



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

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**National food safety standard - Food Microbiological Testing -
Testing for Shiga toxin-producing Escherichia coli**

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1 Scope

This standard specifies the testing methods for Shiga toxin-producing *Escherichia coli* (STEC) in food.

This standard is applicable to the testing for Shiga toxin-producing *Escherichia coli* in food.

2 Equipment and materials

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

2.6 Constant temperature water bath of 50 degrees C~55 degrees C, and constant temperature metal

bath of 99 degrees C+/-1 degrees C.

2.12 Sterile conical flask with stopper: The capacities are 250 mL, 500 mL, and 1000

mL.

2.17 Micropipette and tip: 0.2 uL~2 uL, 2 uL~20 uL, 20 uL~200 uL, and 100 uL~1000

uL.

2.18 10 uL sterile inoculating loop.

2.19 1.5 mL sterile EP tubes, sterile microcentrifuge tubes, sterile 15 mL centrifuge tubes.

2.20 0.22 um pore size sterile filter membrane.

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

3.6 Brain heart infusion broth (BHI)-50% glycerol: see A.6.

3.7 0.85% sterile saline.

3.8 5% Chelex-100 solution: see A.7.

3.9 E-buffer solution: see A.8.

3.10 1 mol/L hydrochloric acid solution.

3.11 1xTE buffer: see A.9.

3.12 Sterile PBS buffer: see A.10.

5 Operating steps

5.1 Sample preparation

5.1.1 Sample storage After sampling, the sample shall be sent to the laboratory for testing as soon as possible.

If it cannot be tested in time, the sample to be tested shall be stored according to the sample storage requirements. If there are no special requirements, it shall be refrigerated in a refrigerator at 2 degrees C~5 degrees C, and the test shall be completed within 24 hours.

5.1.2 Solid or semi-solid samples Aseptically weigh and take 25 g of the test sample, add it into a sterile homogenizing bag with a filter containing 225 mL of EC (or mTSB), and homogenize with a slap homogenizer for 1 to 2 minutes; or put it into a sterile homogenizing cup containing 225 mL of EC (or mTSB), use a rotating blade homogenizer to homogenize at 8000 r/min~10000 r/min for 1 min~2 min to make a 1:10 homogeneous sample solution.

5.1.3 Liquid samples Aseptically take 25 mL of the test sample and add it into a sterile homogenizing bag or sterile conical flask containing 225 mL of EC (or mTSB), and mix thoroughly to make a 1:10 sample solution.

5.2 Enrichment Culture the sample solution prepared in 5.1 at 44 degrees C+/-1 degrees C for 18 h~24 h. At the same time, set up a control test; use the standard strain of Escherichia coli carrying stx1 and/or stx2 to be cultured in EC (or mTSB) as a positive control, and the standard strain of Escherichia coli not carrying stx1 and stx2 to be cultured in EC (or mTSB) as a negative control, and carry out a blank medium control.

5.3 Initial screening of stx virulence genes

5.3.1 Test environment and process control The environmental conditions and process

control of real-time fluorescence quantitative PCR experiments shall be carried out in accordance with the requirements specified in GB/T 27403-2008.

5.3.2 DNA template preparation in fluorescence, and the corresponding Ct values are < 30 .

5.3.3.6 Real-time fluorescence quantitative PCR result determination In compliance with the principle of result validity in 5.3.3.5, when the sample to be tested is tested, a) If the Ct values of stx1 and stx2 are ≥ 40 , it is judged as stx-negative;

b) If the Ct value of stx1 or stx2 is < 35 , and the Ct value of 16S rDNA gene is < 35 , it is judged as stx-positive;

c) If the Ct values of stx1 and stx2 are both ≥ 35 and < 40 , the real-time fluorescence quantitative PCR amplification experiment shall be repeated.

After the second amplification experiment, if the Ct value of stx1 or stx2 is < 40 and the Ct value of the 16S rDNA gene is < 35 , it is judged as stx-positive;

if the Ct values of stx1 and stx2 are ≥ 40 , it is judged as stx-negative.

For the sample judged as stx-negative, the result is reported as "No Shiga toxin-producing *Escherichia coli* is detected in the 25 g(mL) of the sample" according to the process.

The sample enrichment solution judged as stx-positive shall be subjected to acid treatment and immunomagnetic bead enrichment treatment respectively according to the provisions of 5.4 and 5.5.

5.4 Acid treatment for enrichment solution Pipette 450 μ L of stx-positive sample enrichment solution confirmed in 5.3 into a 1.5 mL sterile EP tube, centrifuge at 10000g for 2 min, discard the supernatant, and resuspend the bacterial precipitate in 450 μ L of E-buffer solution; add 25 μ L of 1 mol/L hydrochloric acid (pH 2.0~2.5), shake at room temperature for 1 hour, streak inoculate on STEC chromogenic medium or MacConkey agar medium plate with 10 μ L sterile inoculation loop, and culture at 36 degrees C $\pm$ 1 degrees C for 18 h~24 h.

Pipette 100 μ L of the above acid treatment solution to 900 μ L of the E-buffer solution to dilute it, vortex and mix, then use a 10 μ L sterile inoculation loop to inoculate it on the STEC chromogenic medium or MacConkey agar medium plate, and incubate at 36 degrees C $\pm$ 1 degrees C for 18 h~ 24 h.

5.5 Immunomagnetic bead enrichment processing for 7 important serotypes of STEC

5.5.1 In addition to the acid treatment described in 5.4, the enrichment solution of the sample judged as stx-positive in 5.3 also needs to be enriched with immunomagnetic beads at the same time. According to the instructions provided by the manufacturer, 7 important serotypes (O26, O45, O103, O111, O121, O145, O157) STEC are enriched, and standard strains with corresponding serotypes are set as positive controls. When there is a deviation between the manufacturer's instructions and the following description, operate according to

the manufacturer's instructions and use one pipette for each sample to avoid cross-contamination.

5.5.2 Number the microcentrifuge tubes according to the sample and standard strain.

Use 7 microcentrifuge tubes for each sample (one tube for each of the 7 types of immunomagnetic beads) and place them on the magnetic plate rack. Mix the 7 STEC immunomagnetic bead suspension reagents by gentle shaking, then add them to the corresponding numbered microcentrifuge tubes.

5.5.3 Pipette 8 mL of stx-positive enrichment solution into a 15 mL centrifuge tube, centrifuge at 10,000 g for 3 min, and discard the supernatant; add 3 mL of sterile PBS to resuspend the precipitate and vortex to mix, centrifuge at 10,000 g for 3 min, repeat the PBS washing step 2 to 3 times, discard the supernatant, and set aside the bacterial precipitate for later use.

5.5.4 Add 800 μ L of sterile PBS buffer to resuspend the bacterial precipitate produced in 5.5.3, vortex and mix thoroughly, then pipette 100 μ L of the bacterial suspension into the 7 microcentrifuge tubes with immunomagnetic beads mentioned in 5.5.2, and cover the tubes. Change the pipette tip for each sample, and the standard strain must be tested separately from the sample to avoid cross-contamination. Each sample needs to be enriched with 7 serotypes of immunomagnetic beads.

5.5.5 Binding: Invert and mix several times. Incubate on a mixer at 37 °C for 60 min, with a gentle speed setting to allow the target bacteria to fully contact with the immunomagnetic beads.

5.5.6 Capture: After the incubation, insert the magnetic plate into the magnetic plate rack to capture the magnetic beads. Keep tilting the magnetic plate rack within 3 minutes to ensure that all the immunomagnetic beads in the suspension and on the lid are collected, and round or oval brown aggregates can be seen in the middle of the wall of the microcentrifuge tube.

5.5.7 Magnetic bead purification: Carefully open the centrifuge tube cover and gently remove the supernatant from the opposite side of the immunomagnetic bead aggregates.

When the liquid level is close to the immunomagnetic bead aggregates, the liquid shall be slowly sucked away until the liquid level is away from the vicinity of the aggregates to ensure that the immunomagnetic bead aggregates are not sucked away. If the drawn supernatant contains magnetic beads, put it back into the microcentrifuge tube and repeat step 5.5.6. This step is a critical step in purification and needs to be completed. Cultures that produce acid or not on the triple sugar iron slant, produce acid on the bottom layer, are indole-positive, and are H₂S-negative and urease-negative, are *Escherichia coli*. Cultures that do not produce acid on the bottom layer of the triple sugar iron slant, or are positive for any of H₂S, KCN, and urease, are not *Escherichia coli*.