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Voluntary - Public

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**GAIN Report Number:** CH9111

## China - Peoples Republic of

**Post:** Beijing

### National Food Safety Standard - Vitamin B12

**Report Categories:**

FAIRS Subject Report

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**Report Highlights:**

On November 20, 2009, China notified the WTO of "National Food Safety Standard of the People's Republic of China for Determination of Vitamin B12 in Foods for Infants and Young Children, Raw Milk and Dairy Products" as SPS/N/CHN/158. The date for submission of final comments to the WTO is January 1, 2010. The proposed date of entry into force has not been specified.

**Executive Summary:**

On November 20, 2009, China notified the WTO of "National Food Safety Standard of the People's Republic of China for Determination of Vitamin B12 in Foods for Infants and Young Children, Raw Milk and Dairy Products" as SPS/N/CHN/158. The date for submission of final comments to the WTO is January 1, 2010. The proposed date of entry into force has not been specified

Thanks go to the consortium of industry and 3<sup>rd</sup> country Embassies in Beijing for their assistance in

translating and reviewing this standard.

This report contains an UNOFFICIAL translation of National Standard on Determination of Vitamin B12 in Foods for Infants and Young Children, Raw Milk and Dairy Products.

**General Information:**

BEGIN TRANSLATION

GB National Food Safety Standard

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**Determination of Vitamin B12 in Foods for Infants and Young Children, Raw Milk and Dairy Products**

**Draft for Comment**

Issued on xx-xx-xxxx

Implemented on xx-xx-xxxx

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Issued by the Ministry of Health

of the People's Republic of China

**1. Scope:**

This standard is formulated a microbial method for determination of vitamin B12.

This standard is applicable to Determination of Vitamin B12 in Foods for Infants and Young Children, Raw Milk and Dairy Products.

**2. Principle:**

*Lactobacillus leichmannii* is very sensitive to the existence of vitamin B12. So it can be used for determination of vitamin B12 in sample.

**3. Reagent, strain and culture medium**

All reagents, if no special specification, refer to analytic reagent; All experiment water, if no special

order, refers to level 3 water.

- 3.1. NaCl solution: 9g/L.
- 3.2. ethanol: volume fraction is 25%
- 3.3. disodium hydrogen phosphate, anhydrous
- 3.4. Sodium Metabisulfite, anhydrous
- 3.5. Citric Acid(with a hydrate)
- 3.6. Strain: *Lactobacillus leichmannii*
- 3.7. Culture medium
  - 3.7.1 *Lactobacillus* agar culture medium: Photolysis peptone 15g, yeast extract 5g, glucose 10g, tomato juice 100ml, monopotassium phosphate 2g, Poly-sorbose Monooleate 1g, agar 10g, add distilled water to total 1000ml, adjust PH to  $6.8 \pm 0.2 (25^{\circ}\text{C})$ .
  - 3.7.2 Vitamin B12 determine medium: Acid hydrolysis of vitamin-free casein 15g, glucose 40g, asparagineAsn 0.2g, sodium citrate 20g, ascorbic acid 4g, L-cystine 0.4g, DL-tryptophan 0.4g, adenine sulphate 20mg, hydrochloric acid guanine 20mg, uracil 20mg, xanthine 20mg, riboflavin 1mg, thiamine hydrochloride 1mg, biotin 10 $\mu\text{g}$ , nicotinic acid 2mg, p-Aminobenzoate 2mg, calcium pantothenate 1mg, pyridoxine hydrochloride alcohol 4mg, Pyridoxal hydrochloride 800 $\mu\text{g}$ , folic acid 200 $\mu\text{l}$ ,  $\text{KH}_2\text{PO}_4$  1g,  $\text{K}_2\text{HPO}_4$  1g,  $\text{FeSO}_4$  0.4g,  $\text{MnSO}_4$  20mg, sodium chloride 20mg, ferrous sulfate 20mg, Poly-sorbose Monooleate 2g, add distilled water to total 1000ml, adjust PH to  $6.8 \pm 0.2 (25^{\circ}\text{C})$ .
- 3.8 Vitamin B12: Reference Standard

#### **4. Apparatus**

Common laboratory equipment and spectrophotometer

#### **5. Preparation**

##### 5.1 Preparation of strain

5.1.1 Transfer of cells from cylindrical *Lactobacillus* agar culture medium of *Lactobacillus leichmannii* to new culture medium. Incubate it and transfer once again. Then transfer cells from agar to *Lactobacillus* broth culture medium and incubation.

5.1.2 Centrifuge culture 2-3minutes at 2000r/min then decant supernate. Re-suspend cells by 10ml NaCl solution and centrifuge it again. Repeat above steps 3-4 times, then transfer 0.4ml suspension into 10ml NaCl solution, for determination.

5.1.3 The transmission of the suspension (5.1.2) at 550nm tested by spectrophotometer with water as blank reference should between 60%-80%.

##### 5.2 Preparation of standard

5.2.1 Vitamin B12 stock solution:100ng/ml.

Accurately weigh vitamin B12 standard and dilute with ethanol(3.2) to make concentration exactly 100ng/ml.

5.2.2 Vitamin B12 stock solution:1ng/ml.

Dilute the stock solution (5.2.1) with ethanol(3.2) to total 1000ml.

5.2.3 Work standard solution: two different concentrations of vitamin B12.

High concentration: 0.02ng/ml.

Dilute 5ml stock solution (5.2.2) with distilled water to total 250ml.

Low concentration: 0.01ng/ml.

Dilute 5ml stock solution (5.2.2) with distilled water to total 500ml.

## 6. Determination

6.1 Preparation of sample:

6.1.1 Dissolve disodium hydrogen phosphate, anhydrous (3.3) 1.3g, Sodium Metabisulfite, anhydrous (3.4) 1.0g, Citric Acid (with a hydrate) (3.5) 1.2g with 100ml distilled water.

6.1.2 Weight a amount of samples(equivalent contain 50-100ng vitamin B12), mixed with above solution (6.1.1), then add 150ml distilled water. Hydrolyze for 10min at 121°C, then cool-off and adjust to PH4.5. Dilute with distilled water to make mass concentration of vitamin B12 between 0.01-0.02ng/ml and Sodium Metabisulfite less than 0.03mg./ml.

6.2 preparation of solution of standard curve

Add distilled water, standard solution and vitamin B12 determine medium into according to the table1, and make Triplicate.

Table1:

| Tube No:   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|--|---|---|---|---|---|---|---|---|---|----|
| Distilled water:(ml)   | 5 | 5 | 4 | 3 | 2 | 1 | 0 | 2 | 1 | 0  |
| Standard solution#:(ml)  | 0 | 0 | 1 | 2 | 3 | 4 | 5 | 3 | 4 | 5  |
| Medium:(ml)  | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5  |
| #:add low concentration standard solution in No.3-7; add high concentration standard solution in No.8-10 |   |   |   |   |   |   |   |   |   |    |

6.3 Assay solution:

Add distilled water, sample solution and vitamin B12 determine medium into according to the table2, and make Triplicate.

Table2:

| Tube No:              | 1 | 2 | 3 | 4 |
|-----------------------|---|---|---|---|
| Distilled water :(ml) | 4 | 3 | 2 | 1 |
| Sample solution: (ml) | 1 | 2 | 3 | 4 |

|             |   |   |   |   |
|-------------|---|---|---|---|
| Medium:(ml) | 5 | 5 | 5 | 5 |
|-------------|---|---|---|---|

#### 6.4 Inoculation

Sterilize all tubes(6.2 and 6.3) for 5mins at 121°C, then cool-off this tubes rapidly. Drip a drop of suspension(6.1.2) into each above tube by capillary pipette(except standard No1). Mixing and incubate for 19-20h at 37°C±0.5°C.

#### 6.5 assay

Assay the T of the maximum concentration tube with using a blank inoculation as reference. And assay this tube again after 2h. If the difference between this two result is  $\leq 2\%$ , that mean you can take out all the tubes and assay their T and take down record.

### 7. Calculations

With the amount of vitamin B12 in standard solution as X-axis, the data of T as Y-axis, do working curve. Check the average value of vitamin B12 from the working curve according to the T of sample, the calculate the actual content of vitamin B12 in per 100g/ml sample according to this formula:

Content of vitamin B12 in sample  $[\mu\text{g}/100\text{g}(\text{ml})] = x/m \times F/1000 \times 100$

X: the average value of vitamin B12 checked from the working curve, ng;

F: dilution factor

m: mass or volume of the sample.

### 8. Allowable error

The difference between the values of the twice tests to the same sample should  $\leq 10\%$ .