

**National Standard of the People's Republic of China**

GB 4789.10—2010

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**National food safety standard**  
**Food microbiological examination: Staphylococcus aureus**

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of the People's Republic of China**



## Preface

This standard replaces GB/T 4789.10-2008 Microbiological examination of food hygiene- Detection of *Staphylococcus aureus* and GB/T4789.37-2008 Microbiological examination of food hygiene- Enumeration of *Staphylococcus aureus*.

The main revised content of this standard to GB/T 4789.10-2008 and GB/T 4789.37-2008 are as follows:

- The Chinese and English name of the standard has been changed.
- The scope has been amended.
- Procedure of sample preparation has been standardized.
- Explanation of *Coefficient 1.1* in the Calculation Formula has been added.
- The name of *Trypticase soy broth* has been standardized and changed to *10% sodium chloride Trypticase soy broth*.
- Method 2 - Baird-Parker enumeration of *Staphylococcus aureus* and Method 3 –MPN enumeration of *Staphylococcus aureus* have been added.

*Appendix A, Appendix B and Appendix C* of this standard are normative annexes.

This standard replaces all previous standard as follows:

- GB 4789.10-1984, GB 4789.10-1994, GB/T 4789.10-2003, GB/T 4789.10-2008.
- GB/T 4789.37-2008.



# National food safety standard

## Food microbiological examination: *Staphylococcus aureus*

### 1. Scope

This standard stipulates the examination method for *staphylococcus aureus* in foods.

*Method 1* in this standard applies to qualitative examination of *staphylococcus aureus* in foods. *Method 2* applies to enumeration of *staphylococcus aureus* in foods relatively containing more *Staphylococcus aureus*. *Method 3* applies to enumeration of *staphylococcus aureus* in foods relatively containing fewer *Staphylococcus aureus* but more other microorganism.

### 2. Apparatus and Materials

In addition to the conventional apparatus for sterilization and culture in microbiological laboratory, other apparatus and materials are as follows:

2.1 Constant temperature incubator:  $36 \pm 1^\circ\text{C}$

2.2 Refrigerator:  $2\sim 5^\circ\text{C}$

2.3 Constant temperature water bath:  $37\sim 65^\circ\text{C}$

2.4 Balance weighing to 0.1g

2.5 Homogenizer

2.6 Oscillator

2.7 Sterile pipette: 1ml with graduation of 0.01ml, 10 ml with graduation of 0.1ml, or micropipette and pipette tips

2.8 Sterile conical flask: 100 ml and 500ml

2.9 Sterile petri dish: diameter 90mm

2.10 Injector: 0.5mL

2.11 pH meter or pH colorimetric tubes or precise pH test paper

### 3. Culture medium and Reagent

3.1 10% sodium chloride trypticase soy broth: See A.1 in *Appendix A*.

3.2 7.5% sodium chloride broth: See A.2 in *Appendix A*.

3.3 Plasma agar plate: See A.3 in *Appendix A*.

3.4 Baird-Parker agar plate: See A.4 in *Appendix A*.

3.5 Brain heart infusion broth (BHI): See A.5 in *Appendix A*.

3.6 Rabbit plasma: See A.6 in *Appendix A*.

3.7 Diluent: Phosphate buffer solution: See A.7 in *Appendix A*.

3.8 Nutrient agar small slant: See Chapter A.8 in *Appendix A*.

3.9 Gram stain solution: See Chapter A.9 in *Appendix A*.

3.10 Sterile normal saline: See Chapter A.10 in *Appendix A*.

## Method 1 Qualitative examination of staphylococcus aureus

### 4. Examination Procedures

Refer to *Figure 1* for the examination procedures of staphylococcus aureus.

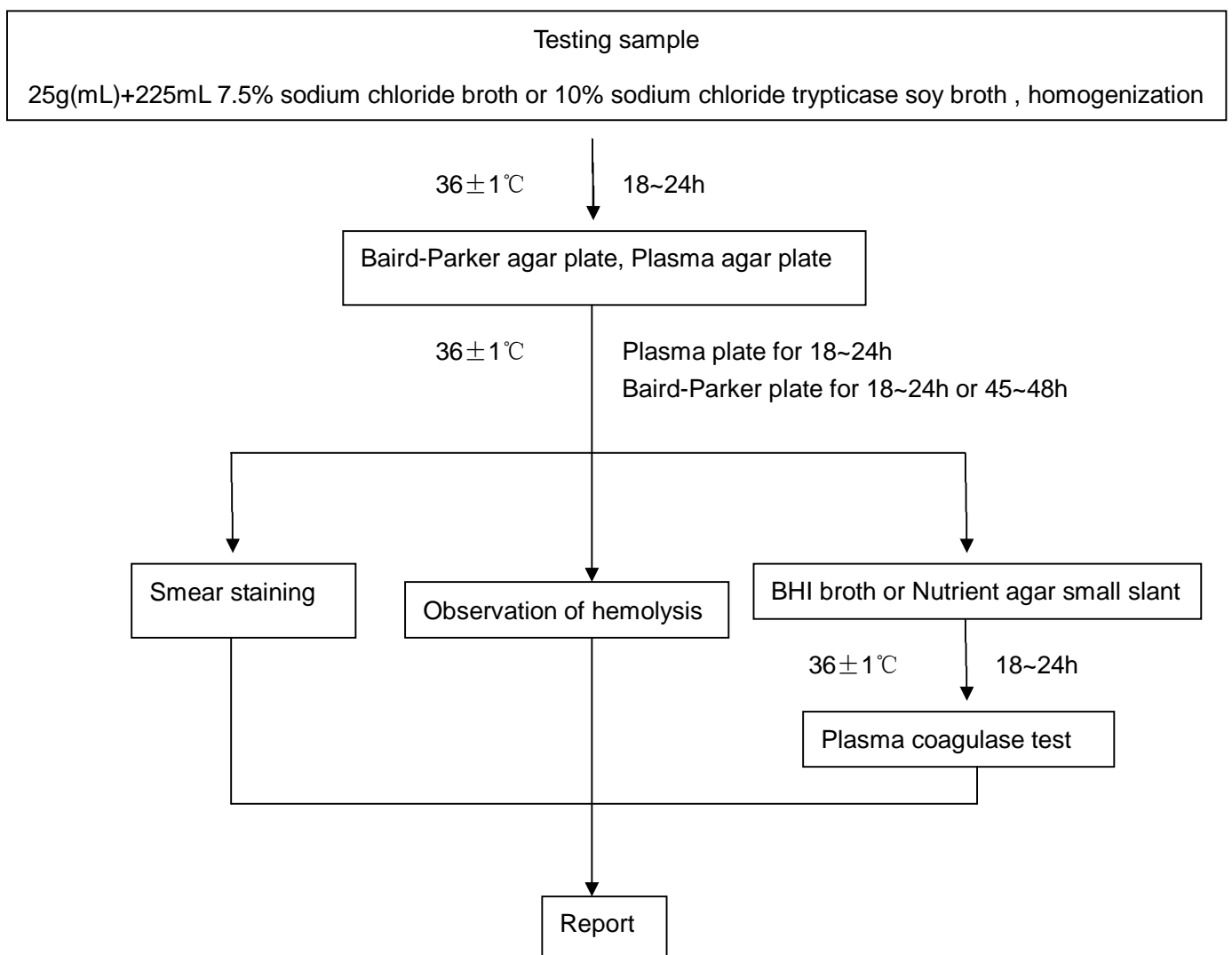


Figure 1 Examination procedures of staphylococcus aureus

### 5. Operating Procedures

#### 5.1 Treatment of sample

Take 25g sample into an sterile homogenization cup containing 225mL 7.5% sodium chloride broth or 10% sodium chloride trypticase soy broth, homogenize at 8000r/min~10000 r/min for 1~2min, or take

sample into a sterile homogenization bag containing 225mL 7.5% sodium chloride broth or 10% sodium chloride trypticase soy broth, and flap with a rattling-type homogenizer for 1~2min. For liquid sample, take 25mL sample into a sterile conical flask containing 225mL 7.5% sodium chloride broth or 10% sodium chloride trypticase soy broth (proper amount of sterile glass beads may be put into the flask in advance), shake and mix it homogeneously.

## 5.2 Enrichment and Isolation incubation

5.2.1 Incubate the above homogeneous sample solution at  $36 \pm 1^\circ\text{C}$  for 18~24h. *Staphylococcus aureus* becomes turbid growth in 7.5% sodium chloride broth, it also becomes turbid growth in 10% LST if sample is serious polluted.

5.2.2 Respectively streak inoculate above cultures on Baird-Parker agar plate and blood plate, incubate at  $36 \pm 1^\circ\text{C}$  for 18~24h or 45~48h.

5.2.3 When *Staphylococcus aureus* are on the Baird-Parker agar plate, diameters of colonies are 2mm~3mm and the color is gray to black with a border of light color. The colonies are surrounded by a turbid zone, which have transparent rings on their outer layer. When contacting the colony with inoculating needle, the hardness is similar to butter to gum. Non-fat soluble colonies may occur occasionally, but they have no turbid zones or transparent rings. Comparing with the typical colony, the colony isolated from long-kept frozen or dry foods will generate lighter black, and maybe rougher and drier appearance. Colonies forming on the blood plate are large, round, smooth, salient, humid, in golden yellow (sometimes in white), complete transparent hemolytic circles surround the colonies. Take above mentioned colonies for microscopic examination of Gram Stain and plasma coagulase test.

## 5.3 Identification

5.3.1 Staining and microscopic examination: *Staphylococcus aureus* are gram-positive coccus, arranged in grape shape. They have no spores and capsules with diameter of 0.5~1 $\mu\text{m}$ .

5.3.2 Plasma coagulase test: Pick one or more suspicious colonies from Baird-Parker agar plate or blood plate, inoculate into 5mL BHI and nutrient agar small slant respectively, incubate at  $36 \pm 1^\circ\text{C}$  for 18~24h.

Take 0.5mL fresh-prepared rabbit plasma into a small test tube, add 0.2~0.3mL BHI culture, oscillate and shake the tube, put it into incubator or water bath at  $36 \pm 1^\circ\text{C}$ , observe every half-hour within 6h. If coagulation occurs (clots when the tube is tilted or inverted) or coagulation volume is larger than 50% original volume, it is judged as positive. Meanwhile, broth culture for plasma coagulase test, containing both positive and negative *Staphylococcus* strains, is used as control. Commercial reagent can also be used for plasma coagulase test according to manual.

If result is suspicious, select colonies from nutrient agar small slant into 5mL BHI, incubate at  $36 \pm 1^\circ\text{C}$  for 18~24h and repeat.

## 5.4 Detection for staphylococcal enterotoxin

To identify if suspected food poisoning sample or *Staphylococcus aureus* strains may generate staphylococcal enterotoxin, should refer to *Appendix B* to examine staphylococcal enterotoxin.

## 6. Results and Report

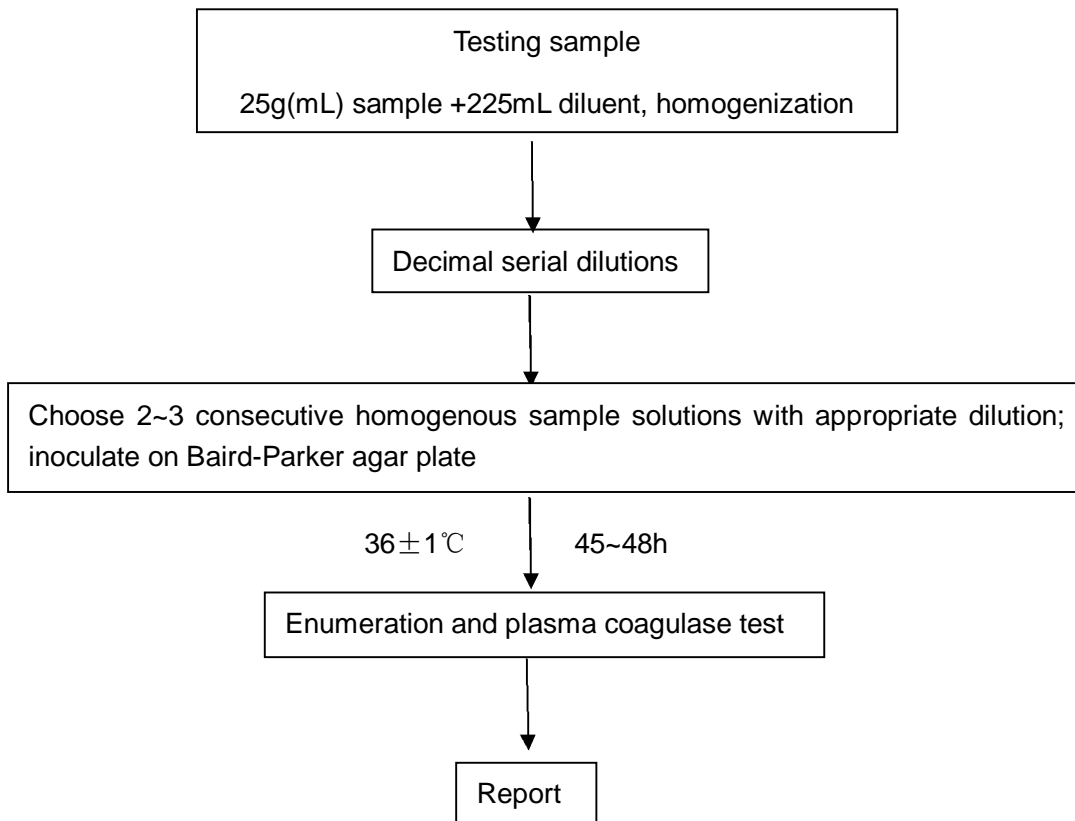
6.1 Result determination: *STAPHYLOCOCCUS aureus* can be determined if meet 5.2.3, 5.3 and plasma coagulase test show positive.

6.2 Result report:: *Staphylococcus aureus* is **detected** or **not detected** In 25g (or 25mL) sample.

## Method 2 Baird-Parker enumeration of staphylococcus aureus

### 7. Examination procedures

Refer to *Figure 2* for the enumeration procedures of *Staphylococcus aureus*.



**Figure 2 Baird-Parker enumeration procedures of staphylococcus aureus**

### 8. Operating Procedures

#### 8.1 Dilution of sample

8.1.1 Solid and semi-solid sample: Take 25g sample into an sterile homogenization cup containing 225mL phosphate buffer or normal saline, homogenize at 8000r/min~10000 r/min for 1~2min, or take sample into a sterile homogenization bag containing 225mL diluent, and flap with a rattling-type homogenizer for 1~2min, to get homogeneous sample solution of 1: 10.

8.1.2 Liquid sample: Use sterile pipette to take 25mL sample into a sterile conical flask containing 225mL phosphate buffer or normal saline (proper amount of sterile glass beads should be put into the flask in advance), mix well to get homogeneous sample solution of 1: 10.

8.1.3 Use 1mL sterile pipette or micropipette to take 1mL homogeneous sample solution of 1:10, feed it into a sterile tube containing 9mL diluent along the tube wall ( note: pipette or pipette tip do not touch the diluent), shake or repeatedly insufflate and flap with 1mL sterile pipette, mix well to get homogeneous sample solution of 1:100.



8.1.4 Make decimal serial dilutions of homogeneous sample solution according 8.1.3, change a new 1mL sterile pipette or pipette tip once per diluted.

## 8.2 incubation of sample

Choose 2~3 consecutive homogenous sample solutions with appropriate dilution (the initial sample solution may be chose) according the evaluation of pollution to sample. When making decimal serial dilutions, take 1mL homogenous sample solution and respectively inoculate 0.3mL, 0.3ml, 0.4ml of the solution on 3 Baird-Parker agar plates, then smear all over the plate with L-shaped rod noticing do not touch the edge. If there are beads of water on Baird-Parker agar plates, dry them in incubator at 25~50℃ till the beads of water disappear before use.

## 8.3 Incubation

8.3.1.1 Generally keep the plates still for 10min after smear. If the sample solution can not be absorbed easily, inoculate the plates in incubator at  $36 \pm 1$  °C for 1h, invert the plates after the solution is absorbed. Incubate at  $36 \pm 1$  °C for 45~48h.

## 8.4 Enumeration of typical colonies and confirmation

8.4.1 When staphylococcus aureus are on the Baird-Parker agar plate, diameters of colonies are 2mm~3mm and the color is gray to black with a border of light color. The colonies are surrounded by a turbid zone, which have transparent rings on their outer layer. When contacting the colony with inoculating needle, the hardness is similar to butter to gum. Non-fat soluble colonies may occur occasionally, but they have no turbid zones or transparent rings. Comparing with the typical colony, the colony isolated from long-kept frozen or dry foods will generate lighter black, and maybe rougher and drier appearance.

8.4.2 Choose plates with typical staphylococcus aureus colonies and with total colonies count of 3 plates for same dilution between 20~200CFU.

a) If only one dilution plates' count is between 20~200CFU and typical colonies can be found on them, then enumerate typical colonies on these dilution plates.

b) If the lowest dilution plates' count is less than 20CFU and typical colonies can be found on them, then enumerate typical colonies on these dilution plates.

c) If a certain dilution plates' count is greater than 200CFU and typical colonies can be found on them, but typical colonies can not be found on next dilution plates, then enumerate typical colonies on these dilution plates.

d) If a certain dilution plates' count is greater than 200CFU and typical colonies can be found on them, and typical colonies can be found on next dilution plates but the count is not between 20~200CFU, then enumerate typical colonies on these dilution plates.

Calculate as formula (1) for all above.

e) If two consecutive dilution plates' count is between 20~200CFU, then calculate as formula (2).

8.4.3 Optionally choose 5 colonies from typical ones (choose all if less than 5), do plasma coagulase test respectively.

## 9. Calculation

Formula (1):

$$T = \frac{AB}{Cd} \dots\dots\dots (1)$$

In the formula:

- T— count of staphylococcus aureus colonies in sample
- A— total count of typical colonies for a certain dilution
- C— count of colonies positive in plasma coagulase test for a certain dilution
- D— count of colonies used for plasma coagulase test for a certain dilution
- d— dilution factor

Formula (2):

$$T = \frac{A_1B_1/C_1 + A_2B_2/C_2}{1.1d} \dots\dots\dots (2)$$

In the formula:

- T— count of staphylococcus aureus colonies in sample
- A1— total count of typical colonies for the first dilution (low dilution multiple)
- A2— total count of typical colonies for the second dilution (high dilution multiple)
- B1— count of colonies positive in plasma coagulase test for the first dilution (low dilution multiple)
- B2— count of colonies positive in plasma coagulase test for the second dilution (high dilution multiple)
- C1— count of colonies used for plasma coagulase test for the first dilution (low dilution multiple)
- C2— count of colonies used for plasma coagulase test for the second dilution (high dilution multiple)
- 1.1— calculation coefficient
- d— dilution factor (the first dilution)

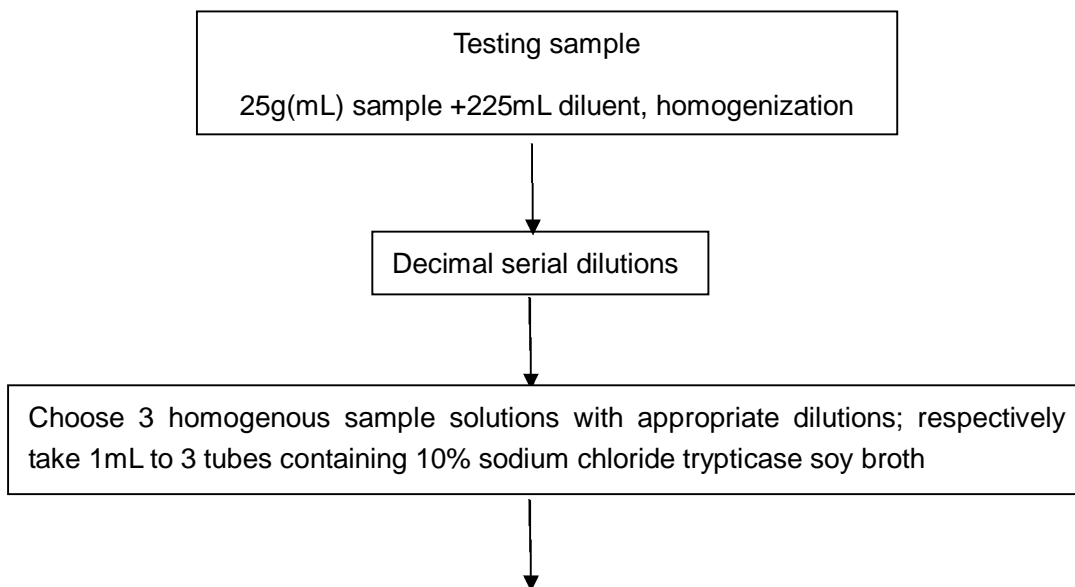
**10. Results and Report**

According to the count of typical staphylococcus aureus colonies on Baird-Parker agar plate and result calculated as formulas in 9, report count of staphylococcus aureus per g (mL) sample, expressed as CFU/g(mL). If T=0, report as “<1 X the lowest dilution multiple”.

**Method 3 MPN Enumeration of staphylococcus aureus**

**11. Examination procedures**

Refer to *Figure 2* for MPN enumeration procedures of staphylococcus aureus.



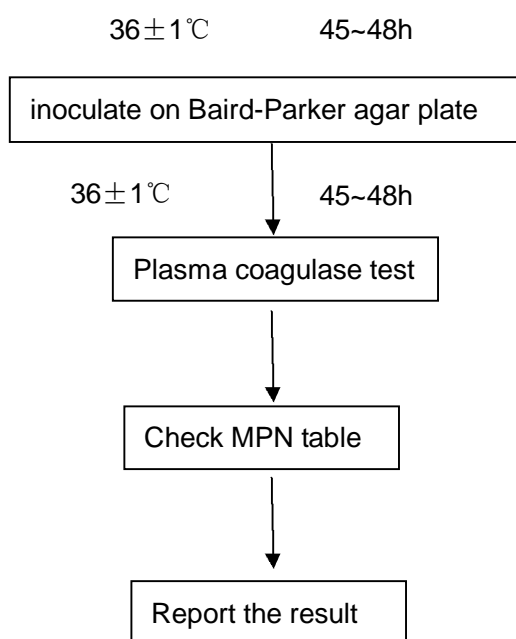


Figure 3 MPN enumeration procedures of *Staphylococcus aureus*

## 12. Operating Procedures

### 12.1 Dilution of sample

According to 8.1

### 12.2 Inoculation and incubation

12.2.1 Choose 3 homogenous sample solutions with appropriate dilution (the initial sample solution may be chose) according the evaluation of pollution to sample. When making decimal serial dilutions, respectively take 1mL homogenous sample solution into 3 tubes containing 10% sodium chloride trypticase soy broth for each dilution and incubate at  $36\pm 1^{\circ}\text{C}$  for 45~48h.

12.2.2 Use incubating loop to pick one loop from the tubes containing colonies, inoculate on Baird-Parker agar plate and incubate at  $36\pm 1^{\circ}\text{C}$  for 45~48h.

### 12.3 Confirmation of typical colonies

12.3.1 See 8.4.1

12.3.2 Pick at least one colony from typical colonies to BHI broth and nutrient agar slant, incubate at  $36\pm 1^{\circ}\text{C}$  for 18~24h. Do plasma coagulase test according to 5.3.2.

## 13. Results and Report

Calculate the amount of the corresponding tubes that the plasma coagulase test of the colony is positive. Check MPN retrieval table (see appendix C), report the most probable number of staphylococcus aureus per g (mL) sample, expressed as MPN/g (mL).

**Appendix A**  
**(Normative Appendix)**  
**Culture Medium and Reagent**

**A.1 10% sodium chloride Trypticase soy broth****A.1.1 Composition**

Trypticase (or tryptone)	17.0g
Phytone (or soya peptone)	3.0g
NaCl	100.0g
K <sub>2</sub> HPO <sub>3</sub>	2.5g
Sodium pyruvate	10.0g
Glucose	2.5g
Distilled water	1000mL
pH 7.3±0.2	

**A.1.2 preparation method**

Mix and heat above compositions, slowly agitate and dissolve, adjust pH, separately put 225mL into each bottle and conduct autoclave sterilization at 121℃ for 15min.

**A.2 7.5% NaCl broth****A.2.1 Composition**

Peptone	10.0g
Beef extract	5.0g
NaCl	75g
Distilled water	1000mL
pH 7.4	

**A.2.2 Preparation method**

Heat and dissolve the above compositions, adjust pH, separately put 225mL into each bottle and conduct autoclave sterilization at 121□ for 15min.

**A.3 Blood agar plate****A.3.1 Composition**

Agar of soybean powder (pH 7.4~7.6)	100mL
De-fiber sheep blood (or rabbit blood)	5~10mL

**A.3.2 Preparation method**

Heat and dissolved agar, then cool it to 50 □, add de-fiber sheep blood (or rabbit blood) via aseptic operation, shake well and pour on the plate.

**A.4 Baird-Parker agar plate****A.4.1 Composition**

Tryptone	10.0g
Beef extract	5.0g
Yeast extract	1.0g

Sodium pyruvate	10.0g
Glycine	12.0g
LiCl·6H <sub>2</sub> O	5.0g
Agar	20.0g
Distilled water	950mL

pH 7.0±0.2

#### A.4.2 Formulation method for bacteria-increasing agent

Mix 50mL 30% yolk saline and 10mL 1% potassium tellurite solution, which has been sterilized and filtered, store the mixture in the refrigerator.

#### A.4.3 Preparation method

Add all the components into the distilled water, heat and boil heat to completely dissolving, adjust pH, put every 95mL into each bottle, conduct autoclave sterilization at 121℃ for 15min. For use, heat and melt the agar, cool it to 50℃, add 5mL yolkpotassium tellurite solution as bacteria-increasing agent, which has been preheated to 50℃, in each 95mL, shake and pour it on the plate. Culture medium shall be dense opaque. It can't be stored in the refrigerator for more than 48h before use.

### A.5 BHI broth

#### A.5.1 Composition

Tryptone	10.0g
NaCl	5.0g
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O	2.5g
Glucose	2.0g
Beef heart infusion	500mL

pH 7.4±0.2

#### A.5.2 Preparation method

Heat and dissolve, adjust pH, put into 16mmX160mm test tube, take 5mL broth into each bottle and conduct autoclave sterilization at 121℃ for 15min.

### A.6 Rabbit plasma

Take 3.8g sodium citrate, add 100mL distilled water, dissolve and filtrate for bottling, conduct autoclave sterilization at 121℃ for 15min.

Preparation of rabbit plasma: Take 3.8% sodium citrate, add 4 proportions of rabbit blood (100%), mix well and settle (or conduct centrifugation at 3000r/min for 30min) to reduce blood cells and obtain plasma.

### A.7 Phosphate buffer solution

#### A.7.1 Composition

KH <sub>2</sub> PO <sub>4</sub>	34.0g
Distilled water	500mL

pH 7.2

#### A.7.2 preparation method

Stock solution: Weigh 34.0g KH<sub>2</sub>PO<sub>4</sub>, dissolve it in 500mL distilled water, use 175mL 1mol/L NaOH solution to adjust pH to 7.2, dilute it to 1000mL with distilled water and then store in refrigerator.

Diluent: Take 1.25mL stock solution, dilute it to 1000mL with distilled water, separately put into the suitable containers and conduct autoclave sterilization at 121℃ for 15min.

### A.8 Nutrient agar small slant

#### A.8.1 Composition

Peptone	10.0g
Beef extract	3.0g
NaCl	5.0g

Agar	15.0~20.0g
Distilled water	1000mL
pH 7.2~7.4	

### A.8.2 Preparation method

Dissolve all the components into the distilled water except agar, add 2mL 15% NaOH for dissolution, correct PH to 7.2~7.4, add agar, heat and boil to melt the agar, separately put into 13mmX130mm tubes and conduct autoclave sterilization at 121℃ for 15min.

## A.9 Gram stain solution

### A.9.1 Crystal violet stain solution

#### A.9.1.1 Composition

Crystal violet	1.0g	
95% ethanol	20.0mL	
1% aqueous solution of diammonium oxalate	80.0mL	

#### A.9.1.2 Preparation method

Completely dissolve crystal violet in ethanol and then mix it with diammonium oxalate solution.

### A.9.2 Gram iodine solution

#### A.9.2.1 Composition

I	1.0g
KI	2.0g
Distilled water	300mL

#### A.9.2.2 Preparation method

Firstly mixed I with KI will, add a little distilled water, shake well and then add distilled water to 300mL after complete dissolution.

### A.9.3 Hansa yellow counterstain solution

#### A.9.3.1 Composition

Hansa yellow	0.25g
95% ethanol	10.0mL
Distilled water	90.0mL

#### A.9.3.2 Preparation method

Dissolve Hansa yellow in the ethanol and then dilute it with distilled water.

### A.9.4 Staining method

- Fix the smear on the flame, drip crystal violet stain solution, stain it for 1min and wash with distilled water.
- Drip Gram iodine solution, react for 1min and wash with water.
- Drip 95% ethanol, decolorize for about 15~30s until the staining solution is washed (avoid excessive decolorization) and wash with water.
- Drip hansa yellow counterstain solution, counter for 1min, wash with distilled water, dry and conduct microscopic examination.

## A.10 Sterile normal saline

### A.10.1 Composition

NaCl	8.5mL
Distilled water	1000mL

**Appendix B**  
**(Normative Appendix)**  
**Inspection Method for Staphylococcal Enterotoxin**

**B.1 Reagents and materials**

Except additional provision, All reagents should be analytically pure, the testing water be conformed to the provision of GB/T 6682 Grade 1 Water.

B.1.1 ELISA assay kit for A, B, C, D, E type of staphylococcal enterotoxin

B.1.2 pH test paper: range 3.5~8.0, precision 0.1.

B.1.3 0.25mol/L, pH 8.0 Tris buffer solution: Dissolve 12.1g Tris in 800mL de-ionized water, add 42mL concentrated HCl after cooling to ambient temperature, adjust pH to 8.0.

B.1.4 Phosphate buffer solution with pH 7.4: Take 0.55g NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O (or 0.62g NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O), 2.85g Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O (or 5.73g Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O), 8.7g NaCl to 1000mL distilled water, mix well.

B.1.5 n-heptane

B.1.6 10% sodium hypochlorite solution

B.1.7 Enterotoxin toxin-producing culture medium

B.1.7.1 Composition

Peptone	20.0g
Pancreatic digest of casein	200mg (amino acid)
NaCl	5.0g
K <sub>2</sub> HPO <sub>4</sub>	1.0g
KH <sub>2</sub> PO <sub>4</sub>	1.0g
CaCl <sub>2</sub>	0.1g
MgSO <sub>4</sub>	0.2g
Nicotinic acid	0.01g
Distilled water	1000mL

pH7.2~7.4

B1.7.2 Preparation method

Mix all the components in the water and adjust pH to 7.2~7.4, conduct autoclave sterilization at 121 °C for 30min.

B.1.8 Nutrient agar

B.1.8.1 Composition

Peptone	10.0g
Beef extract	3.0g
NaCl	5.0g
Agar	15.0~20.0g
Distilled water	1000mL

B.1.8.2 Preparation method

Dissolve all the components except agar into the distilled water, add 2mL 15% NaOH for dissolution, correct PH to 7.2~7.4, add agar, heat and boil to melt the agar, separately put into bottles and conduct autoclave sterilization at 121 °C for 15min.

**B.2 Instrument and Appartus**

B.2.1 balance weighing to 0.01g

B.2.2 homogenizer

B.2.3 Centrifuge: 3000 g~5000 g

B.2.4 Centrifuge tube: 50mL

B.2.5 Filter: aperture of membrane 0.2  $\mu$  m

B.2.6 Trace sampler: 20~200  $\mu$  L, 200~1000  $\mu$  L

B.2.7 Multi-channel trace sampler: 50~300  $\mu$  L

B.2.8 Microplate Washer (Optional)

B.2.9 Microplate Reader

### **B.3 Instrument and Appartus**

This method can be completed by using ELISA assay kit for A,B,C,D,E type of staphylococcal enterotoxin. This method is based on the reaction of enzyme-linked immunosorbent assay (ELISA). A~E holes of each microporous article of the 96-microtiter-plate were coated with A, B, C, D, E type of staphylococcal enterotoxin antibody. H hole is for the positive quality control and has been coated with mixed type of staphylococcal enterotoxin antibodies. F and G holes are for negative controls, coated with antibodies of non-immunized animals. If the samples contain staphylococcal enterotoxin, the dissociative staphylococcal enterotoxin combines with the specific antibody coated on the micropores to form antigen-antibody complex and the remaining compositions are washed off during washing the plate. The antigen-antibody complex (two anti-) combines with peroxidase markers and non-binding enzyme markers are washed off during washing. Add enzyme substrate and color reagent and incubate, the enzyme Catalytic substrates on the enzyme markers decompose and change the colorless reagent to be blue. Color can turns to yellow from blue by adding reaction stopping solution and indicates the end of enzyme reaction. Measure the absorbance value of micropore solution with 450 nm wavelength microplate reader. Staphylococcal enterotoxin in samples is proportional to the absorbance.

### **B.4 Inspection procedures**

B.4.1 Method for detection of staphylococcal enterotoxin in the culture of isolated strains.

Inoculate tested strains on nutrient agar slant (tube 18mm\*180mm) and incubate at 37°C for 24h, wash the lawn with saline and put it into the toxin-producing culture medium, one bottle for each strain, incubate at 37°C for 48h with oscillation of 100 times/min, take the liquid and centrifugate at 8000r/min for 20min, heat at 100°C for 10min, take upper clear liquid and dilute, take 100  $\mu$  L diluted sample solution and tested.

B.4.2 Method for extraction and detection of staphylococcal enterotoxin from food sample

B.4.2.1 Milk and milk powder

Dissolve 25g milk powder into 125mL, 0.25M, pH8.0 Tris buffer solution and mix, same to liquid milk, prepare according to the following procedures. Centrifugate at 15°C, 3500g for 10min, receive the skim milk by removing the surface layer of fat, dilute with distilled water(1:20), take 100  $\mu$  L diluted sample solution and tested.

B.4.2.2 Foods containing fat not exceeding 40%

Weigh 10g sample and mince, add 15mL pH4.7 PBS solution and homogenize, shake for 15min. Centrifugate at 15°C, 3500g for 10min, removing the surface layer of fat if necessary. Take upper clear liquid and filter to degerming, take 100  $\mu$  L filtrate to be tested.

B.4.2.3 Foods containing fat exceeding 40%

Weigh 10g sample and mince, add 15mL pH4.7 PBS solution and homogenize, shake for 15min. Centrifugate at 15°C, 3500g for 10min, take 5.0mL upper suspension to another Centrifuge tube, add 5ml n-heptane, mix well for 10min, Centrifugate at 15°C, 3500g for 5min, remove all the above organic phase ( layer of n-heptane), note not remain any of n-heptane, filter the aqueous phase of the lower part to degerming, take 100  $\mu$  L filtrate to be tested.



B.4.2.4 Other foods are treated appropriately according to the above methods of food treatment.

### B.4.3 Inspection

B.4.3.1 All operations should be under ambient temperature (20~25℃), Temperature of all reagents in ELISA assay kit for A,B,C,D,E type of staphylococcal enterotoxin should rise to ambient room before use. Change new pipette tips when sucking up different reagents and sample solution during determination. Immerse the used pipette tips and exhausted liquid in 10% sodium hypochlorite solution overnight.

B.4.3.2 Put required numbers of microporous articles into the framework (one sample needs one microporous article), Add sample solutions into A~G holes of microporous article, 100 μ L for each hole, Add 100 μ L into H hole, flap the microporous article gently to mix it homogeneously. Sealed with adhesive paper to prevent solution evaporation, incubate at ambient temperature for 1h.

B.4.3.3 Dump the liquid in the holes into container containing 10% sodium hypochlorite solution and flap some times on absorbent paper to ensure no liquid remained in holes. Use multi-channel sampler to add 250 μ L eluant in each hole, dump again and flap on absorbent paper to dry, repeat 4 times of above washing plate operation. This step also can be completed by Microplate Washer.

B.4.3.4 Add 100μL enzyme labelled antibody in each hole, flap the microporous article gently to mix it homogeneously, incubate at ambient temperature for 1h.

B.4.3.5 Repeat washing plate procedure 4.3.3.

B.4.3.6 Add 50μL TMB substrate and color former into each micro-hole, flap gently to mix it homogeneously, incubate at ambient temperature and in dark, light-avoiding place for 1h.

B.4.3.7 Add 100μL 2mol/L Sulfuric acid stopping solution, lap gently to mix it homogeneously, use microplate reader to measure OD value of each hole under 450nm in 30min.

### B.4.4 Calculation of the result and expression

#### B.4.4.1 Quality control

Calculated result of OD value for positive quality control should greater than 0.5, OD value for negative quality control should less than 0.3, test results should not be approved if the results do not meet the above requirements simultaneously. Exclude the interference of endogenous peroxidase for positive results.

#### B.4.4.2 Calculation of critical value

F hole and G hole of each microporous article are for negative quality control, the average value of the OD values for the two holes plus 0.15 is for critical value.

Sample: Negative quality control 1=0.08

Negative quality control 2=0.10

Average value=0.09

Critical value-0.09+0.15=0.24

#### B.4.4.3 Result expression

Judge sample holes for which value are less than OD value to be negative, expressed as not detect a certain type of staphylococcal enterotoxin in sample. Judge sample holes for which value are greater than or equal to OD value to be positive, expressed as detect a certain type of staphylococcal enterotoxin in sample.

## B.5 Biosafety

Because the existence of other potentially infectious material in sample can not be eliminated, Do treat the rejected materials in strict accordance with GB19489.

**Appendix C**  
**(Normative Appendix)**

**Most probable number (MPN) retrieval table of staphylococcus aureus**

**C.1 Most probable number (MPN) retrieval table of staphylococcus aureus**

See Table C.1 to retrieve most probable number (MPN) of staphylococcus aureus in 1g (mL) sample

**Table C.1 Most probable number (MPN) retrieval table of staphylococcus aureus**

Amount of positive tubes			MPN	95% confidence interval		Amount of negative tubes			MPN	95% confidence interval	
0.10	0.01	0.001		Lower limit	Upper limit	0.10	0.01	0.001		Lower limit	Upper limit
0	0	0	<3.0	-	9.5	5	2	0	21	4.5	42
0	0	1	3.0	0.15	9.6	5	2	1	28	8.7	94
0	1	0	3.0	0.15	11	5	2	2	35	8.7	94
0	1	1	6.1	1.2	18	5	3	0	29	8.7	94
0	2	0	6.2	1.2	18	5	3	1	36	8.7	94
0	3	0	9.4	3.6	38	3	0	0	23	4.6	94
1	0	0	3.6	0.17	18	3	0	1	38	804	110
1	0	1	7.2	1.3	18	3	0	2	64	17	180
1	0	2	11	3.6	38	3	1	0	43	9	180
1	1	0	7.4	1.3	20	3	1	1	75	17	200
1	1	1	11	3.6	38	3	1	2	120	37	420
1	2	0	11	3.6	42	3	1	3	160	40	420
1	2	1	15	4.5	42	3	2	0	93	18	420
1	3	0	16	4.5	42	3	2	1	150	37	420
2	0	0	9.2	1.4	38	3	2	2	210	40	430
2	0	1	14	3.6	42	3	2	3	290	90	1000
2	0	2	20	4.5	42	3	3	0	240	42	1000
2	1	0	15	3.7	42	3	3	1	460	90	2000
2	1	1	20	4.5	42	3	3	2	1100	180	4100
2	1	2	27	8.7	94	3	3	3	>1100	420	-

Note1: 3 dilutions [0.1 g(mL)、0.01 g(mL) and 0.001 g(mL)] are adopted in this table, inoculate 3 tubes for each dilution.

Note2: If the tested-sample quantities are changed to 1 g (mL)、0.1 g(mL) and 0.01 g(mL), numbers listed in this table should be decreased for 10 times, If the tested-sample quantities are changed to 0.01 g(mL)、0.001 g(mL) and 0.0001 g(mL), numbers listed in this table should be increased for 10 times, and so on with others.