



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

GB 5413.14-2010

**National food safety standard -- Determination of vitamin B12 in
foods for infants and young children, milk and milk products
(English Translation)**

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Foreword

This Standard replaces GB/T 5413.14-1997 Milk Powder and Formula Foods for Infant and Young Children - Determination of Vitamin B12 Content.

Compared with GB/T 5413.14-1997, this Standard mainly has the following changes.

--- Change the standard name into Determination of Vitamin B12 in Foods for Infants and Young Children, Milk and Milk Products.

This Standard's Appendix A is normative.

The historical editions replaced by this Standard are as follows.

--- GB 5413-1985, GB/T 5413.14-1997.

National Food Safety Standard Determination of Vitamin B12 in Foods for Infants and Young Children, Milk and Milk Products

1 Scope

This Standard specifies the method for determination of vitamin B12 in foods for infants and young children, milk and milk products.

This Standard is applicable to determination of vitamin B12 in foods for infants and young children, milk and milk products.

2 Normative References

The following documents are essential to the application of this document. For the dated documents, only the versions with the dates indicated are applicable to this document; for the undated documents, only the latest version (including all the amendments) are applicable to this Standard.

3 Principle

Utilize *Lactobacillus leichmannii*'s specificity and sensitivity to vitamin B12;

quantitatively determine content of vitamin B12 in sample. In the medium for determination, SUPPLY all nutrients except vitamin B12, so the transmittance generated by microbial growth shall be corresponding to the content of Vitamin B12 in standard curve work solution and unknown to-be-measured solution.

4 Reagents and Materials

All reagents, if not specified otherwise, refer to analytic reagent; all experiment water, if not specified otherwise, refers to grade 2 water in GB/T 6682.

4.1

Strain. *Lactobacillus leichmannii* ATCC 7830.

4.2 Vitamin B12 (Vitamin B12 or Cyanocobalamin) standard products. its molecular

formula is $C_{63}H_{88}CoN_{14}O_{14}P$; purity $\geq 99\%$.

4.3 Culture medium

4.3.1

Lactobacillus agar culture medium. see Appendix A.

4.5 Ethanol solution. volume fraction is 25%.

4.6 Anhydrous sodium hydrogen phosphate (Na_2HPO_4).

4.7 Anhydrous sodium metabisulfite ($Na_2S_2O_5$).

4.8 Citric Acid (with a hydrate) ($C_6H_8O_7 \cdot H_2O$).

4.9.1 Vitamin B12 stock solution (10 μ g/mL). accurately weigh vitamin B12 standard

product (4.2); dilute with ethanol solution (4.5) to fix the volume to that vitamin B12

concentration is 10 μ g/mL.

4.9.2 Vitamin B12 intermediate solution (100ng/mL). dilute 5 mL of stock solution (4.9.1) with ethanol solution (4.5) to 500mL.

4.9.4 Standard curve work solution. absorb twice the 5mL of Vitamin B12 work solution (4.9.3) respectively; place them into 250mL and 500mL flasks; use water to fix volume to the scale. The concentration of high-concentration solution is 0.02ng/mL; while the concentration of low-concentration solution is 0.01ng/mL.

4.9.3 can be stored for three months. Solutions in 4.9.4 shall be prepared just before use.

5 Apparatuses and Instruments

In addition to the microbiological laboratory conventional sterilization and cultivation devices, other devices and materials are as follows.

5.1

Balance. sensitivity is 0.1mg.

5.2

pH meter. accuracy ≤ 0.01 .

5.6

Thermostatic incubator. 36°C $\pm$ 1°C.

5.7

Refrigerator. 2°C ~ 5°C.

5.8

Sterile pipette. 10 mL (with 0.1 mL scale) or micro-pipette and sucker.

5.9

Dispenser. 0 mL~10 mL.

5.14

Quantitative filter paper. diameter is 90 mm.

5.15

Tube. 18 mmx180 mm.

6.2 Treatment of specimen

6.2.1

Weigh 1.3g of anhydrous sodium hydrogen phosphate (4.6), 1.0g of anhydrous sodium metabisulfite (4.7), 1.2 g of citric acid (with a hydrate) (4.8); use 100 mL of water to dissolve.

6.2.2 Weigh certain amount of sample (accurate to 0.1mg), it contains about 50 ~

100ng of vitamin B12; use 10mL of above solution (6.2.1) to mix; then add 150mL of water. Hydrolyze for 10min at 121°C; then cool down and adjust pH to 4.5 ± 0.2 .

6.3 Preparation of standard curve

According to the sequence in Table 1, ADD water, standard curve work solution (4.9.4) and vitamin B12 determination used culture medium (4.3.3) INTO culture tubes, make triplicate.

6.4 Preparation of solution under test

Tube No.

6.5 Sterilization

Cap all tubes (6.3 and 6.4); sterilize for 5min at 121°C (products' culture medium shall be sterilized according to the labelled instructions)

6.6 Inoculation

Cool down above-mentioned tubes rapidly to below 30°C. Use dropper or pipette to add a drop (about 50μL) of bacterial solution (6.1.2) into each of above tubes (in which, except the blank S1 in the standard curve tubes).

6.7 Incubating

Put the tube into the thermostatic incubator, then incubate for 19 ~ 20h at $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

6.8 Determination

After the completion of incubating, visually inspect each tube; in not-inoculated tube, the incubating liquid should be clear. If there occurs turbid, then the determination is invalid.

6.8.1

Take inoculated blank tube as a reference, determine the transmittance of maximum-concentration standard curve tube. Re-determine this tube again after 2h. If the transmittance's difference between these two results is $\leq 2\%$, then take out all the tubes