution, according to formula (1) calculate the volume of the solution, it requires mid salt stock solution at a concentration of 500 µg/mL.

Stock solution volume (mL) = xx cm or simplified to. stock solution volume (mL) = cm x (1) where.

M-- quality standards of folic acid, in milligrams (mg);

C-- purity standards of folic acid in grams per hundred grams (g/100 g).

The solution was diluted with water to the scale, size and thoroughly mixed with water to a straw computational requirements, into a brown reagent bottle $2^{\circ}\text{C} \sim 4^{\circ}\text{C}$ ice Box refrigerated storage period of 4 months.

4.14.2 folic acid intermediate standard solution (50 µg/mL). 10 mL absorb folic acid stock standard solution (4.14.1) in 100 mL brown volumetric flask with water to volume, mix thoroughly, $2^{\circ}\text{C} \sim 4^{\circ}\text{C}$ refrigerator, shelf life of one month.

4.14.3 folic acid working standard solution (0.05 ng/mL, 0.1 ng/mL). draw 1 mL folate intermediate standard solution (4.14.2) in 100 mL Brown volumetric flask, add water to volume and mix. Then suck the liquid 1 mL to 100 mL brown volumetric flask, volume, and mix. From Liquid 5 mL were drawn in 250 mL and 500 mL brown volumetric flask, with phosphate buffer I (4.3.1) to volume, mix, it is a high concentration of working standard solution (0.1 ng/mL) and low concentrations of working standard solution (0.05 ng/mL). Pro