# NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA



# GB 4789.42-2025

## National food safety standard - Food microbiological examination - Examination of Norovirus

食品安全国家标准 食品微生物学检验 诺如病毒检验

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## 1 Scope

GB 4789.42-2025 is the Chinese national food safety standard for detecting norovirus in food by real-time fluorescent RT-PCR. Norovirus causes more foodborne illness than any bacterium, spreads from an infectious dose of a few virions, and cannot be cultured, so detection is necessarily molecular; the difficulty is recovering enough intact RNA from a food matrix that contains inhibitors, which is why the method carries a process control virus through the whole extraction. The standard sets the equipment and materials, the media and reagents, the examination procedure, the operating steps and the results and reporting, with annexes giving the real-time RT-PCR primers and probes, the reaction system and parameters, the process control virus and its primers and probes, the preparation of external amplification control RNA, and the removal of RNase. It applies to shellfish, raw vegetables, hard surface foods, soft fruits and packaged drinking water, and takes effect on 16 September 2025.

This Standard specifies the real-time fluorescence RT-PCR examination method for Norovirus in foods. This Standard is applicable to the detection of Norovirus in shellfish, raw vegetables, hard surface foods, soft fruits and packaged drinking water.

## 2 Equipment and Materials

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

2.1 Real-time fluorescence PCR instrument.

2.2 Low-temperature centrifuge. centrifugal force > 12,000g, temperature 4 C, sleeve volume suitable for

1.5 mL/50 mL centrifuge tubes.

2.3 Sterile blades or equivalent homogenizer.

2.4 Vortex mixer.

**2.5 Electronic balance. with a division value of**

0.1 g and

0.1 mg.

2.6 Full-temperature oscillator or equivalent device. temperature range 2 C ~ 60 C.

2.7 Constant-temperature water bath or constant-temperature metal bath. temperature range is room temperature ~ 100 C.

2.8 Centrifuge. centrifugal force > 5,000g, sleeve volume suitable for 50 mL/15 mL centrifuge tubes.

2.9 Low-temperature refrigerator. 80 C.

2.10 Micropipette.

2.11 pH meter or precision pH test paper.

2.12 Sterile homogenizing bag. with a filter and a volume of 400 mL.

2.13 Sterile cotton swab.

2.14 Sterile shellfish peeling knife.

2.15 Rubber pad.

2.16 Sterile scissors.

2.17 Sterile tweezers.

## 3 Culture Medium and Reagents

Unless otherwise specified, all reagents used for the tests are analytically pure; the water used for the tests is RNase-free ultrapure water (see E.2.1).

3.1 G I and G II genome Norovirus primers and probes. see Appendix A.

3.2 Process control virus primers and probes. see Appendix C.

3.3 Process control virus. Mengo virus or Escherichia coli bacteriophage MS2, see Appendix C.

3.4 External amplification control RNA. see Appendix D.

3.5 Tris/glycine/beef extract (TGBE) buffer solution. see E.2.2. 3.6 5 x PEG/NaCl solution. see E.2.3.

3.7 Phosphate buffer solution (PBS). see E.2.4.

3.8 Chloroform/n-butanol mixture. see E.2.5.

3.9 Proteinase K solution. see E.2.6. 3.10 75% ethanol. see E.2.7.

3.11 Trizol reagent. see E.2.8.

3.12 Hydrochloric acid (HCl) solution (6 mol/L). see E.2.9.

3.13 Sodium hydroxide (NaOH) solution (1 mol/L). see E.2.10.

3.14 Pectinase. Aspergillus niger pectinase, activity 5 U/mg; or Aspergillus aculeatus pectinase, activity 3,800 U/mL.

3.15 Magnesium chloride.



## 4 Examination Procedures

See Figure 1 for the Norovirus examination procedures.

## 5 Operation Steps

5.1 Sample Processing The samples to be tested shall generally be transported in an environment below 4 C. The laboratory shall detect the samples as soon as possible after receiving them. If the samples cannot be detected within 24 hours, they shall be stored in a 80 C refrigerator, taken out before the test, and placed at room temperature, until the samples are completely thawed. Cross- contamination shall be prevented during sample processing and PCR detection. Each sample can be set up for 2 ~ 3 parallel processing. During sample processing, pay attention to replacing test consumables, such as scissors, tweezers and pipette tips to prevent cross-contamination between samples.

### 5.2.1 Soft fruits and raw vegetables

5.2.1.1 Cut 25 g of soft fruits (a general term for soft and juicy fleshy fruits developed from the ovary, such as strawberries and grapes) or raw vegetables (for example, lettuce) into small pieces of about

2.5 cm x 2.5cm under sterile conditions (if the fruit or vegetable is smaller than this volume,

it can be left uncut).

5.2.1.2 Transfer the sample pieces to a sterile homogenizing bag (with a filter and a volume of 400 mL), add 10 L of process control virus, and then, add 40 mL of TGBE buffer solution (for soft fruit samples, it is necessary to add 30 U Aspergillus niger pectinase, or 1,140 U Aspergillus aculeatus pectinase).

5.2.1.3 Use a full-temperature oscillator, at room temperature and 60 times/min, oscillate for 20 minutes. For acidic soft fruits (soft fruits with a pH of less than

7.0 after the ash from the incineration of soft fruits is dissolved in water, such as strawberries, waxberries and raspberries), during the oscillation process, it is necessary to determine the pH every 10 minutes. When the pH is lower than 9.0, use 1 mol/L NaOH to adjust the pH to 9.5.Each time the pH is adjusted, extend the oscillation time by 10 minutes.

5.2.1.4 Transfer the extracting solution to a 50 mL centrifuge tube. If the volume is relatively large, use 2 centrifuge tubes. 10,000g, 4 C, centrifuge for 30 min. Take the supernatant to a sterile test tube or conical flask and use 6 mol/L HCl to adjust the pH to 7.0.

5.2.1.5 Add 5 PEG/NaCl solution that is

0.25 times the volume of the extracting solution to the extracting solution, so that the final content of PEG/NaCl in the extracting solution is 100 g/L PEG,

0.3 mol/L NaCl. At room temperature, shake it well for 1 min, then, at 4 C and 60 times/min, oscillate it for 60 min or let it stand at 4 C overnight. 10,000g, 4 C, centrifuge for 30 min and discard the supernatant. 10,000g, 4 C, centrifuge for 5 min to compact the precipitate and discard the supernatant.

5.2.1.6 Add 500 L of PBS to re-suspend the precipitate. If the sample is raw vegetables, directly transfer the re-suspension to an RNase-free centrifuge tube, determine and record the volume of the re-suspension for subsequent RNA extraction. If the sample is soft fruit, transfer the re-suspension to a chloroform-resistant sterile centrifuge tube, add 500 L of chloroform/n- butanol mixture, conduct vortex to mix it, let it stand at room temperature for 5 min, 10,000 g, 4 °C, centrifuge for 15 min, transfer the supernatant to an RNase-free centrifuge tube, determine and record the volume of the supernatant for subsequent RNA extraction.

### 5.2.2 Hard surface foods

5.2.2.1 Use PBS to moisten a sterile cotton swab and vigorously wipe the food surface (such as carrots, melons, nuts, 50 cm2 wiping area 100 cm2), and record the wiping area. Add 10 L of process control virus to the cotton swab. If the food surface is too rough, the cotton swab may be damaged. Multiple cotton swabs may be used, but only one of the cotton swabs needs to be added with the process control virus.