National Standard of the People's Republic of China

GB4789.10—2016

National Standards For Food Safety

Food Microbiological Analysis
Staphylococcus aureus determination

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Forward

This standard replaces GB4789.10-2010 "National Food Safety Standard for Microbiological Examination of Staphylococcus aureus", SN/T0172-2010 "Methods for the Examination of Staphylococcus aureus in Imported and Exported Foods", and SN/T2154-2008 "Methods for the Detection of Coagulase Positive Staphylococcus in Imported and Exported Foods - Rabbit Plasma Fibrinogen Agar Culture Medium Technology".

Compared with GB4789.10-2010, the main changes in this standard are as follows:

——The bacterial enrichment solution used in the experiment is uniformly 7.5% sodium chloride broth.

1 Scope

This standard specifies the method of detecting staphylococcus aureus in food.

The first method is applicable to the qualitative inspection of staphylococcus aureus in food. The second method is applicable to the higher content of staphylococcus aureus counting of staphylococcus aureus in food. The third method is applicable to the lower content of staphylococcus aureus counting of staphylococcus aureus in food.

2 Equipment and materials

In addition to routine sterilization and culture equipment in microbiology laboratory, other equipment and materials are as follows:

2.1 Constant temperature incubator: 36℃ ±1℃.

2.2 Refrigerator: 2~5℃.

2.3 Constant Temperature Water Bath Box: 36℃~56℃.

2.4 Balance: 0.1g of sensibility.

2.5 Homogenizer.

2.6 Oscillator.

2.7 Sterile pipette: 1mL (with 0.01mL scale), 10mL (with 0.1mL scale) or micropipette and pipette tip.

2.8 Sterile Erlenmeyer flask: capacity 100mL, 500mL.

2.9 Sterile Petri Dish: 90mm in diameter.

2.10 Coated rod.

2.11 pH meter or pH colorimetric tube or precision pH test paper.

3 Medium and Reagents

3.1 7.5% sodium chloride broth: see A.1.

3.2 Blood agar plate: see A.2.

3.3 Baird-Parker agar plate: see A.3.

3.4 Brain heart extract broth (BHI): See A.4.

3.5 Rabbit plasma: See A.5.

3.6 Diluent: phosphate buffer: see A.6.

3.7 Nutritional agar small slope: see A.7.

3.8 Gram staining solution: see A.8.

3.9 Sterile normal saline: see A.9.

The first method- Qualitative inspection of Staphylococcus aureus

4 Inspection procedures

The qualitative inspection procedure of Staphylococcus aureus is shown in Figure 1.

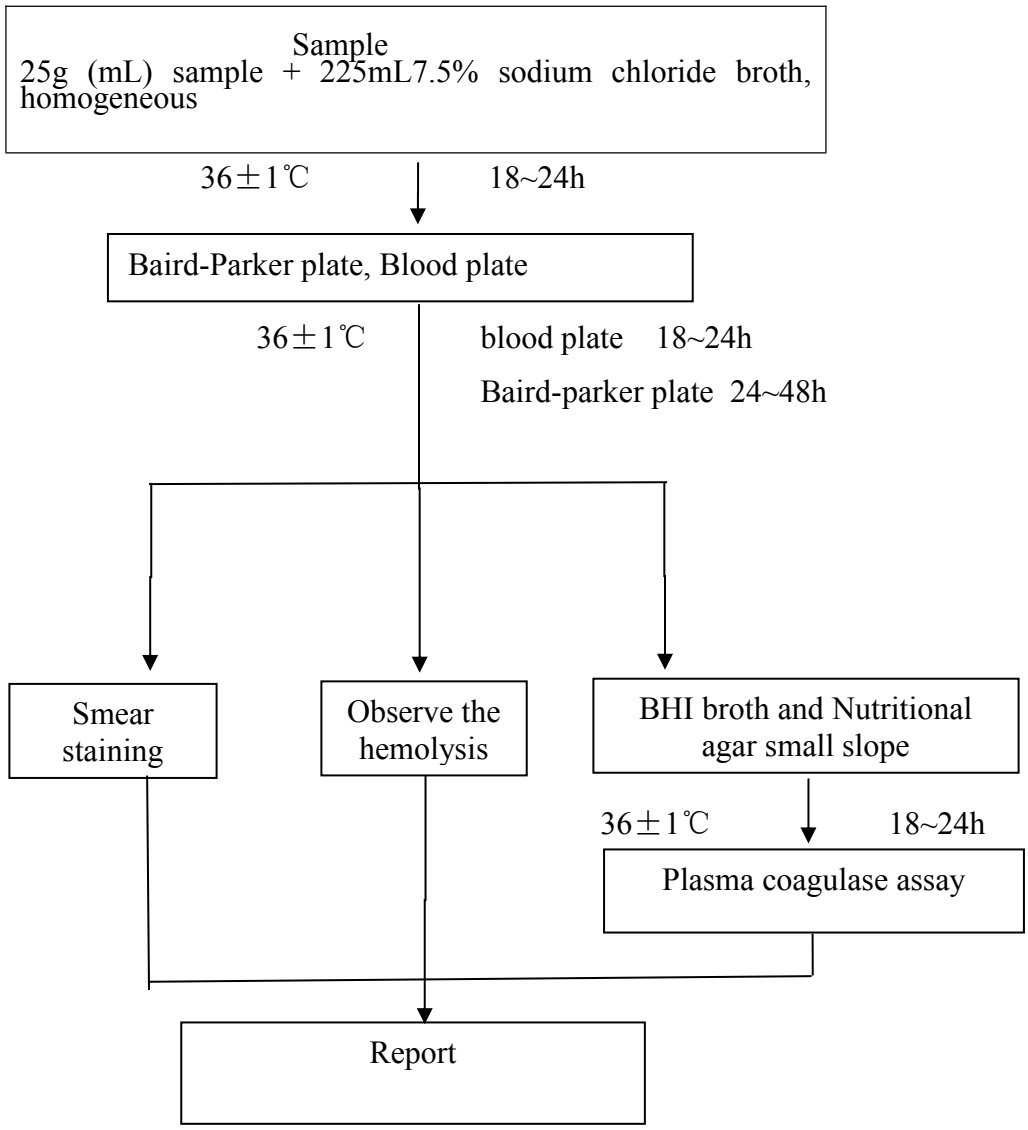


Fig. 1 Test procedure of staphylococcus aureus

5 Operation steps

5.1 handling of samples

Weigh 25g sample into a sterile homogenization cup containing 225mL of 7.5% sodium chloride broth, homogenized at 8000r/min~10000r/min for1min~2min, or put into a sterile homogenization bag containing 225 mL of 7.5% sodium chloride broth, and tap it with a tapping homogenizer for 1 min~2min. If the sample is liquid, draw 25mL of sample to a sterile Erlenmeyer flask containing 225mL of 7.5% sodium chloride broth (the bottle can be preset numbers of sterile glass beads), shake to mix well.

5.2 Enrichment

The above sample solution was incubated at 36 ℃±1 ℃ for 18h~24h. Staphylococcus aureus is cloudy grow in 7.5% sodium chloride broth.

5.3 Separation

The enriched culture was streaked to the Baird-Parker plate and the blood plate, and the blood plate was incubated at 36 ±1 ℃ for 18h~24h. Baird-Parker plates were incubated at 36 ±1℃ for 24h~48h.

5.4 Preliminary identification

Staphylococcus aureus is round on the Baird-Parker plate, the surface is smooth, convex, moist, and the colony diameter is 2mm~3mm, and the color is grayish black to black, shiny, and often has light-colored (non-white) edges, surrounded by an opaque circle (precipitation), and often has a clear outside band. When use inoculation needle to touch the colony, it has a buttery sticky feel. Sometimes strains that do not decompose fat can be seen, except there is no opaque circle and clear outside, other appearances are basically the same. The colonies separated from frozen or dehydrated foods stored for a long time has light color compare with typical colonies, and the appearance may be rough and the texture is relatively dry. On the blood plate, the formed colonies are large, round, smooth, convex, moist, golden yellow (sometimes white Color), a completely transparent hemolytic circle can be seen around the colony. Pick up the above mentioned suspected colonies for Gram stain microscopy and plasma coagulase test.

5.5 Confirmation

5.5.1 Staining microscopic examination: Staphylococcus aureus is Gram-positive cocci, arranged in a staphylococcus-like shape, without spores and capsules, with a diameter 0.5μm~1μm.

5.5.2 Plasma coagulase test: pick at least 5 suspicious colonies on Baird-Parker plate or blood plate (less than 5 select all), and separately inoculated to 5mL BHI and nutrient agar small slope, cultured at 36 ℃ ± 1 ℃ 18h ~ 24h.

Take 0.5 mL of freshly prepared rabbit plasma and put it in a small test tube, then add 0.2ml ~ 0.3ml of BHI culture, shake and mix well, set in a 36 ℃±1 ℃ thermostat or water bath, observe once every half an hour and observe for 6 hours. If it appears to be solidified (that is, when the test tube is tilted or inverted, it will appear clot) or solidified volume greater than half of the original volume, was judged as a positive result. Plasma coagulase test positive and negative Staphylococcus broth culture of the strain served as a control. Commercial reagents can also be used to perform plasma coagulase test according to the instructions.

If the result is suspicious, pick the colonies on the small slope of nutrient agar to 5mL BHI and incubate at 36℃±1℃ for 18h~48h, and repeat the test.

5.6 Inspection of staphylococcal enterotoxin (optional)

For the identification of suspicious food poisoning samples or Staphylococcus aureus strains producing staphylococcal enterotoxin, the staphylococcal enterotoxin should be tested according to Appendix B

6 Results and reports

6.1 Judgment of results: According to 5.4 and 5.5, it can be judged as Staphylococcus aureus.

6.2 Results report: Staphylococcus aureus was detected or not detected in 25g (mL) sample.

The second method -Staphylococcus aureus plate counting method

7 Inspection procedures

The test procedure of the Staphylococcus aureus plate counting method is shown in Figure 2.

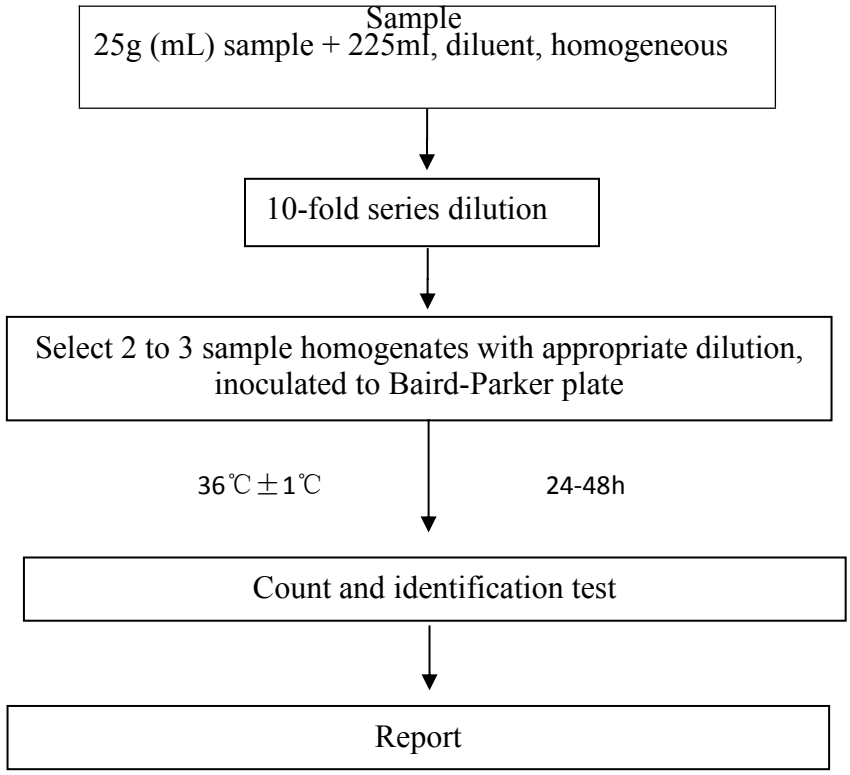


Figure 2 test procedure of Staphylococcus aureus plate counting method

8 Operation steps

8.1 Dilution of samples

8.1.1 Solid and semi-solid samples: Weigh 25g of sample and place it in a sterile homogenization cup containing 225mL of phosphate buffer or normal saline, homogenize 8000r/min~10000r/min for 1min~2min, or put it into a sterile homogeneous bag containing 225mL of diluent, use a hit homogenizer to beat for 1min~2min to make a 1:10 sample homogenate.

8.1.2 Liquid sample: draw 25mL sample with a sterile pipette and place it in a sterile conical flask(preparing an appropriate number of sterile glass beads in the flask) containing 225mL phosphate buffer or normal saline. mix well to make a 1:10 sample homogenate.

8.1.3 Use a 1mL sterile pipette or micropipette to draw 1mL of 1:10 sample homogenate, and slowly inject to a tube along the wall which containing 9ml phosphate buffer or normal saline (note that the pipette or tip does not touch the diluent surface), shake the test tube or change another 1ml sterile pipette to blow repeatedly to mix evenly to make a 1:100 sample solution.

8.1.4 According to the operating procedure of 8.1.3, prepare a 10-fold serially diluted sample

homogenate. For each incremental dilution, change to a 1ml sterile pipette or suction head.

8.2 Inoculation of samples

According to the estimation of the sample contamination status, choose 2~3 suitable dilutions of sample homogenate (liquid samples may include the original solution). While performing 10-fold incremental dilution, each dilution draws 1ml of sample homogenate and inoculates 0.3mL, 0.3mL, 0.4mL respectively. Add to three Baird-Parker plates, then coat the entire plate with a sterile coating stick, taking care not to touch the edge of the plate. Before use, if the water drops on the surface of the Baird-Parker plate can be dried in an incubator at 25 ℃~50 ℃ until the water drops on the surface of the plate disappear.

8.3 Cultivation

Under normal circumstances, after coating, the plate is allowed to stand for 10 min.If the sample liquid is not easy to absorb, the plate can be placed in an incubator at 36 ℃±1 ℃ for 1h, wait for the sample to be absorbed and invert the plate. And incubate at 36℃±1 ℃ for 24 ~ 48 hours.

8.4 Counting and confirmation of typical colonies

8.4.1 Staphylococcus aureus is round on the Baird-Parker plate, the surface is smooth, convex, moist, and the diameter of the colony is 2 mm~3mm, the color is grayish black to black, shiny, often with light-colored (non-white) edges, surrounded by an opaque circle (precipitation), and often has a clear outside. When the colony is touched with the inoculation needle, it has a buttery sticky feel. Sometimes strains that do not break down fat are visible, except that there is no opaque circle and clear band, the appearance is basically the same. Colonies isolated from long-term storage of frozen or dehydrated foods are often has light-colored compare with the typical colonies, and the appearance may be rough, the texture is relatively dry.

8.4.2 Select a plate with typical staphylococcus aureus colonies, and the total number of all colonies of 3 plates of the same dilution is 20CFU~200CFU. And count the number of typical colonies on plates between 20CFU~200CFU.

8.4.3 Select at least 5 suspicious colonies (less than 5 select all) from typical colonies for identification test. Do staining microscopy and plasma coagulation enzyme test (see 5.5), at the same time, streak inoculation to blood plate culture at 36℃±1℃ for 18h~24h to observe the colony morphology. The staphylococcus aureus colonies are large, round, smooth, raised, moist,

and golden yellow (sometimes white), and a completely transparent hemolytic circle can be seen around the colonies.

9 Result calculation

9.1 If there is only one dilution plate with a typical colony number between 20CFU~ 200CFU, count the typical colonies on the dilution plate, and calculate according to formula (1).

9.2 If the number of typical colonies on the lowest dilution plate is less than 20CFU, count the typical colonies on the dilution plate and calculate according to formula (1).

9.3 If the typical number of colonies in a certain dilution plate is greater than 200CFU, but there are no typical colonies on the next dilution plate, count the typical colonies on the dilution plate and calculate according to formula (1).

9.4 If the number of typical colonies of a certain dilution plate is greater than 200CFU, and there are typical colonies on the next dilution plate, but not within the range of 20CFU~200CFU, count the typical colonies on the dilution plate and calculate according to formula (1).

9.5 If the typical number of colonies on two plates with continuous dilution is between 20CFU and 200CFU, calculate according to formula (2).

9.6 Calculation formula

Formula(1):

$$T = AB/Cd \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

In the formula:

T ———The number of staphylococcus aureus colonies in the sample;

A ———Total number of typical colonies at a certain dilution;

B ———Number of colonies identified as positive at a certain dilution;

C ———The number of colonies used for identification test at a certain dilution;

d — Dilution factor.

Formula (2):

$$T = \frac{A_1 B_1 / C_1 + A_2 B_2 / C_2}{1.1d} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

In the formula:

T ———The number of staphylococcus aureus colonies in the sample;

A 1 ———The total number of typical colonies in the first dilution (low dilution factor);

B 1 ———The number of colonies identified as positive at the first dilution (low dilution factor);

C 1 ———The first dilution (low dilution factor) is used to identify the number of colonies in the test;

A 2 ———The total number of typical colonies in the second dilution (high dilution factor);

B 2 ———The number of colonies identified as positive by the second dilution (high dilution factor);

C 2 ———The second dilution (high dilution factor) is used to identify the number of colonies in the test;

1.1—Calculation coefficient;

d — Dilution factor (first dilution).

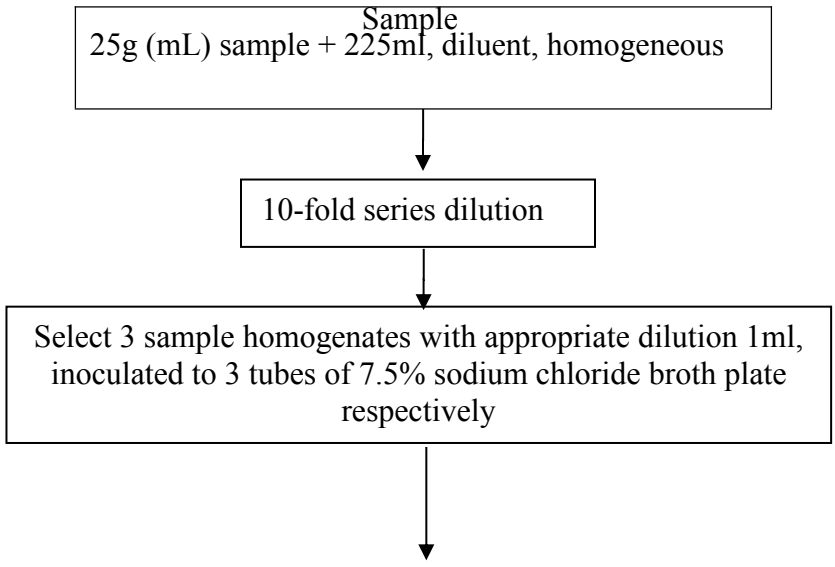
10 Report

According to the calculation result of the formula in 9, report the number of Staphylococcus aureus per g (mL) sample, expressed in CFU/g (mL), if the T value is 0, then report as less than 1 times the lowest dilution factor.

Third method MPN count of Staphylococcus aureus

11 Inspection procedures

The MPN count test procedure for Staphylococcus aureus is shown in Figure 3.



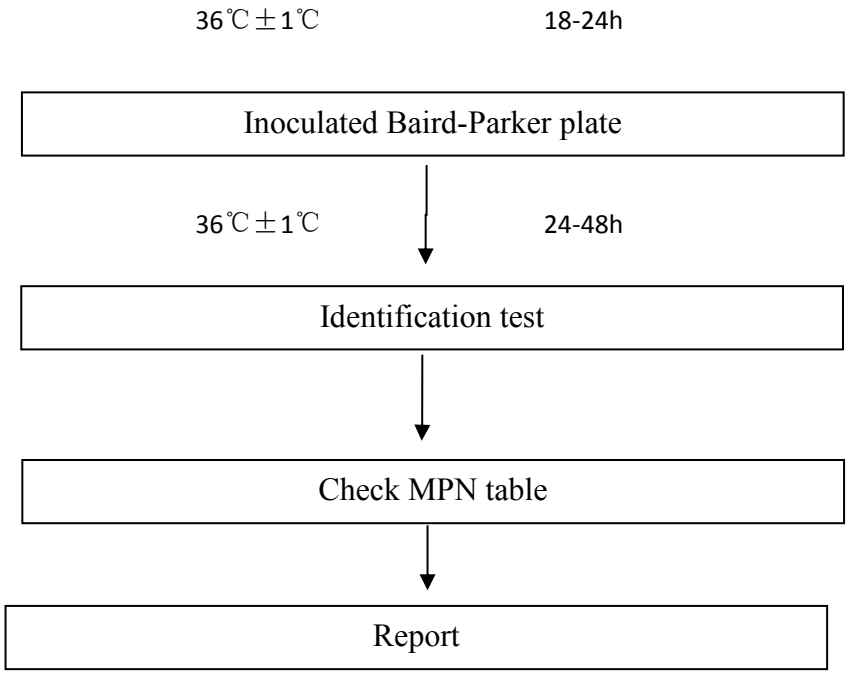


Figure 3 Staphylococcus aureus MPN test procedure

12 Operation steps

12.1 Dilution of samples

Follow 8.1.

12.2 Inoculation and cultivation

12.2.1 According to the estimation of the contamination status of the sample, select 3 sample dilutions of suitable dilution (liquid samples may include the original solution),

At the same time of 10-fold incremental dilution, each dilution was inoculated with 1ml of sample homogenate to 7.5% sodium chloride broth tube (if the inoculation amount exceeded 1mL, then use double feed 7.5% sodium chloride broth), inoculate 3 tubes of each dilution, and cultured at 36 ℃ ± 1 ℃ for 18h ~ 24h.

12.2.2 Use the inoculating loop to take one loop of the culture from the cultured 7.5% sodium chloride broth tube and transfer to the Baird-Parker plate at 36℃±1℃ for 24h~48h.

12.3 Confirmation of typical colonies

Follow 8.4.1, 8.4.3.

13 Results and reports

According to the number of test tubes confirmed to be positive for Staphylococcus aureus,

check the MPN search table (see Appendix C) and report the most likely number of staphylococcus aureus in each g (mL) sample, and expressed in MPN/g (mL).

Appendix A

Media and reagents

A.1 7.5% sodium chloride broth

A.1.1 Ingredients

Peptone	10.0g
Beef paste	5.0g
Sodium chloride	75g
Distilled water	1000mL

A.1.2 Preparation

Heat and dissolve the above ingredients, adjust the pH to 7.4±0.2, divide into aliquots, 225mL per bottle, and autoclave at 121 ℃ for 15min.

A.2 Blood agar plate

A.2.1 Ingredients

Soy flour agar (pH7.5±0.2)	100mL
Defibrillated sheep blood (or rabbit blood)	5mL~10mL

A.2.2 Preparation

Heat and melt the agar, cool to 50 ℃, add defibrinated sheep blood aseptically, shake well, and pour into the plate.

A.3 Baird-Parker agar plate

A.3.1 Ingredients

Tryptone	10.0g
Beef paste	5.0g
Yeast extract	1.0g
Sodium pyruvate	10.0g
Glycine	12.0g
Lithium chloride (LiCl·6H 2 O)	5.0g
Agar	20.0g
Distilled water	950mL

A.3.2 Mixing method of bacteria enhancer

50mL of 30% yolk saline was mixed with 10mL of 1% potassium tellurite solution sterilized by 0.22μm pore size filter membrane, store in the refrigerator.

A.3.3 Preparation

Add all the ingredients to distilled water, heat and boil until completely dissolved, and adjust the pH to 7.0±0.2. Divided into aliquots,per bottle. 121℃ high pressure sterilize for 15min. Heat the

melted agar immediately before use, cool to 50 ℃, and add the yolk potassium tellurite bacterium preheated to 50 ℃ for every 95ml culture, shake and pour into the plate. The medium should be dense and opaque. Store in the refrigerator for no more than 48h before use.

A.4 Brain Heart Extract Broth (BHI)

A.4.1 Ingredients

Tryptone	10.0g
Sodium chloride	5.0g
Disodium hydrogen phosphate (12H 2 O)	2.5g
Glucose	2.0g
Ox Heart Extract	500mL

A.4.2 Preparation

Heat to dissolve, adjust the pH to 7.4±0.2, dispense 16mm×160mm test tubes, 5mL each tube 121℃ high pressure sterilize for 15min.

A.5 Rabbit plasma

Take 3.8g of sodium citrate, add 100ml distilled water, dissolve and filter, bottle, and autoclave at 121 ℃ for 15min. Rabbit plasma preparation: take one part of 3.8% sodium citrate solution, plus 4 parts of rabbit whole blood, mix well and let it stand (or centrifuge at 3000r/min for 30min) to make the blood cells drop, get plasma.

A.6 Phosphate buffer

A.6.1 Ingredients

Potassium dihydrogen phosphate (KH2PO4)	34.0g
Distilled water	500mL

A.6.2 Preparation

Stock solution: Weigh 34.0 g of potassium dihydrogen phosphate dissolved in 500ml distilled water, adjust PH to 7.2 with1mol/L sodium hydroxide solution about 175ml, diluted with distilled water to 1000ml and stored in the refrigerator.

Diluent: Take 1.25ml of the stock solution, dilute it to 1000ml with distilled water, and aliquot it in a appropriate container, autoclave at 121℃ for15min.

A.7 Nutritional agar small slope

A.7.1 Ingredients

Peptone	10.0g
Beef paste	3.0g
Sodium chloride	5.0g
Agar	15.0g~20.0g
Distilled water	1000mL

A.7.2 Preparation

Dissolve all components except agar in distilled water, add about 2ml of 15% sodium hydroxide solution and adjust the pH to 7.3±0.2. Add agar, heat and boil to dissolve the agar, divide into 13mm×130mm test tubes, autoclave at 121 ℃ for 15min.

A.8 Gram staining solution

A.8.1 Crystal violet staining solution

A.8.1.1 Ingredients

purple crystal	1.0g
95% ethanol	20.0mL
1% ammonium oxalate aqueous solution	80.0mL

A.8.1.2 Preparation

The crystal violet was completely dissolved in ethanol, and then mixed with ammonium oxalate solution.

A.8.2 Gram's iodine solution

A.8.2.1 Ingredients

Iodine	1.0g
Potassium iodide	2.0g
Distilled water	300mL

A.8.2.2 Preparation

Mix the iodine and potassium iodide first, add a little distilled water and shake well. After completely dissolved, add distilled water to 300mL.

A.8.3 Sand yellow counter staining solution

A.8.3.1 Ingredients

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

A.8.3.2 Preparation

Dissolve sand yellow in ethanol and then dilute with distilled water.

A.8.4 Dyeing method

a) The smear is fixed on the flame, and dropwise the crystal violet dye solution, dyed for 1 min, and washed with water.

b) Add Gram's iodine solution dropwise for 1min and wash with water.

c) Add 95% ethanol dropwise to decolorize for about 15s~30s, until the staining solution is washed away, do not excessively decolorize, wash with water.

d) Add redyeing solution dropwise, redyeing for 1 min, wash with water, wait for drying and microscopic examination.

A.9 Sterile saline

A.9.1 Ingredients

Sodium chloride	8.5g
Distilled water	1000mL

A.9.2 Preparation

Weigh 8.5g of sodium chloride dissolved in 1000ml of distilled water, autoclave at 121 ℃ for 15min.

Appendix B

Staphylococcal enterotoxin test

B.1 Reagents and materials

Unless otherwise specified, the reagents used are of analytical grade, and the test water should meet the requirements of GB/T6682 for primary water.

B.1.1 A, B, C, D, E type Staphylococcus aureus enterotoxin typing ELISA detection kit.

B.1.2 pH test paper, the range is 3.5~8.0, the accuracy is 0.1.

B.1.3 Tris buffer at 0.25mol/l, pH 8.0: Dissolve 121.1 g of Tris in 800ml of deionized water and cool for the room temperature, add 42ml concentrated HCL to adjust the pH to 8.0.

B.1.4 Phosphate buffer pH 7.4: Weigh 0.55g of NaH2PO4·H2O (or NaH2 PO4·2H 2 O 0.62g), Na2HPO4·2H2O 2.85g (or Na2HPO4·12H2O 5.73g), NaCl 8.7g dissolved in 1000mL distilled water, fully mixed.

B.1.5 Heptane.

B.1.6 10% sodium hypochlorite solution.

B.1.7 Enterotoxin production medium

B.1.7.1 Ingredients

Peptone	20.0g
Pancreatic digested casein	200mg (amino acid)
Sodium chloride	5.0g
Dipotassium hydrogen phosphate	1.0g
Potassium dihydrogen phosphate	1.0g
Calcium chloride	0.1g
Magnesium sulfate	0.2g
Niacin	0.01g
Distilled water	1000mL

pH7.3±0.2

B.1.7.2 Preparation

Mix all ingredients in water, adjust pH after dissolution, autoclave at 121 ℃ for 30min.

B.1.8 Nutritional agar

B.1.8.1 Ingredients

Peptone	10.0g

Beef paste	3.0g
Sodium chloride	5.0g
Agar	15.0g~20.0g
Distilled water	1000mL

B.1.8.2 Preparation

Dissolve all components except agar in distilled water, add 15% sodium hydroxide solution to adjust the pH to 7.3±0.2. Add agar and heat to boil to dissolve the agar. Dispense flask and autoclave at 121 ℃ for 15min.

B.2 Instruments and equipment

B.2.1 Electronic balance: 0.01g.

B.2.2 Homogenizer.

B.2.3 Centrifuge: speed 3000g~5000g.

B.2.4 Centrifuge tube: 50mL.

B.2.5 Filter: The membrane pore size is 0.2μm.

B.2.6 Micro sampler: 20μL~200μL, 200μL~1000μL.

B.2.7 Micro multichannel sampler: 50μL~300μL.

B.2.8 Automatic plate washer (optional).

B.2.9 Microplate reader: wavelength 450nm.

B.3 Principle

This method can be completed by A, B, C, D, E type Staphylococcus aureus enterotoxin typing enzyme-linked immunosorbent assay kit. The method basis on the enzyme-linked immunosorbent reaction (ELISA). Wells A~E of each microwell strip of 96-well microplate were coated with A, B, C, D, E staphylococcal enterotoxin antibody, hole H is positive quality control, has been coated with mixed type staphylococcal enterotoxin antibody, F and G holes are negative control, coated with antibodies from non-immunized animals. If there is staphylococcal enterotoxin in the sample, the free staphylococcal enterotoxin is coated with each microwell The specific antibody binds to form an antigen-antibody complex, and the remaining unbound components are washed away during the plate washing process, the antigen-antibody complex is then combined with Peroxidase label (secondary antibody) is bound, unbound enzyme label is washed away during the plate washing process, enzyme substrate and color reagent are added and incubated, the enzyme on the enzyme label catalyzes the decomposition of the substrate, turning the colorless developer into blue, adding the reaction termination solution can change

the color from blue to yellow, and the enzyme reaction is stopped finally, the absorbance value of the microporous solution is measured with a microplate reader at a wavelength of 450nm, and the staphylococcal enterotoxin in the sample was directly proportional to the absorbance value.

B.4 Detection steps

B.4.1 Method for detecting staphylococcal enterotoxin from the culture of isolated strains

The strain to be tested was inoculated with a nutrient agar slant (test tube 18mm×180mm) and cultured at 36 ℃ for 24h, and the colonies were washed with 5ml physiological saline. Pour into 60ml poison production medium, shake at 36 ℃ for 48h, shaking speed is100times/min, suction the bacterial solution and centrifuge at 8000r/min for 20min, heating at 100 ℃ for 10min, taking the supernatant, and taking 100μL of the diluted sample for testing.

B.4.2 Methods for extracting and detecting staphylococcal toxins from food

B.4.2.1 Milk and milk powder

Dissolve 25g of milk powder in 125mL, 0.25M, pH 8.0 Tris buffer, after mixing, follow the same steps as liquid milk, the milk was centrifuged at 15°C and 3500g for 10 minutes. The fat layer formed on the surface is removed to become skimmed milk. Dilute it with distilled water to 1:20. Take 100ul of the diluted sample solution for testing.

B.4.2.2 Foods with a fat content not exceeding 40%

Weigh 10g of the sample and pulverize it, add 15ml of pH 7.4 PBS solution for homogenization. Shake for 15 minutes. Centrifuge for 10min at 15℃ at 3500g. If necessary, remove the upper fat layer. Take the supernatant for filtration and sterilization. Take 100ul of the filtrate for testing.

B.4.2.3 Foods with a fat content exceeding 40%

Weigh 10g of the sample and pulverize it, add 15mL of pH 7.4 PBS solution for homogenization. Shake for 15 minutes. Centrifuge for 10min at 3500g at 15℃. Pipette 5mL of the upper suspension, transfer to another centrifuge tube, then add 5mL of heptane, and mix well for 5min. And at 15℃, centrifuge at 3500g for 5min. The upper organic phase (heptane layer) was discarded, taking care not to leave heptane in the process. Lower water phase layer is filtered and sterilized. Take 100ul of the filtrate for testing.

B.4.2.4 Other foods may refer to the above food processing methods as appropriate.

B.4.3 Testing

B.4.3.1 All operations should be performed at room temperature (20 ℃～25 ℃), A, B, C, D, E Staphylococcus aureus enterotoxin typing all reagents in the ELISA test kit should be raised to room temperature before use. Draw different reagents and sample solutions during the

measurement the tips should be replaced when used, and the used tips and waste should be soaked in 10% sodium hypochlorite solution overnight before treatment.

B.4.3.2 Insert the required number of microwell strips into the frame (one microwell strip is required for one sample). Add the sample solution to A~G of the microwell strip100ul per well. Add 100ul of positive control to well H, pat the microplate with hand to mix well, seal the microwell with adhesive paper to prevent the solution volatilize and incubate at room temperature for 1h.

B.4.3.3 Pour the liquid in the hole into a container containing 10% sodium hypochlorite solution, and tap on the absorbent paper several times to ensure that no residue remains in the hole liquid. Inject 250ul of washing solution into each well with a multi-channel sampler, then pour off and pat dry on absorbent paper. Repeat the above washing operation 4 times. This step can also be completed by an automatic plate washer.

B.4.3.4 Add 100μL of enzyme-labeled antibody to each well, pat the microplate with hands to mix well, and incubate at room temperature for 1h.

B.4.3.5 Repeat the plate washing procedure of B.4.3.3.

B.4.3.6 Add 50ul of TMB substrate and 50ul of chromogenic agent to each microwell, gently mix and incubate at room temperature in a dark place away from light for 30min.

B.4.3.7 Add 100μL of 2mol/L sulfuric acid stop solution, pat gently to mix, measure with a microplate reader at 450nm within 30min, measure the OD value of each microwell solution.

B.4.4 Calculation and presentation of results

B.4.4.1 Quality control

The OD value of the positive test result should be greater than 0.5, and the OD value of the negative control should be less than 0.3. If the above requirements cannot be met at the same time, the test results are not recognized. For positive results, the interference of endogenous peroxidase should be excluded.

B.4.4.2 Calculation of critical value

The F and G holes of each micropore strip are negatively controlled, and the average value of the two negatively controlled OD values plus 0.15 is the critical value.

Example: Yin nature control 1=0.08

Negative control 2=0.10

Mean=0.09

Critical value=0.09+0.15=0.24

B.4.4.3 Expression of results

A sample with an OD value less than the critical value is judged to be negative, expressed as a certain type of Staphylococcus aureus enterotoxin is not detected in the sample. The OD value is equal or above the critical value are judged as positive, which means that a certain type of Staphylococcus aureus enterotoxin is detected in the sample.

B.5 Biosecurity

Since there is no exclusion of other potentially infectious substances in the sample, it is necessary to strictly follow GB19489 "Requirements" for waste disposal.