NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 4789.9-2025

National food safety standard - Food microbiological examination - Examination of Campylobacter jejuni and Campylobacter coli

食品安全国家标准 食品微生物学检验 空肠弯曲菌和结肠弯曲菌检验

Issued on: September 2, 2025 **Implemented on: March 2, 2026**

Issued by: State Administration for Market Regulation;
Standardization Administration of the PRC

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1 Scope

GB 4789.9-2025 is the Chinese national food safety standard for detecting Campylobacter jejuni and Campylobacter coli in food. Campylobacter is the leading bacterial cause of gastroenteritis in most countries that count it, and the reservoir is poultry: the organism is present in the intestines of most broiler flocks and reaches the consumer on the carcass. It is also awkward in the laboratory, being microaerophilic, thermophilic and quick to lose viability in ordinary storage, so the method has to control the atmosphere and the timing carefully. The standard sets the equipment and materials, the culture media and reagents, the testing procedure, the operating steps and the results and reporting, with an annex giving the media and reagents. It takes effect on 2 March 2026.

This Standard specifies the test methods for Campylobacter jejuni and Campylobacter coli in food. This Standard applies to the testing of Campylobacter jejuni and Campylobacter coli in food.

2 Equipment and materials

In addition to the standard sterilization, culture, and molecular detection equipment used in a microbiology laboratory, other equipment and materials are as follows:

2.1 Refrigerator. 2 °C ~ 8 °C, -18 °C ~ -20 °C.

2.2 Incubator. 25 °C ± 1 °C, 36 °C ± 1 °C, 42 °C ± 1 °C.

2.3 Flapping homogenizer.

2.4 Sterile homogenizing bag with filter.

2.5 Electronic balance. Sensitivity

0.1 g, sensitivity

0.01 g, sensitivity

0.001 g.

2.6 Constant temperature water bath or metal bath. 36 °C ~ 100 °C.

2.8 Sterile petri dishes. 60 mm and 90 mm in diameter.

2.9 Sterile hydrophilic filter membrane. 47 mm in diameter, 0.45 μm pore size.

2.10 Microaerobic culture apparatus. Provides microaerophilic conditions (5 % oxygen, 10 % carbon dioxide, and 85 % nitrogen).

2.11 Microscope. 10x ~ 1000x.

2.12 Low-temperature high-speed centrifuge. 8000 g ~ 20000 g, 4 °C.

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

2.14 Real-time fluorescence PCR instrument.

2.15 Real-time fluorescence PCR reaction tube.

2.16 Micropipettes and tips. 0.1 μL ~ 2.5 μL, 0.5 μL ~ 10 μL, 2 μL ~ 20 μL, 20 μL ~ 200 μL, 100 μL ~ 1000 μL.

2.17 Sterile centrifuge tubes. 50 mL, 100 mL, or larger capacity.

2.20 Sterile pipettes. 1 mL (with

0.01 mL graduations), 10 mL (with

0.1 mL graduations).

2.21 Microbial biochemical identification system.

2.22 Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

3 Culture media and reagents

3.1 0.1 % peptone water. See Annex A, A.1.

3.2 Sterile physiological saline. See A.2.

3.3 Campylobacter enrichment broth. See A.3.

3.4 Columbia blood agar. See A.4.

3.5 Karmalai blood agar. See A.5.

3.6 Oxidase reagent. See A.6. 3.7 3 % hydrogen peroxide (H2O2) solution. See A.7.

3.8 Indoleacetic acid paper disc. See A.8. 3.9 1 mol/L sodium thiosulfate (Na2S2O3) solution. See A.9.

3.10 Sodium hippurate hydrolysis reagent. See A.10.

3.11 Microbial biochemical identification kit or microbial biochemical identification system reagent.

3.12 Real-time fluorescence PCR testing kit or testing reagent (2 x real-time fluorescence PCR buffer, dNTPs, Taq DNA polymerase, and sterile deionized water).

3.13 Campylobacter jejuni reference strains [NPRC(S) 01.13897] or other equivalent strains.

3.14 Campylobacter coli reference strains [NPRC(S) 01.13898] or other equivalent strains.

3.15 Campylobacter upsaliensis reference strains [NPRC(S) 01.13899] or other equivalent strains.

3.16 Campylobacter lari reference strains [NPRC(S) 01.13900] or other equivalent strains.

4 Testing procedure

The testing procedure for Campylobacter jejuni and Campylobacter coli are shown in Figure 1.

5.1 Sample preparation

5.1.1 General samples Take 25 g (mL) of sample [50 g for fruits, vegetables, and aquatic products (excluding shellfish)] and add it to a homogenizing bag with filter containing 100 mL of sterile 0.1 % peptone water, homogenize using a flapping homogenizer for 1 min ~ 2 min (or thoroughly knead and oscillate for 5 min ~ 10 min), filter the homogenate into a centrifuge tube, centrifuge the filtrate at 12000 g at 4 °C for 15 min and discard the supernatant (multiple centrifugations can be performed for enrichment), resuspend the precipitate with 1 mL of sterile physiological saline, take 1 mL of the suspension and add it to 9 mL of Campylobacter enrichment broth for enrichment culture.

5.1.2 Samples of whole poultry, etc. Thoroughly rub the inside and outside of the sample with 200 mL ~ 400 mL of sterile 0.1 % peptone water, oscillate for 5 min ~ 10 min, then filter through sterile gauze or a homogenizing bag with filter into a centrifuge tube, centrifuge the filtrate at 12000 g 4 °C for 15 min and discard the supernatant (multiple centrifugations can be performed for enrichment), resuspend the precipitate with 1 mL of sterile physiological saline, take 1 mL of the suspension and add it to 9 mL of Campylobacter enrichment broth

for enrichment culture.

5.1.3 Shellfish (shelled) Take 100 g ~ 200 g of sample and place it into a sterile homogenizing bag, homogenize using a flapping homogenizer for 1 min ~ 2 min, take 25 g of the contents and add it to a homogenizing bag with filter containing 100 mL of sterile 0.1 % peptone water, homogenize using a flapping homogenizer for 1 min ~ 2 min (or oscillate thoroughly for 5 min ~ 10 min) and filter through a filter into a centrifuge tube, centrifuge the filtrate at 12000 g at 4 °C for 15 min and discard the supernatant (multiple centrifugations can be performed for enrichment), resuspend the precipitate with 1 mL of sterile physiological saline, take 1 mL of the suspension and add it to 9 mL of Campylobacter enrichment broth for enrichment culture.

5.1.4 Liquid egg white or whole egg mixture Take 25 g (mL) of sample and add it to 75 mL of sterile 0.1 % peptone water, mix the sample thoroughly, transfer it to a centrifuge tube, centrifuge at 20000 g at 4 °C for 15 min and then discard the supernatant (multiple centrifugations can be performed for enrichment), resuspend the precipitate with 1 mL of sterile physiological saline, take 1 mL of the suspension and add it to 9 mL of Campylobacter enrichment broth for enrichment culture.

5.1.5 Fresh milk and other dairy products For liquid dairy products, take 50 g directly; for solid dairy products, chop them and take 50 g, add them to a homogenizing bag with filter containing 100 mL of sterile 0.1 % peptone water, homogenize using a flapping homogenizer for 15 s ~ 30 s and then filter through a filter into a centrifuge tube. Centrifuge the liquid dairy product or filtrate at 20000 g at 4 °C for 30 min and then discard the supernatant (multiple centrifugations can be performed for enrichment), resuspend the precipitate with 1 mL of sterile physiological saline (avoid introducing the lipid layer), take 1 mL of the suspension and add it to 9 mL of Campylobacter enrichment broth for enrichment culture.

5.1.6 Samples requiring surface swab testing Wipe the surface of the test sample (at least 100 cm^2) with a sterile swab moistened with sterile physiological saline, cut off the swab tip and place it into 9 mL of Campylobacter enrichment broth for enrichment culture.

5.1.7 Water sample Take 4 L of water (for chlorinated water, add 5 mL of 1 mol/L sodium thiosulfate solution per liter of water before centrifugation), centrifuge at 12000 g at 4 °C for 30 min, discard the supernatant (multiple centrifugations can be performed for enrichment), resuspend the precipitate with 1 mL of sterile physiological saline, and take 1 mL of the suspension and add it to 9 mL of Campylobacter enrichment broth for enrichment culture.

5.2 Enrichment Loosen the cap of the screw-cap tube containing the enrichment broth, under microaerophilic conditions, incubate at 42 °C ± 1 °C for 24 h ± 2 h.

5.3 Separation

5.3.1 Membrane application After Columbia blood agar plates and Carmalai blood agar