



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

GB 4789.10-2016

**National food safety standard - Food microbiological
examination - Staphylococcus aureus
(English Translation)**

Issued on: December 23, 2016

Implemented on: June 23, 2017

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

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Foreword

This Standard replaces GB 4789.10-2010 "National food safety standard - Food microbiological examination. Staphylococcus aureus", SN/T 0172-2010

"Determination of Staphylococcus aureus in foods for import and export", SN/T 2154-

2008 "Determination of coagulase-positive staphylococci in import and export food -

Technique using rabbit plasma fibrinogen agar medium".

Compared with GB 4789.10-2010, the main changes of this standard are as follows.

- The enrichment fluid for test is modified to 7.5 % sodium chloride broth.

National food safety standard -

Food microbiological examination -

Staphylococcus aureus

1 Scope

This Standard specifies the examination method for Staphylococcus aureus in foods. Method I of this Standard applies to the qualitative examination of Staphylococcus aureus in foods; Method II applies to the counting of Staphylococcus aureus in foods with high Staphylococcus aureus content; Method III applies to the counting of Staphylococcus aureus in foods with low Staphylococcus aureus content.

2 Equipment and materials

In addition to routine sterilization and culture equipment for microbiological laboratories, other equipment and materials are as follows.

2.7 Sterile pipette. 1 mL (with 0.01 mL scale), 10 mL (with 0.1 mL scale) or micro pipette

and tip.

3 Medium and reagents

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

3.9 Sterile saline. see A.9.

Method I Qualitative examination of Staphylococcus aureus

4 Examination procedure

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

5.1 Sample treatment

WEIGH 25 g of sample to a sterile homogeneous cup containing 225 mL of 7.5 % sodium chloride broth, HOMOGENIZE for 1 min to 2 min at 8000 r/min to 10000 r/min; or PLACE in a sterile homogeneous bag containing 225 mL of 7.5 % sodium chloride broth, BEAT with a bag mixer for 1 min ~ 2 min. If the sample is liquid, pipette 25 mL of sample to a sterile Erlenmeyer flask (it may preset an appropriate number of sterile glass beads in the flask) containing 225 mL of 7.5 % sodium chloride broth.

5.2 Enrichment

PLACE the homogenized sample above at $36\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ to culture for 18 h ~ 24 h.

Staphylococcus aureus is growing turbidly in 7.5 % sodium chloride broth.

5.3 Separation

The enriched culture is inoculated into Baird-Parker plate and blood plate. The blood plate shall be cultured at $36\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for 18 h ~ 24 h. The Baird-Parker plate shall be cultured at $36\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for 24 h ~ 48 h.

5.4 Preliminary identification

Staphylococcus aureus on the Baird-Parker plate is round, smooth-surface, raised, moist, with colony diameter of 2 mm ~ 3 mm, color of gray black to black color, shiny, and usually light (non-white) edge, around the opaque circle (precipitation) and a clear band outside. When the inoculating needle touches the colony, there is a butter-like sticky feeling.

5.5.1 Stain microscopic examination. Staphylococcus aureus is Gram's positive cocci,

arranged in grape-like, no spores, no capsule, with the diameter of about $0.5\text{ }\mu\text{m} \sim 1\text{ }\mu\text{m}$.

5.5.2 Plasma coagulase test. PICK at least 5 suspicious colonies (select all when there

are less than 5) on Baird-Parker plate or blood plate; INOCULATE them to 5 mL of BHI and nutrient agar slants respectively; CULTURE at $36\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for 18 h ~ 24 h.

5.6 Staphylococcal enterotoxin examination (optional)

For identification of suspicious food poisoning samples or Staphylococcus aureus strains producing staphylococcal enterotoxin, Staphylococcal enterotoxin shall be tested according to Annex B.

6.1 Result determination. when complying with 5.4, 5.5, it can be determined as Staphylococcus aureus.

6.2 Result report. Staphylococcus aureus is detected or not detected in 25 g (mL) of

sample.

7 Examination procedure

The examination procedure of Staphylococcus aureus plate counting method is shown

8.1.1 Solid and semi-solid sample. WEIGH 25 g of sample in a sterile homogeneous

cup containing 225 mL of phosphate buffer or saline, HOMOGENIZE at 8000 r/min ~

10000 r/min for 1 min ~ 2 min; or PLACE in a sterile homogeneous bag containing 225

mL of dilution, BEAT with a bag mixer for 1 min ~ 2 min to make into 1.10 sample homogenate.

8.1.2 Liquid sample. PIPETTE 25 mL of sample with a sterile pipette in a sterile

Erlenmeyer flask containing 225 mL of phosphate buffer or saline (an appropriate number of sterile glass beads shall be preset in the flask), MIX well, to make into 1.10 sample homogenate.

8.1.3 PIPETTE 1 mL of 1.10 sample homogenate with a 1 mL sterile pipette or micro

pipette and slowly INJECT it along the tube wall to a sterile test tube containing 9 mL of phosphate buffer or saline (note that the pipette or tip shall not touch the surface of the diluent), SHAKE the test tube or USE a 1mL sterile straw to repeatedly blow to make it evenly mixed, to make into 1.100 sample homogenate.

8.2 Sample inoculation

According to the estimation of the contamination status of the sample, select sample homogenates of 2 ~ 3 appropriate dilutions (for liquid sample, it can include the original solution).