



**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**

食品安全国家标准 食品微生物学检验 大肠埃希氏菌O157H7NM检验

(English Translation)

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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.

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

4 Test Procedures

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

5.1 Enrichment

Take 25 g (or 25 ml) of test specimen by sterile operation to add to a homogeneous bag containing 225 ml of mEC+n broth and homogenize continuously for 1 min ~ 2 min on a vibrating homogenizer;

5.2 Isolation

Take the mEC+n broth enriched, conduct streak inoculation on the CT-SMAC plate and Escherichia coli O157 chromogenic agar plate, culture for 18 h ~ 24 h at $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and observe the colonial morphologies. On the CT-SMAC plate, the typical colonies

Test specimen 25 g (or 25 ml) + 225 ml mEC+n broth, homogenized

5.3 Preliminary biochemical test

Take 5 ~ 10 suspicious colonies respectively from the CT-SMAC and Escherichia coli O157 chromogenic agar plates, inoculate TSI agar respectively, and meanwhile,

inoculate MUG-LST broth, use *Escherichia coli* strain (ATCC25922 or equivalent standard strains) as positive control and *Escherichia coli* O157.H7 (NCTC12900 or equivalent standard strains) as negative control, and culture for 18 h ~ 24 h at 36°C ± 1°C. Conduct oxidase test and Gram staining if necessary. I

5.4.1 Serological test

Pick analytically pure colonies on the nutrient agar plate and use O157 and H7 diagnostic serum or O157 latex agglutination reagent for the slide agglutination test. For the non-agglutination serum because of H7 factor, puncture the inoculation semi-solid agar, check the dynamics, and determined it as a nondynamic strain when all dynamic tests turn out to be negative after continuous 3 passages. If diagnostic serums or latex agglutination reagents produced by different companies are used, the product manuals shall be followed.

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;

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.

7 Test Procedures

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

8.1 Enrichment

As in 5.1.

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 μ L of *Escherichia coli* O157 immunomagnetic beads suspension in each tube.

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.

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.