



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

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**National food safety standard -- Microbiological examination of
food -- Examination of diarrheagenic Escherichia coli**

食品安全国家标准 食品微生物学检验 致泻大肠埃希氏菌检验

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Table of Contents

Foreword...	3
1 Scope...	4
2 Terms, Definitions and Abbreviations...	4
3 Apparatus and Materials...	6
4 Media and Reagents...	7
5 Examination Procedures...	8
6 Operation Steps...	10
7 Result Report...	17
Appendix A Media and Reagents...	18

1 Scope

This standard specifies the examination method of diarrheagenic *Escherichia coli* in foods.

This standard applies to the examination of diarrheagenic *Escherichia coli* in foods.

2.1 Terms and definitions

For the purpose of this standard, the following terms and definitions apply.

2.1.1 Diarrheagenic *Escherichia coli*

A kind of *Escherichia coli* can cause diarrhea-based symptom in human body via contaminated food. The common diarrheagenic *Escherichia coli* mainly includes enteropathogenic *Escherichia coli*, enteroinvasive *Escherichia coli*, enterotoxigenic

Escherichia coli, Shiga toxin-producing *Escherichia coli* (including enterohemorrhagic *Escherichia coli*) and enteroaggregative *Escherichia coli*.

2.1.2 Enteropathogenic *Escherichia coli*

The *Escherichia coli* that can cause adhesion and wiping damage of host intestinal mucosa epithelial cell and does not produce Shiga toxin. This bacterium is the main pathogenic bacteria causing infant diarrhea, with high infectivity, which can be fatal in severe cases.

2.1.3 Enteroinvasive *Escherichia coli*

The *Escherichia coli* that can invade intestinal epithelial cell to cause dysentery-like diarrhea. This bacterium is free from power, lysine decarboxylic reaction and lactose

fermentation, while with both biochemical reaction and antigenic structure approximate to that of *Shigella dysenteriae*. The key genes intruding epithelial cell are antigen encoding gene and controlling gene of invasive plasmid, such as ipaH-gene and ipaR-gene (also referred to as invE-gene).

2.1.4 Enterotoxigenic *Escherichia coli*

The *Escherichia coli* that can secrete heat-stable enterotoxin or/and heat-labile enterotoxin. This bacterium may cause infant and tourist diarrhea, which shows mild water-like diarrhea generally, and may shows serious cholera-like symptom, with low fever or no fever. Diarrhea is often self-limiting and can be self-healing in 2d~3d generally.

2.1.5 Shiga toxin-producing *Escherichia coli* (Enterohemorrhagic *Escherichia coli*)

The *Escherichia coli* that can secrete Shiga toxin and cause adhesion and wiping damage of host intestinal mucosa epithelial cell. Some Shiga toxin-producing

Escherichia coli can cause clinically hemorrhagic colitis (HC) and bloody diarrhea in human, and may be further developed to become hemolytic uremic syndrome (HUS), this kind of Shiga toxin-producing *Escherichia coli* refers to enterohemorrhagic

Escherichia coli.

2.1.6 Enteroaggregative *Escherichia coli*

Enteroaggregative *Escherichia coli* does not invade intestinal epithelial cell, but can cause intestinal fluid accumulation. It neither produces heat-stable enterotoxin/heat-labile enterotoxin nor Shiga toxin. It is only characterized by enteroaggregative adhesion on Hep-2 cell, thus also referred to as Hep-2 cell adhesive

Escherichia coli.

2.2 Abbreviations

For the purposes of this standard, the following abbreviations apply.

2.2.7 EAEC: Enteroaggregative *Escherichia coli*

2.2.8 escV: gene encoding LEE-encoded type III secretion system factor

2.2.9 eae: gene encoding intimin for *Escherichia coli* attaching and effacing

2.2.10 bfpB: bundle-forming pilus B;

2.2.11 stx1: Shiga toxin one

6.1.1 Solid or semi-solid sample

For solid or semi-solid sample, weigh 25g of examined sample by aseptic operation, add it into a homogenizing cup with 225mL of nutrient broth to homogenize by a spinning blade-type homogenizer at 8000r/min~10000r/min for 1min~2min; or add the examined sample into a homogenizing bag with 225mL of nutrient broth to homogenize by a slap-type homogenizer for 1min~2min.

6.1.2 Liquid sample

Weight 25mL of examined sample by aseptic technique, add it into an aseptic conical flask with 225mL of nutrient broth (proper amount of aseptic glass beads may be preset in the flask), and then oscillate the flask to mix them well.

6.2 Enrichment

Culture the homogeneous sample solution prepared in 6.1 at $36^{\circ}\text{C}\pm 1^{\circ}\text{C}$ for 6h. Take 10 μL of the solution, inoculate it into 30mL enterobacteria enrichment broth tube, and then culture at $42^{\circ}\text{C}\pm 1^{\circ}\text{C}$ for 18h.

6.3 Isolation

Carry out streak inoculation for the enrichment broth into MAC and EMB agar plates, culture them at $36^{\circ}\text{C}\pm 1^{\circ}\text{C}$ for 18h~24h, and then observe colony characteristics. On the MAC agar plate, the typical colony decomposing lactose is brick red to peach while the colony not decomposing lactose is colorless or light pink. On the EMB agar plate, the typical colony decomposing lactose is purple black with or without metallic luster in center while the colony not decomposing lactose is colorless or light pink.

6.4 Biochemical test

6.4.1

Select 10~20 suspicious colonies from the plates (select all colonies in case of 10 below), therein lactose-fermented, lactose non-fermented and delayed fermented colonies shall be picked up to inoculate TSI slants respectively. Simultaneously, inoculate these cultures to peptone water, urea agar (pH 7.2) and KCN broth respectively. Culture them at $36^{\circ}\text{C}\pm 1^{\circ}\text{C}$ for 18h~24h.

6.4.2

In the case that TSI slant produces acid or fails to produce acid, bottom layer produces acid, indole is positive, as well as both H₂S and urase are negative, the culture is *Escherichia coli*. In the case that TSI slant and bottom layer fails to produce acid, or any one of H₂S, KCN and urea is positive, the culture is non-*Escherichia coli*.

If necessary, gram stain and oxidase test shall be carried out. The *Escherichia coli* is gram negative bacillus and oxidase is negative.

6.4.3

If biochemical identification kit or microbial identification system is adopted, the depurated colonies shall be picked from nutrient agar plate to prepare bacterial suspension with appropriate turbidity by aseptic diluent, and then identified by biochemical identification kit or microbial identification system.

6.5.1 Adopt the colonies with biochemical reaction in conformity to *Escherichia coli* characteristic to carry out PCR confirmatory test.

Note: PCR laboratory location planning, basic working principles and attentions shall refer to the "Construction Standard of Center for Disease Control and Prevention" (JIANBIAO 127-2009) and the Appendix (Working Guideline of Clinical Gene

Amplification Examination Laboratory in Medical Institution) of "Management

Method for Clinical Gene Amplification in Medical Institution" issued by the

National Health and Family Planning Commission of the People's Republic of China (the original Ministry of Health of the People's Republic of China) (2010).

6.5.2 Apply 1μL inoculating loop to scrape the colonies cultured for 18h~24h from

nutrient agar plate or slant, suspend them in 200μL of 0.85% aseptic normal saline, sufficiently scatter and prepare bacterial suspension, centrifuge at 13000r/min for

3min, and then discard the supernatant. Add 1mL of aseptic deionized water to sufficiently mix the thallus well, and maintain in 100°C water bath or metal bath for

10min. After cooling by ice bath, centrifuge at 13000r/min for 3min, collect supernatant and dilute it by aseptic deionized water in the proportion of 1: 10, and then adopt 2μL of which to serve as the template for PCR detection. All the treated

DNA templates are directly used for PCR reaction or stored temporarily at 4°C and subjected to PCR reaction; otherwise, they shall be preserved at -20°C below for standby (within one week). Bacterial genomic extraction kit may also be adopted for extracting bacterial DNA, which shall be operated according to the instruction of bacterial genomic extraction kit.

6.5.3 For each PCR reaction, EPEC, EIEC, ETEC, STEC/EHEC and EAEC

standard strains shall be adopted as positive control. Meanwhile, *Escherichia coli*

ATCC 25922 or equivalent standard bacterial strain shall be adopted as negative control,