GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 4789.11-2014

National Food Safety Standard – Food Microbiological Examination – Examination of β-type Hemolytic Streptococcus

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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 β-type Hemolytic Streptococcus

1 Scope

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

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

2 Terms and Definitions

2.1 β-type hemolysis

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

2.2 β-type hemolytic streptococcus

It can produce β-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:

a) Constant temperature incubator: 36°C±1°C;

b) Refrigerator: 2°C~5°C;

c) Anaerobic culture apparatus;

d) Balance: with sensibility of 0.1g;

e) Homogenizer and matched homogenizing bag;

f) Microscope: 10X~100X;

plate is 2 mm~3 mm in diameter, grey-white, semi-transparent, smooth, round, with protruded surface, neat edge, and β-type hemolysis is formed.

6.3 Identification

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°C±1°C for 18 h~24 h.

6.3.2 Gram stain microscopy

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

6.3.3 Catalase test

Select suspicious colonies to clean glass slide; drop-add appropriate amount of 3% hydrogen peroxide solution, it is deemed as positive if any bubble is produced immediately. The catalase of β-type hemolytic streptococcus is negative.

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°C±1°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°C±1°C for 10 min; thus the mixture will self-solidify (the solidification degree shall be until that the content is stagnant when the test tube is upside-down). Cultivate under 36°C±1°C for 24 h continuously; it is deemed as positive if the solidified body is fully dissolved again; otherwise, it is negative, the β-type hemolytic streptococcus is positive.

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.

Nalidixic acid sodium solution	10.0 mL

A.1.3.2 Preparation

Mix the compositions listed in A.1.3.1 well under aseptic condition, pack separately for standby.

A.2 Columbia CNA blood agar

A.2.1 Compositions

Casein Tryptone	12.0 g
Digestion solution of animal tissue protein	5.0 g
Yeast extract	3.0 g
Beef extract	3.0 g
Corn starch	1.0 g
Sodium chloride	5.0 g
Agar	13.5 g
Polymyxin	0.01 g
Nalidixic acid	0.01 g
Distilled water	1000.0 mL

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

A.4.2 Gram's iodine solution

A.4.2.1 Compositions

Iodine	1.0 g
Kalium iodide	2.0 g
Distilled water	300.0mL

A.4.2.2 Preparation

Firstly mix the iodine with potassium iodide, add a little distilled water into the mixture and shake sufficiently, wait till the mixture is dissolved completely, and add distilled water to 300mL.

A.4.3 Safranin solution

A.4.3.1 Compositions

Safranin	0.25 g
95% ethanol	10.0mL
Distilled water	90.0mL

A.4.3.2 Preparation

Dissolve safranin into the ethanol, and dilute with distilled water.

A.4.4 Staining procedure

Fix the smear over the flame of alcohol lamp, drop-add crystal violet staining solution for staining for 1 min, then wash with water; drop-add gram's iodine solution and wash with water after 1 min; drop-add 95% ethanol for decoloring for 15 s~30 s until the staining solution is washed off (don't decolor excessively), then wash with water; drop-add counterstain for 1 min, wash with water and dry for microscopic examination.

A.5 Tryptone soybean broth (TSB)

A.5.1 Compositions

Tryptone	17.0 g
Soybean peptone	3.0 g
Sodium chloride	5.0 g
Potassium dihydrogen phosphate (anhydrous)	2.5 g

water, mix well and pack separately for standby.

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