



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

GB 4789.11-2014

**National Food Safety Standard -- Microbiological Examination
of Food Hygiene -- Examination of Streptococcus Hemolyticus
(English Translation)**

Issued on: December 1, 2014

Implemented on: May 1, 2015

**Issued by: National Health and Family Planning Commission of the
People's Republic of China**

Table of Contents

Foreword...	3
1 Scope...	4
2 Terms and Definitions...	4
3 Equipment and Materials...	4
4 Culture Media and Reagents...	5
5 Inspection Procedure...	5
6 Operation Procedure...	6
7 Results and Reports...	7
Appendix A Culture Media and Reagents...	8

Foreword

This Standard replaces "Microbiological Examination of Food Hygiene - Examination of Hemolytic Streptococcus" (GB/T 4789.11-2003).

Compared with GB/T 4789.11-2003, this Standard has the following main changes.

- The standard name was modified;
- The "Scope" was modified;
- The "Terms and Definitions" was added;
- The "Equipment and Materials" was modified;
- The "Culture Media and Reagents" was modified;
- The procedure of sample dilution with aseptic normal saline and the bacitracin sensitivity test were deleted;
- The catalase test was added;
- Appendix A was added.

National Food Safety Standard -

Food Microbiological Examination -

Examination of beta-type Hemolytic Streptococcus

1 Scope

This Standard specifies the inspection method for beta-type hemolytic streptococcus in foods.

This Standard is applicable to inspection of beta-type hemolytic streptococcus in foods.

2.1 beta-type hemolysis

Totally transparent hemolytic rings are formed around the bacterial colony, and the erythrocytes are fully dissolved.

2.2 beta-type hemolytic streptococcus

It can produce beta-type hemolytic streptococcus pyogenes (or A-group) and streptococcus agalactiae (or B-group).

3 Equipment and Materials

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

4.4 Gram stain solution. see A.4 of Appendix A.

4.8 3% hydrogen peroxide (H₂O₂) solution. see A.8 of Appendix A.

5 Inspection Procedure

The inspection procedure of hemolytic streptococcus is detailed in Figure 1.

6.1 Sample Treatment and Bacteria Enrichment

Weigh 25 g (mL) of sample according to aseptic operation; put into a homogenizing bag that contains 225 mL of mTSB;

6.2 Separation

The enrichment broth is subject to streak inoculation to the Columbia CNA blood agar plate and anaerobic cultivation under 36°C±1°C for 18 h~24 h, then observe the colony shape.

6.3.1 Separable pure cultivation

Select five suspicious colonies (if less than five, select all) to inoculate with Columbia blood agar plate and TSB enrichment broth respectively, then cultivate under $36^{\circ}\text{C}\pm 1^{\circ}\text{C}$ for 18 h~24 h.

6.3.2 Gram stain microscopy

Select suspicious colony for gram staining microscopic examination. The beta-type hemolytic streptococcus is gram positive, spherical or oval, and generally shaped in short chain.

6.3.4 Streptokinase test (optional item)

Suck 0.2 mL of potassium oxalate blood plasma into 0.8 mL of sterilized normal saline and mix well; then add 0.5 mL of TSB culture solution of suspicious colony after cultivation under $36^{\circ}\text{C}\pm 1^{\circ}\text{C}$ for 18 h~24 h and 0.25 mL of 0.25% calcium chloride solution; oscillate and shake well; place into the water bath of $36^{\circ}\text{C}\pm 1^{\circ}\text{C}$ for 10 min;

6.3.5 Other inspections

Identify the suspicious colony with biochemical identification kit or biochemical identification card.

7 Results and Reports

Upon the above test results, report the detected or un-detected hemolytic streptococcus in every 25 g (mL) of samples.

Appendix A

Culture Media and Reagents

A.1 Modified tryptone soybean broth (mTSB) culture medium

A.1.1 Basal medium (Tryptone soybean broth [TSB])

A.1.2 Antibiotic solution

A.1.2.1 Polymyxin solution

Weigh 10 mg of polymyxin B into 10 mL of sterile purified water, shake to mix well, filter and remove bacteria after fully dissolved.

A.1.2.2 Nalidixic acid sodium solution

Weigh 10 mg of nalidixic acid into 10 mL of sodium hydroxide solution (0.05mol/L), shake to mix well, filter and remove bacteria after fully dissolved.

A.1.3 Complete medium

A.2.2 Preparation

Dissolve the compositions listed in A.2.1 into the distilled water, heat to dissolve, calibrate the pH value to 7.3 ± 0.2 and sterilize at 121°C for 12 min; after cooling to around 50°C , add 50 mL of aseptic defibrinated sheep blood, shake well and pour to the plate.

A.3 Columbia blood agar

A.3.1 Basal medium

A.3.1.1 Compositions

Animal tissue zymolyte 23.0 g

Starch 1.0 g

Sodium chloride 5.0 g

Agar 8.0 g~18.0 g

Distilled water 1 000.0mL

A.4 Gram stain solution

A.4.1 Basal medium of crystal violet staining solution

A.4.1.1 Compositions

Crystal violet 1.0 g

95% ethanol 20.0mL

1% ammonium oxalate solution 80.0 mL

A.4.1.2 Preparation

Fully dissolve the crystal violet into ethanol, and then mix with ammonium oxalate solution.

A.4.2 Gram's iodine solution

A.4.2.1 Compositions