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National Dairy Standard - Antibiotics

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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 the Microbiological Examination of Foods: Examination of Residues of Antibiotics in Milk" as SPS/N/CHN/195. 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 the Microbiological Examination of Foods: Examination of Residues of Antibiotics in Milk" as SPS/N/CHN/195. 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 3rd country Embassies in Beijing for their assistance in

translating and reviewing this standard.

This report contains an UNOFFICIAL translation of National Food Safety Standard of the People's Republic of China for the Microbiological Examination of Foods: Examination of Residues of Antibiotics in Milk.

BEGIN TRANSLATION

General Information:

BEGIN TRANSLATION

ICS 07.100.30
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The National Standard of People's Republic of China

GB4789.27—××××

Replace GB4789.27-2008

Microbiological examination in foods—

Examination of residue of antibiotics in raw milk

(Draft for approval)

Issued on xx-xx-xxxx Implemented on xx-xx-xxxx

Issued by the Ministry of Health

of the People's Republic of China

Preface

This standard replaces GB/T 4789.27-2008 (Microbiological examination in foods-Examination of

residue of antibiotics in raw milk)

In comparison with GB/T 4789.27-2008, the major changes of this standard are as follows:

—The name of this standard has been changed to "Microbiological examination in foods— Examination of residue of antibiotics in raw milk"

—Appendix A in this standard is a normative appendix

This standard is proposed by and interpreted by Ministry of Health of P.R. China.

This standard replaces all previous standards, those issued editions are:

— GB 4789.27-1984, GB4789.27-1994, GB/T4789.27-2003, GB4789.27-2008

Microbiological examination in foods— Examination of residue of antibiotics in raw milk

1. Scope

This standard prescribes the test method of residue of antibiotics in raw milk.

The first method in this standard applies to the examination of the antibiotics which can inhibit *Streptococcus thermophilus* in raw milk; The second method applies to the antibiotics which can inhibit *Bacillus stearothermophilus var. calidolactis* in raw milk, this method also can applies to examine the antibiotics in reconstituted milk, pasteurized sterilized milk, or milk powder.

Method 1: Inhibitory method by Streptococcus thermophilus

2. Principle

Streptococcus thermophilus should be added in the test sample after the sample is sterilized at 80°C. Streptococcus thermophilus grows during the incubation. Add the metabolizing substrate 2,3,5-triphenyltetrazolium chloride (TTC), the Streptococcus thermophilus will still be growing if no antibiotics or the antibiotics are below the detection limit. TTC will be deoxidized to a red substance. On the contrary, if the antibiotics are beyond the detection limit, the bacteria will be inhibited and no deionization reaction will occur (color will not change).

3. Apparatus and materials

Except the general facilities of sterilization and incubation in microbiological laboratory, other requirements of instruments and materials are as below:

3.1. Refrigerators : controlled at 2°C – 5°C, -20°C – -5°C
3.2. Incubators: controlled at 36°C ± 1°C
3.3. Water bathes with lids: controlled at 36°C ± 1°C, 80°C ± 2°C
3.4. Balance, weighing to 0.1g, 0.001g
3.5. 1 mL (with the scale of 0.01mL), 10.0mL (with the scale of 0.1mL) Sterilized pipettes, or microsyringe and the tips
3.6. Sterilized tubes: 18 mm x 180 mm
3.7. Thermometer 0°C – 100°C
3.8. Vortex mixer

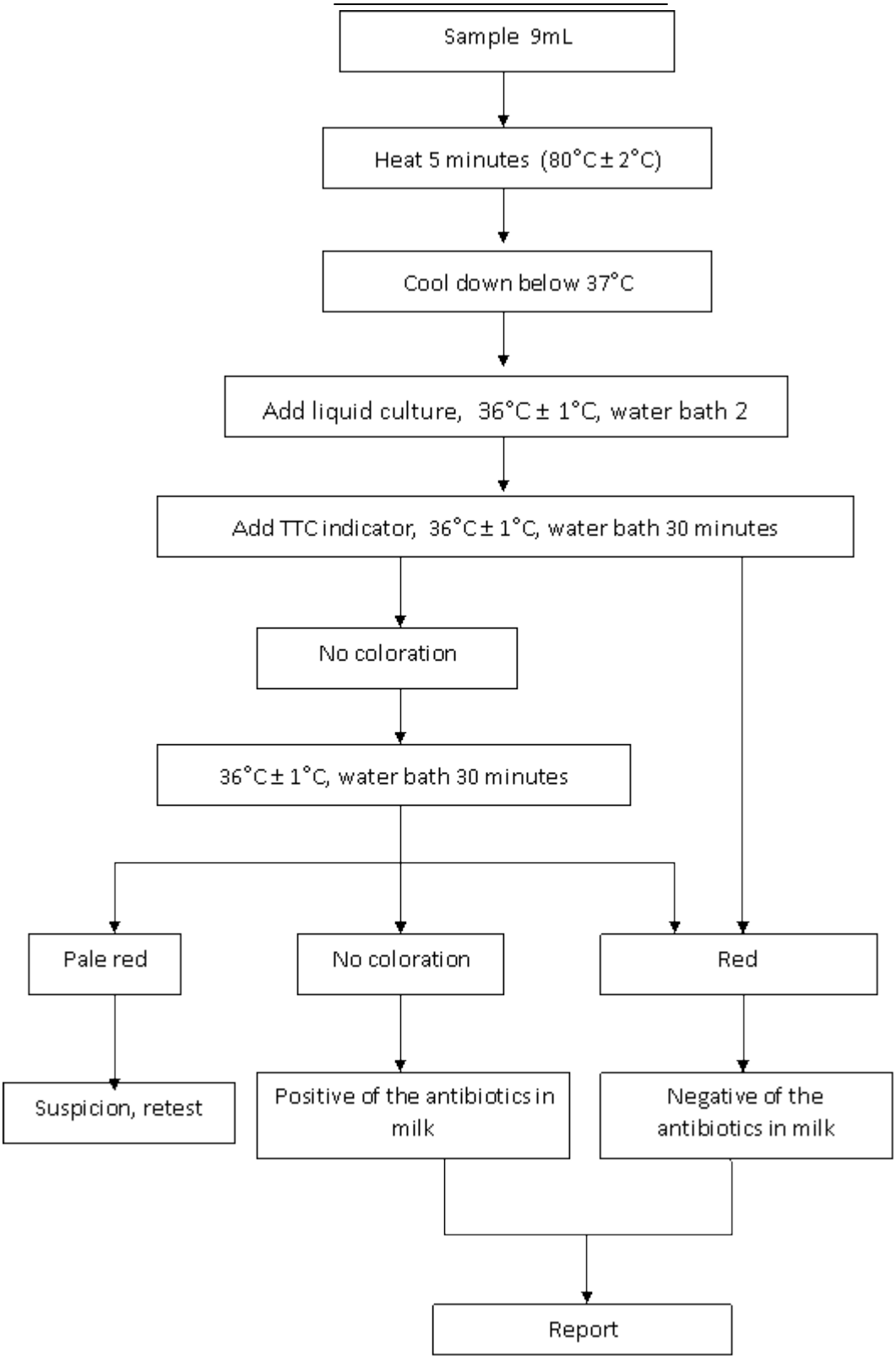
4. Culture, medium and reagents

4.1. Culture: Streptococcus thermophilus
4.2. Sterilized skimmed milk: see chapter A.1
4.3. 2,3,5-triphenyltetrazolium chloride (TTC) solution: see chapter A.2
4.4. Penicillin G reference solution: see chapter A.3

5. Test flow-diagram

Test procedure to detect the residues of antibiotics in raw milk refers to Figure 1.

Figure 1 Detection procedure of antibiotics in raw milk



6. Test procedure

6.1. Activation of culture

Transfer an inoculating loop of culture (Streptococcus thermophilus) into 9 mL of sterilized skimmed milk, incubate at 36°C ± 1°C for 12-15 hours, storage in a Refrigerator 2°C - 5°C. Transfer the culture from the previous liquid culture by every 15 days.

6.2. Working liquid culture

Add activated liquid culture into sterilized skimmed milk, incubate at 36°C ± 1°C for 15hours ± 1hour. Dilute the liquid culture with the same volume of skimmed milk to a working liquid culture.

6.3. Incubation

Add 9 mL sample into a test tube (18 mm x 180 mm), duplicate sample, negative control and positive control are required. For positive control, use 9 mL Penicillin G reference solution; for negative control, use 9 mL sterilized skimmed milk. All tubes are heated in water bath at 80°C ± 2°C for 5 minutes, cool down below 37°C. Add working liquid culture 1 mL, centrifuge the tube carefully to mix the culture, incubate in water bath at 36°C ± 1°C for 2 hours. Add 0.3mL 4% TTC solution and mix on the vortex mixer for 15 seconds or shake the tube. Incubate in a water bath at 36°C ± 1°C for 30 minutes (lightproof is necessary). Check the coloration reaction. If the color is no change, incubate again for 30 minutes and determine. To prevent the interference of the light, the determination of the color should be quick.

6.4. Determination

Observe the color with a white background. If milk in the tube does not change the color, it indicates that antibiotics are present in the milk, result is positive. If the milk in the tube changes to red; result is negative. If the color is suspicious, retest and determine.

7. Report

If the color changes to red in final observation, report as antibiotics negative in milk, if the color of milk remains the original white, report as antibiotics positive in milk.

The detection limits of this method for normal antibiotics are as below:

Penicillin 0.004 IU, Streptomycin 0. 5 IU, Gentamicin 0.4 IU, Kanamycin 5 IU.

Method 2: Inhibitory method by Bacillus stearothermophilus

8. Principle

The medium is prepared by addition of Bacillus stearothermophilus spores and pH indicator (Bromocresol purple). Sample is added in the medium and incubated, if antibiotics are not present in the milk or the level is below the detection limit, the bacteria spores will grow in the medium and utilize the sugar in milk to cause acidification. The pH indicator will change from purple to yellow. On the contrary, if the level of antibiotics is higher than detection limit, the spores will not grow, no change of the pH indicator, color will remain purple.

9. Apparatus and materials

Except the general facilities of sterilization and incubation in microbiological laboratory, other requirement of instruments and materials are as below:

9.1. Refrigerators : controlled at 2°C – 5°C, -20°C – -5°C

9.2. Incubators: controlled at 36°C ± 1°C, 56°C ± 1°C

9.3. Water bathes: controlled at 65°C ± 2°C, 80°C ± 2°C

9.4. Sterilized pipettes or 100 μL, 200 μL microsyringes and tips

9.5. Sterilized tubes: 18 mm x 180 mm, 15 mm x 100 mm
9.6. Thermometer 0°C – 100°C
9.7. Centrifuge: rpm.5000r/min.

10. Culture, medium and reagents

10.1. Culture: *Bacillus stearothermophilus var. calidolactis*
10.2. Sterilized phosphate buffer: see chapter A.4
10.3. Sterilized skimmed milk: see chapter A.1
10.4. Bromocresol Purple Dextrose Peptone Medium: see chapter A.5
10.5. Penicillin G reference solution: see chapter A.3

11. Test flow-diagram

The test procedure to detect the residues of antibiotics in raw milk refers to Figure 2

12. Test procedure

12.1. Spore suspension

Subculture by streaking a loop of Bacillus stearothermophilus var. calidolactis on to the agar, and incubate at 56°C ± 1°C for 24 hours. Select round, ivory white, translucent specific bacteria colonies with a loop on to the agar again, incubate for 24 hours at 56°C ± 1°C, and then transfer to another incubator for 3 days- 4 days incubation at 36°C ± 1°C. Use microscope to confirm if spores grow from more than 95% of the bacteria, then prepare the spore suspension. Use 1 mL - 3 mL Sterilized phosphate buffer to wash the colonies on the surface of each plate (If use Kolle flask, 10 mL -20 mL phosphate buffer for each flask). Centrifuge the washed suspension for 15 minutes at the speed of 5000 r/min. Remain the precipitated spores and add 0.03 mol/L sterilized phosphate buffer (pH 7.2) in it. Suspension is prepared with 10^9 cfu/mL spore count. Put the spore suspension in the water bath for 10 minutes (80°C ± 2°C), seal the suspension to prevent moisture evaporation. Store at 2°C – 5°C for use.

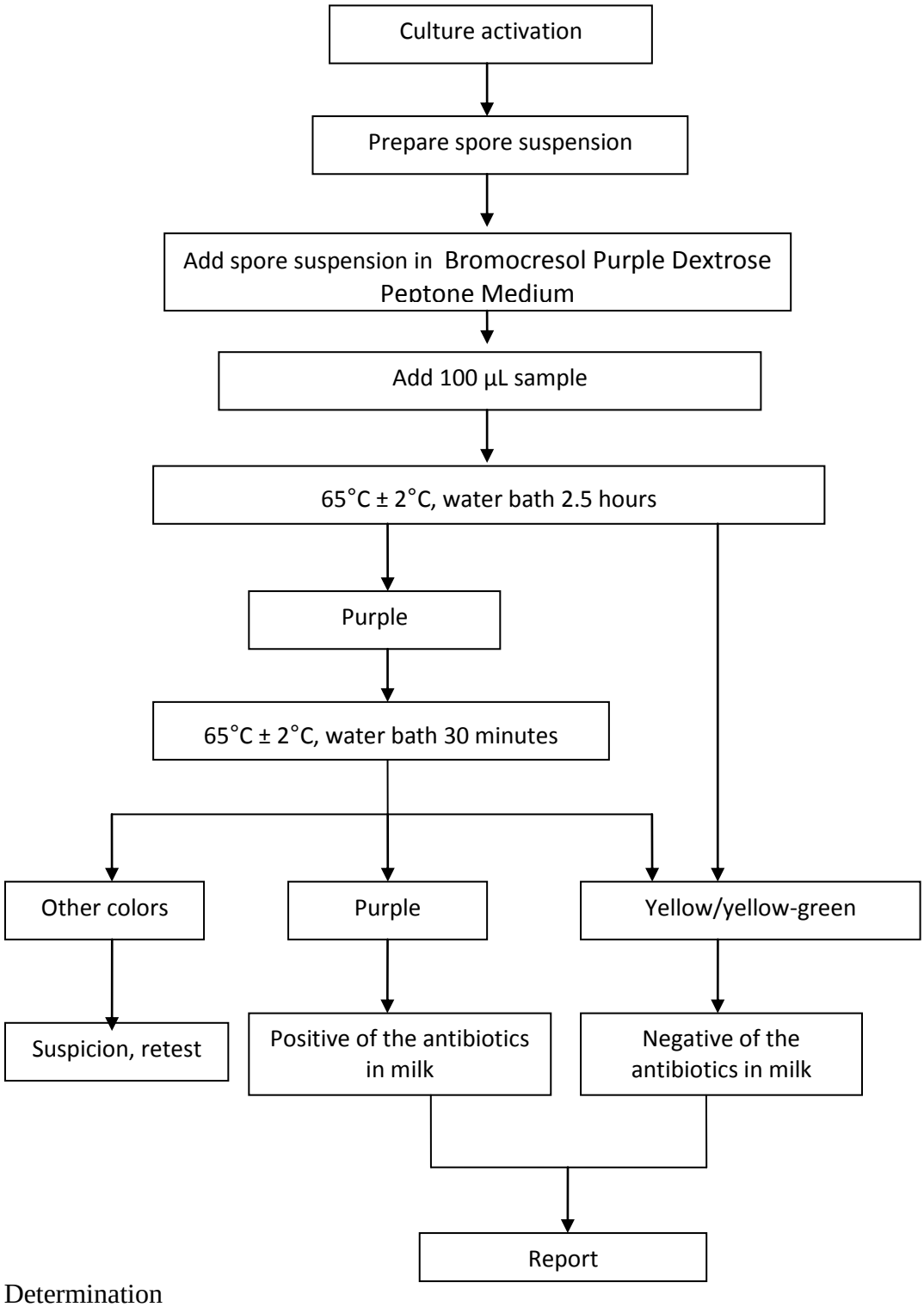
12.2. Test medium:

Add appropriate spore suspension in Bromocresol Purple dextrose peptone medium, mix well to make sure the final concentration of spore in the medium is 8 x10^5 cfu/mL -2 X 10^6 cfu/g. Separate the mixture (spore suspension + bromocresol purple dextrose peptone medium) into small tubes, 200 µL for each tube. Seal the tubes to prevent moisture evaporation. The test medium can be stored at 2°C – 5°C for 6 months.

12.3. Procedure of incubation

Add 100µL sample in the test medium, spin carefully and mix. Duplicate sample, 1 positive control, 1 negative control are required. Positive control is 100µL Penicillin G reference solution, Negative control is 100µL antibiotic free skimmed milk. Incubate the mixture in a water bath for 2.5 hours at 65°C ± 2°C, check if the color of medium changes or not. If no change, incubate again for 30 minutes then determine as a final result.

Figure 2 Detection procedures of antibiotics in raw milk



12.4. Determination

Observe the color of medium in the tube from lateral and bottom. If the color keeps original purple, it is positive; if the color of the medium turns to yellow or yellow-green, it is negative; if the color is between purple and yellow/yellow-green, it is suspicious and need to be incubated again for 30 minutes, then determine as the final result. If the color is still between yellow-purple, it means the antibiotics is close to the detection limit. In this case, it is suggested to run

the test again.

13. Report

Report as antibiotics positive if the color of medium remains purple during final observation.
Report as antibiotics negative if the color of medium turns to yellow or yellow-green.
The detection limits of this method for normal antibiotics are as below:
Penicillin 3μg/L, Streptomycin 50μg/L, Gentamicin 30μg/L, Kanamycin 50μg/L.

Appendix A
(Normative Appendix)
Medium and Reagents

A.1 Sterilized Skimmed Milk

A.1.1 Composition

Antibiotic free skimmed milk

A.1.2 Preparation

Skimmed milk is sterilized at 115°C for 20 minutes or dissolve antibiotic free skimmed milk powder with distilled water (1:10), heat until it is dissolved completely, sterilize at 115°C for 20 minutes

A.2 2,3,5-triphenyltetrazolium chloride (TTC) solution

A.2.1 Composition

2,3,5-triphenyltetrazolium chloride (TTC)	1 g
Sterilized distilled water	5 mL

A.2.2 Preparation

Weight TTC and dissolve in sterilized distilled water, store in a brown bottle at 2°C – 5°C. If the solution turns to semitransparent white or pale brown, it cannot be used anymore. Dilute the solution with 5 multiple water to prepare a 4% solution before use.

A.3 Penicillin G reference solution

A.3.1 Composition

Potassium Penicillin G	30.0 mg
Sterilized phosphate buffer	In a proper volume
Antibiotic free skimmed milk	In a proper volume

A.3.2 Preparation

Weigh Potassium Penicillin G standard sample accurately, dissolve it in sterilized phosphate buffer, the concentration of the Penicillin G reference solution is 100 IU/mL -1000 IU/mL. Dissolve this solution with sterilized antibiotic free skimmed milk to 0.006 IU/mL. Separate the reference solution into small tubes, seal for further use. Store at
-20°C not more than 6 months.

A.4 Sterilized phosphate buffer

A.4.1 Composition

Sodium Dihydrophosphate	2.83 g

Potassium Dihydrophosphate	1.36 g
Distilled water	1000 mL

A.4.2 Preparation

Mix above materials, adjust pH to 7.3 ± 0.1, High pressure sterilization (20minutes, 121°C).

A.5 Bromocresol Purple Dextrose Peptone Medium

A.5.1 Composition

Peptone	10.0 g
Dextrose	5.0 g
2% Bromocresol Purple Ethanol solution	0.6mL
Agar	4.0 g
Distilled water	1000 mL

A.5.2 Preparation

Add peptone, dextrose, agar in distilled water, heat and stir until dissolve entirely. Adjust pH to 7.3 ± 0.1, then add bromocresol purple ethanol solution and mix. High pressure sterilization at 115°C for 30 minutes.