GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 4789.36-2016

National Food Safety Standard - Microbiological Examination of Food Hygiene Examination of Escherichia Coli O157:H7/NM

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Issued by: National Health and Family Planning Commission of the People's Republic of China;
China Food and Drug Administration.

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Foreword

This Standard replaces GB/T 4789.36-2008, *Microbiological Examination of Food Hygiene – Examination of Escherichia Coli O157:H7/NM.*

Compared with GB/T 4789.36-2008, the major changes of this Standard are as follows:

-- the standard name is modified into "*National Food Safety Standard – Microbiological Examination of Food Hygiene – Examination of Escherichia Coli O157:H7/NM.*";

-- the application scope of the standard is modified;

-- the apparatus and materials are modified;

-- the text descriptions of culture media and biochemical reactions are modified;

-- "Method II The Principle of the Immunomagnetic Beads Capture Method" is deleted;

-- "Method III Full-automatic Enzyme-linked Fluorescence Immunoassay Instrument Screening Method" is deleted;

-- "Method IV Full-automatic Pathogenic Bacteria Detecting System Screening Method" is deleted.

National Food Safety Standard - Microbiological Examination of Food Hygiene Examination of Escherichia Coli O157:H7/NM

1 Application Scope

This Standard specifies the test method of Escherichia coli o157:h7/nm in foods.

This Standard applies to the test of Escherichia coli o157:h7/nm in foods.

2 Apparatus and Materials

In addition to the conventional sterilization and culture equipment in the microbiological laboratory, other apparatus and materials are as follows:

2.1 Thermostatic incubator: 36°C ± 1°C.

2.2 Refrigerator: 2°C ~ 5°C.

2.3 Thermostatic water bath: 46°C ± 1°C.

2.4 Balance: sensitivities 0.1 g and 0.01 g.

2.5 Homogenizer.

2.6 Microscope: 10X ~ 100X.

2.7 Sterile pipettes: 1 ml (having graduates of 0.01 ml), 10 ml (having graduates of 0.1 ml) or pipettes and tips.

2.8 Sterile homogeneous cups or sterile homogeneous bags: capacity 500 ml.

2.9 Sterile culture dishes: diameter 90 mm.

2.10 pH meters or precise pH test paper.

2.11 Long-wave UV lamps: 365 nm, power ≤ 6 W.

2.12 Microcentrifuge tubes: 1.5 ml or 2.0 ml.

2.13 Magnetic plate, magnetic plate frame, specimen mixer.

2.14 Microbiological identification system.

3 Culture Media and Reagents

3.1 Modified EC broth (mEC + n): see A.1.

3.2 Modified Sorbitol-MacConkey agar (CT-SMAC): see A.2.

3.3 Trisaccharide iron agar (TSI): see A.3.

3.4 Nutrient agar: see A.4.

3.5 Semi-solid agar: see A.5.

3.6 Lauryl sulphate peptone broth – MUG (MUG-LST): see A.6.

3.7 Oxidase reagent: see A.7.

3.8 Gram stain solution: see A.8.

3.9 PBS-Tween 20 lotion: see A.9.

3.10 Potassium tellurite (analytical reagent).

3.11 Cefixime.

3.12 Chromogenic medium for Escherichia coli O157.

3.13 Diagnostic serum for Escherichia coli O157 and H7 or latex agglutination reagent for O157.

3.14 Identification kit.

3.15 Anti-E. coli O157 immunomagnetic beads.

Method I The Routine Culture Method

4 Test Procedures

See Figure 1 for the test procedures of the routine culture method of Escherichia coli O157: H7/NM.

Simmons citrate	Negative
Lysine decarboxylase	Positive (purple)
Ornithine decarboxylase	Positive (purple)
Cellobiose fermentation	Negative
Raffinose fermentation	Positive
MUG test	Negative (no fluorescence)
Dynamic test	Dynamic or non-dynamic

5.4.2.2 If the biochemical identification kit or microbiological identification system is selected, pick colonies from the nutrient agar plate, use the diluent to make the bacterial suspension of appropriate turbidity and use the biochemical identification kit or microbiological identification system for identification.

5.4.3 Virulence genes test (optional item)

When Escherichia coli O157: H7 or O157: NM is detected in the specimen, it may be tested by inoculating Vero cells or Hela cells and observing the cytopathic effects if the existence of Vero cytotoxin requires further tests; it may also be tested by using the gene probe tests or polymerase chain reaction (PCR) method for Shiga toxin genes (*stx1* and *stx2*), *eae*, *hly* and so on. If kits are used for the test of the above-mentioned genes, the product manuals shall be followed.

6 Result Reporting

Summarize the biochemical and serological test results and report whether Escherichia coli O157:H7 or Escherichia coli O157:NM is detected or not detected in the 25 g (or 25 ml) of specimen.

Method II The Immunomagnetic Beads Capture Method

7 Test Procedures

See Figure 2 for the test procedures of the immunomagnetic beads capture method of Escherichia coli O157:H7/NM.

8.2.2 Number the microcentrifuge tubes in accordance with the specimens and quality-control strains, use one microcentrifuge tube for each specimen, and then insert into the magnetic plate frame. Vibrate the Escherichia coli O157 immunomagnetic beads suspension gently on the vortex mixer, use an opener to open the cap of each microcentrifuge tube, and add 20 μL of Escherichia coli O157 immunomagnetic beads suspension in each tube.

8.2.3 Take 1 ml of mEC+n broth enrichment medium, add to the microcentrifuge tube, put on the cap, and then vibrate gently for 10 s. Replace one specimen adding tip for each specimen, and the quality-control strains shall be separated from the specimen to prevent cross-contamination.

8.2.4 Binding: at 18°C ~ 30°C, rotate the above-mentioned microcentrifuge tube together with the magnetic plate frame on the specimen mixer or use hands to rotate gently for 10 min to make Escherichia coli O157 fully contact with the immunomagnetic beads.

8.2.5 Capture: insert the magnetic plate into the concentrated magnetic beads in the magnetic plate frame. Tilt the magnetic plate frame within 3 min to ensure the immunomagnetic beads in the suspension and on the cap are fully collected. Then, rounded or oval brown accumulation becomes visible in the middle of the wall of the microcentrifuge tubes.

8.2.6 Absorption of supernatant: take one sterile elongated pipette and absorb gently the supernatant by inserting deep into the liquid from the immunomagnetic beads accumulation opposite to the liquid level. When the liquid is absorbed until the liquid level passes through the immunomagnetic accumulation, slow down to ensure no immunomagnetic beads are absorbed away. If the supernatant absorbed contains magnetic beads, place them back in the microcentrifuge tube, and repeat the procedures in 8.2.5. Replace one sterile elongated tube for each specimen.

Fall of immunomagnetic beads: the magnetic beads accumulation of some specimens, especially those specimens containing much fat is liable to fall to the bottom. When absorbing the supernatant, it is hard to prevent the loss of magnetic beads; and under such circumstances, 50 μL ~ 100 μL of supernatant may be reserved in the microcentrifuge tube. If it is operated like this in the later washing process, the effects of fat will become smaller and the purpose of full capture may also be achieved.

8.2.7 Washing: move away the magnetic plate from the frame, add 1 ml of PBS-Tween 20 lotion in each microcentrifuge tube, and rotate on a specimen mixer or use hand to rotate gently for 3 min to wash the immunomagnetic beads mixture. Repeat the above-mentioned procedures 8.2.5 ~ 8.2.7.

8.2.8 Repeat the above-mentioned procedures 8.2.5 ~ 8.2.6.

8.2.9 Immunomagnetic beads suspension: move away the magnetic plate and make the immunomagnetic beads suspend in 100 μL of PBS-Tween 20 lotion once again.

Annex A

Culture Media and Reagents

A.1 Modified EC broth (mEC+n)

A.1.1 Ingredients

Tryptone	20.0 g
Bile salt No.3	1.12 g
Lactose	5.0 g
$K_2HPO_4 \cdot 7H_2O$	4.0 g
KH_2PO_4	1.5 g
NaCl	5.0 g
Novobiocin sodium salt solution (20 mg/ml)	1.0 ml
Distilled water	1 000 ml

A.1.2 Preparation

Except novobiocin, dissolve all ingredients in the water, heat to boiling, calibrate the pH to 6.9 ± 0.1 at 20°C ~ 25°C, and load separately. Conduct autoclaved sterilization for 15 min at 121°C as standby. Prepare novobiocin stock solution of concentration 20 mg/ml and conduct disinfection by filtering. Wait until the temperature of the medium is cooled down to below 50°C, make the final concentration 20 mg/l in accordance with the proportion of 1 ml of novobiocin stock solution added to 1 000 ml of culture medium.

A.2 Modified Sorbitol-MacConkey (CT-SMAC) agar

A.2.1 Sorbitol-MacConkey (SMAC) agar

A.2.1.1 Ingredients

Peptone	20.0 g
Sorbitol	10.0 g
Bile salt No.3	1.5 g
Sodium chloride	5.0 g
Neutral red	0.03 g
Crystal violet	0.001 g
Agar	15.0 g
Distilled water	1 000 ml

A.2.1.2 Preparation

Except agar, crystal violet and neutral red, dissolve all ingredients in the distilled water, heat to boiling, calibrate the pH to 7.2 ± 0.2 at 20°C ~ 25°C, add agar, crystal violet and neutral red, boil to dissolve and load separately. Conduct autoclaved sterilization for 15 min at 121°C.

Lactose	5.0 g
Dipotassium hydrogen phosphate (K_2HPO_4)	2.75 g
Potassium dihydrogen phosphate (KH_2PO_4)	2.75 g
Lauryl sodium sulphate	0.1 g
4-Methylumbelliferyl -β-D-glucuronide (MUG)	0.1 g
Distilled water	1 000 ml

A.6.2 Preparation

Dissolve all ingredients into the distilled water, heat to boiling until fully dissolved, calibrate the pH to 6.8 ± 0.2 at 20°C ~ 25°C, load separately into test tubes with Durham tube, 10 ml each tube, and conduct autoclaved sterilization for 15 min at 121°C.

A.7 Oxidase reagent

A.7.1 Ingredients

N,N'-Dimethyl-p-phenylenediamine dihydrochloride or N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride	1.0 g
Distilled water	100 ml

A.7.2 Preparation

Prepare in small quantities for freshness, store in the refrigerator away from light, and use within 7 days.

A.7.3 Test method

Use sterile cotton swab to take single colonies, add dropwise oxidase reagent and it is oxidase test positive if it appears pink or amaranthine within 10 s. It is oxidase negative if the colour stays unchanged.

A.8 Gram stain solution

A.8.1 Crystal violet stain solution

A.8.1.1 Ingredients

Crystal violet	1.0 g
95% ethyl alcohol	20 ml
1% ammonium oxalate aqueous solution	80 ml

A.8.1.2 Preparation

Dissolve crystal violet fully into the ethyl alcohol, and then mix with the ammonium oxalate solution.

A.8.2 Gram iodine solution

A.8.2.1 Ingredients

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