



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

GB 4789.7-2013

**National food safety standard - Food Microbiological
Examination - *Vibrio parahaemolyticus***

食品微生物学检验 副溶血性弧菌检验

(English Translation)

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Foreword

This Standard replaces GB/T 4789.7-2008 Microbiological examination of food hygiene - Examination of *Vibrio parahaemolyticus*.

Compared with GB/T 4789.7-2008, the main modifications are as follows.

- modified the Chinese name of the standard;
- modified the scope;
- modified the equipment and materials;
- modified the mediums and reagents;
- modified the sample preparation process;
- modified the inspection procedures.

National Food Safety Standard - Food Microbiological
Examination - *Vibrio parahaemolyticus*

1 Scope

This Standard specifies the inspection method for *Vibrio parahaemolyticus* in food.

This Standard is applicable to the inspection of *Vibrio parahaemolyticus* in

food.

2 Normative references

In addition to microbial laboratory routine sterilization and culture equipment, other equipment and materials are as follows.

3 Mediums and reagents

3.1 3% sodium chloride alkaline peptone water. see A.1 of Annex A

3.2 thiosulfate-citrate-bile salt-sucrose (TCBS) agar. see A.2 of Annex A

3.3 3% sodium chloride trypsin soybean agar. see A.3 of Annex A

3.6 3% sodium chloride mannitol test medium. see A.6 of Annex A

3.7 3% sodium chloride lysine decarboxylase test medium. see A.7 of

3.8 3% sodium chloride MR-VP medium. see A.8 of Annex A

3.9 3% sodium chloride solution. see A.9 of Annex A

4 Inspection procedures

See Figure 1 for *Vibrio parahaemolyticus* test procedures.

5.1.1 After the collection of non-frozen sample, immediately store it in a 7°C

~ 10°C refrigerator, and carry out the early inspection as soon as possible.

The frozen sample should be thawed at 45°C for not more than 15 min or at 2°C to 5°C for not more than 18 h.

5.1.2 Take surface tissue, gut or gill for fish and cephalopods. For shellfish, take all contents, including shellfish and body fluids.

5.1.3 Take 25 g (mL) of the sample aseptically. Add 225 mL of 3% sodium chloride alkaline peptone. Homogenize at 8000 r/min for 1 min with a rotary blade homogenizer. Or slap with slam homogenizer for 2 min.

5.2.1 Qualitative detection

Incubate the 1.10 liquid sample prepared in 5.1.3 at $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 8 h to 18 h.

5.3.1 For all enrichment solutions with growth, use the inoculation ring in the

distance of 1 cm below the surface to take a ring of enrichment solution.

Scribe and separate on TCBS plate or Vibrio color medium plate, a test tube for a flat plate. Cultivate it at $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 18 h ~ 24 h.

5.4 Pure culture

Pick up three or more suspicious colonies. Scribe 3% sodium chloride trypsin soybean agar plate. Cultivate at $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 18h ~ 24h.

5.5.1 Oxidase test. select a single culture of pure culture for oxidase test;

Vibrio parahaemolyticus shall be oxidase positive.

5.5.4 Halophilic test. pick purely cultured single suspicious colony;

respectively inoculate 0%, 6%, 8% and 10% peptone water of different concentrations of sodium chloride; cultivate at $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 24h observation; observe the liquid turbidity. Vibrio parahaemolyticus shall not grow or grow weakly in cisplatin without sodium chloride and of 10% sodium chloride. It shall grow vigorously in peptone water of 6% sodium chloride and 8% sodium chloride.

5.6 Determination of identification

Take pure culture and respectively inoculate mannitol test medium containing 3% sodium chloride, lysine decarboxylase test medium, MR-VP medium.

6.1 Preparation

Inoculate two tubes of 3% sodium chloride trypsin soy agar test tube slope. Cultivate at $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 18h ~ 24h. Use 5% glycerol solution containing 3% sodium chloride to wash 3% sodium chloride trypsin soy agar slant culture so

as to obtain a strong bacterial suspension.

6.2 Identification of K Antigen

Take a tube of 6.1 well-prepared suspension. First, use polyvalent K antiserum for detection.

6.3 Identification of O antigen

Transfer another tube of bacteria suspension into the centrifuge tube for 1 h sterilization at 121°C. After sterilization, carry out the centrifugation at 4000 r/min for 15 min. Discard the upper liquid. Wash the precipitate three times with physiological saline, centrifugation at 4000 r/min for 15 min each time. After the last centrifugation, it shall stay a little upper liquid. Well mix the bacteria into a suspension.

7 Kanagawa test (optional)

The Kanagawa test tests whether there is a specific hemolysin on Wagstsuma agar. The positive results of Kanagawa test shall be significantly correlated with the pathogenicity of *Vibrio parahaemolyticus* isolates.

8 Results and reports

Report 25 g (mL) samples of which *Vibrio parahaemolyticus* is detected, according to the detection of suspected colony biochemical traits. If quantitative test is performed, the MPN values shall be reported on the basis of the number of test tubes that are confirmed to be positive for *Vibrio parahaemolyticus* and for each g (mL) of *Vibrio parahaemolyticus*.

Annex A

Medias and reagents

A.1 3% sodium chloride alkaline peptone water

A.2 Thiosulfate-citrate-bile salt-sucrose (TCBS) agar

A.2.1 Ingredient

A.2.2 Method