NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 4789.12-2025

National food safety standard - Food microbiological examination - Examination of Clostridium botulinum toxin and botulinum toxin

食品安全国家标准 食品微生物学检验 产肉毒毒素梭菌及肉毒毒素检验

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1 Scope

GB 4789.12-2025 is the Chinese national food safety standard for detecting botulinum toxin-producing clostridia and botulinum neurotoxin in food. Botulism is rare and catastrophic: the toxin is the most poisonous substance known, a few nanograms will kill, and in China the classic vehicles are home-fermented bean products, cured meats and improperly canned foods. Detection is unusual in that both the organism and its toxin must be sought, and the toxin assay determines the type as well as the presence. 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 reagent formulations. It takes effect on 2 March 2026.

This standard specifies the test methods for botulinum toxin-producing Clostridium and botulinum neurotoxin in food. This standard applies to the testing for botulinum toxin-producing Clostridium and botulinum neurotoxin in food.

2 Terms and definitions

botulinum toxin-producing Clostridium A collective term for a type of Gram-positive anaerobic bacilli that form oval-shaped spores, which are generally larger than a bacterial cell and located at the subterminal end, and are capable of producing botulinum neurotoxin under suitable culture conditions. This includes Clostridium botulinum and Clostridium butyricum, among others.

3 Equipment and materials

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

3.1 Refrigerator. 2 °C~8 °C, -18 °C~-20 °C, -80 °C.

3.2 Balance. The sensitivities are

0.1 g and

0.001 g.

3.3 pH meter, pH colorimetric tubes or precision pH test paper.

3.4 Sterile scissors, tweezers, and reagent spoons.

3.5 Homogenizer or sterile mortar.

3.6 Centrifuges. 3000 g and 14000 g.

3.7 Anaerobic culture apparatus. anaerobic incubator, anaerobic tank, anaerobic bag or apparatus that provides equivalent anaerobic effect.

3.8 Constant temperature incubator. 36 °C±1 °C and 28 °C±1 °C.

3.9 Constant temperature devices. 36 °C±1 °C, 60 °C±1 °C, 80 °C±1 °C, and 100 °C±1 °C.

3.10 Microscope. 100x~1000x.

3.11 Conventional PCR instrument and real-time fluorescence PCR instrument.

3.16 Sterile pipettes. 1 mL (with a graduation of

0.01 mL), 10 mL (with a graduation of

0.1 mL), and 25 mL (with a graduation of

0.1 mL).

3.17 Sterile conical flask. 100 mL.

3.18 Petri dish. 90 mm in diameter.

3.19 Centrifuge tubes.

1.5 mL, 5 mL, and 50 mL.

3.20 Sterile filter. The filter membrane pore sizes are 0.45 μm and 0.22 μm.

3.21 PCR reaction tube.

3.22 Sterile syringe. 1 mL.

3.23 Mice. weighing 15 g~20 g. Each batch of experiments shall use KM (Kunming) or ICR mice of the same strain and single sex. The weight difference between individuals of the same sex shall not exceed ±20% of the average weight, and if they are female animals, they shall not have mated or been pregnant.

4 Culture media and reagents

Unless otherwise specified, reagents used in PCR experiments shall be analytical grade or conform to biochemical reagent standards, and water used for culture medium preparation shall meet the requirements of GB 4789.28.

4.1 Physiological saline. see A.1.

4.2 Cooked meat culture medium. see A.2.

4.27 DNA Molecular Mass Standard. Covering target band length.

5 Testing procedure

The testing procedure for botulinum toxin-producing Clostridium and botulinum neurotoxin is shown in Figure 1.

6 Operating steps

6.1 Sample preparation For solid, semi-solid, and difficult-to-pipette liquid foods, weigh 25 g aseptically and place them in a sterile homogenizing bag or sterile mortar. For block-shaped foods, chop them aseptically. For foods with high water content, add 25 mL of gelatin phosphate buffer. For foods with low water content, such as milk powder and beef jerky, add 50 mL (or more; the volume of the diluent can be adjusted according to the moisture content of the sample, generally diluted to the point where it can be inoculated) of gelatin phosphate buffer. Soak for 30 minutes, then homogenize using a sterile homogenizer for 1 min~2 min or grind with a sterile pestle to prepare a homogenate. Collect the homogenate for later use. For easily pipetted liquid foods, shake well and take 25 mL for later use. NOTE. After sampling, the remaining samples shall be refrigerated at 2 °C~8 °C until the test report is issued. Samples that test positive shall be subjected to unharmful treatment by pressure steam sterilization. It is recommended to sterilize under high-pressure steam at 121 °C for more than 30 minutes.

6.2 Botulinum neurotoxin testing

6.2.1 Preparation of the toxin solution Take about 30 mL of the sample homogenate prepared in

6.1 or 25 mL of the homogenized liquid sample and place it in a sterile centrifuge tube. Centrifuge at 3000 g for 10 min~20 min. Collect the supernatant and divide it into two portions, and place them in sterile test tubes. One portion is used directly for toxin detection, and the other portion is treated with trypsin as described in

6.2.2 and then used for toxin detection.

6.2.2 Preparation of trypsin-treated supernatant Adjust the pH of the supernatant to 6.2±

0.2 with 1 mol/L sodium hydroxide or 1 mol/L hydrochloric acid. Add 1 part trypsin (with an activity of 1.250) solution to 9 parts of the supernatant, mix well, and incubate at 36 °C±1 °C for 1 hour, occasionally and gently shaking the reaction solution.

6.2.3 Toxin detection Three mice are intraperitoneally injected with the centrifuged supernatant and the trypsin-treated supernatant, respectively,