NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 4789.30-2025

National food safety standard - Food microbiological examination - Examination of Listeria monocytogenes

食品安全国家标准 食品微生物学检验 单核细胞增生李斯特氏菌检验

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Standardization Administration of the PRC

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Foreword

This document was issued on 16 March 2025 by the State Administration for Market Regulation; Standardization Administration of the PRC and takes effect on 16 September 2025.

It is a GB standard without the /T suffix: compliance is mandatory in China.

This Standard replaces GB 4789.30-2016 "National food safety standard - Food microbiological test - Listeria monocytogenes test".

Compared with GB 4789.30-2016, this Standard has the following major changes.

- MODIFY the scope of application;

- MODIFY the culture medium and reagents, and ADD the formula of agar Listeria according to Ottaviani and Agosti;

- MODIFY the enrichment solution, selective culture medium, test procedure, identification method, etc. of the first method -- Qualitative test of Listeria monocytogenes;

- MODIFY the sample inoculation, colony count and confirmation, result count, and

result report of the second method -- Plate count method for Listeria monocytogenes;

- MODIFY the sample inoculation of the third method -- MPN count method for Listeria monocytogenes.

National food safety standards

Food microbiology test

Listeria monocytogenes test

1 Scope

GB 4789.30-2025 is the Chinese national food safety standard for detecting Listeria monocytogenes in food. Listeria is the pathogen that makes ready-to-eat food dangerous rather than merely spoiled: it grows at refrigeration temperature, which almost nothing else does, so a low count at production becomes a high one by the end of shelf life, and in pregnant women and the immunocompromised the case fatality is among the highest of any foodborne infection. The standard sets the equipment and materials, the culture media and reagents, and then the examination procedures, operating steps and reporting for the qualitative detection, the enumeration and the confirmation, in each case through selective enrichment, isolation on selective agar and biochemical and molecular confirmation. It replaces GB 4789.30-2016 and takes effect on 16 September 2025.

This Standard specifies the test methods for Listeria monocytogenes in food.

The first method of this Standard applies to the qualitative test of Listeria monocytogenes in food; the second method applies to the counting of Listeria monocytogenes in food with a high content of Listeria monocytogenes; the third method applies to the counting of Listeria monocytogenes in food with a low content of Listeria monocytogenes.

2 Equipment and materials

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

2.1 Refrigerator. 2 degrees C ~ 8 degrees C.

2.2 Constant temperature incubator. 30 degrees C +/- 1 degrees C, 36 degrees C +/- 1 degrees C, 25 degrees C ~ 30 degrees C.

2.3 Homogenizer.

2.4 Microscope. 100x ~ 1000x.

2.5 Electronic balance. the sensitivity is 0.1 g, 0.1 mg.

2.6 Conical flask. 100 mL, 500 mL.

2.7 Sterile pipette. 1 mL (with 0.01 mL scale), 10 mL (with 0.1 mL scale) or pipette (with a scale of 0.1 mL, 1 mL, 10 mL) and sterile pipette tip.

2.8 Sterile culture dish. diameter of 90 mm.

2.9 Sterile test tube. 16 mm x 160 mm.

2.10 Centrifuge. 4000 r/min.

2.11 Sterile centrifuge tube. 30 mm x 100 mm.

2.12 Sterile syringe. 1 mL.

2.13 Oil immersion lens or phase contrast microscope.

2.14 Sterile coating stick.

2.15 Listeria monocytogenes ATCC 19111 or CMCC 54004 or other equivalent strains.

2.16 Listeria innocua ATCC 33090 or other equivalent strains.

2.17 Listeria ivanovii ATCC 19119 or other equivalent strains.

2.18 Listeria seeligeri ATCC 35967 or other equivalent strains.

2.19 Staphylococcus aureus ATCC 25923 or other equivalent strains, requiring the production of beta-hemolytic ring.

2.20 Rhodococcus equi ATCC 6939 or NCTC 1621 or other equivalent strains.

3 Culture medium and reagents

3.1 Tryptic soy broth with 0.6 % yeast extract powder (TSB-YE). see A.1 in Annex A.

3.2 Tryptic soy agar with 0.6 % yeast extract powder (TSA-YE). see A.2.

3.3 Fraser enrichment broth (FB1, FB2). see A.3.

3.4 Agar Listeria according to Ottaviani and Agosti. see A.4.

3.5 PALCAM medium. see A.5.

3.6 Gram staining solution. see A.6.

3.7 SIM motility medium. see A.7.

3.8 Buffered glucose peptone water [for methyl red (MR) and acetyl methyl alcohol

(VP) tests]. see A.8.

3.9 Sheep blood agar. see A.9.

3.10 Sterile phosphate buffer. see A.10.

3.11 Sterile normal saline. see A.11.

3.12 Sugar fermentation tube. see A.12.

4 Test procedure

The qualitative test procedure for Listeria monocytogenes is shown in Figure 1.

5 Operation steps

5.1 Enrichment

Take 25 g (mL) of sample by aseptic operation, place it in a sterile homogenizing cup containing 225 mL of FB1 enrichment broth, and homogenize at 8000 r/min ~ 10000 r/min for 1 min ~ 2 min, or place it in a sterile homogenizing bag containing 225 mL of FB1 enrichment broth, and beat it with a slapping homogenizer for 1 min ~ 2 min to make a 1.10 sample homogenate. If the sample is in liquid form, it can also be mixed by oscillation or stirring. Culture at 30 degrees C +/- 1 degrees C for 24 h +/- 2 h. Mix well, pipette 0.1 mL of FB1 enrichment broth, and inoculate in 10 mL of FB2 enrichment broth. Culture at 30 degrees C +/- 1 degrees C for 24 h +/- 2 h.

5.2 Separation

Take the mixed FB2 enrichment broth and inoculate on agar Listeria according to Ottaviani and Agosti (or other equivalent Listeria chromogenic medium) plates and PALCAM medium plates respectively, culture at 36 degrees C +/- 1 degrees C for 24 h ~ 48 h, and observe the colonies grown on each plate. Typical colonies form round blue-green colonies with a diameter of 1 mm ~ 3 mm on agar Listeria according to Ottaviani and Agosti plates, surrounded by opaque halos. Typical colonies form round gray-green colonies with a diameter of 1 mm ~ 3 mm on PALCAM medium plates, surrounded by brown-black hydrolysis circles. After 48 h of culture, some colonies form black spots