

ICS 67.050
CCS C 53

SN

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

SN/T 5225-2019

**Rapid detection method of five diarrhea-causing Escherichia
coli in imported and exported foods
(English Translation)**

Issued on: January 1, 1970

Implemented on: July 1, 2020

Issued by: General Administration of Customs

Table of Contents

Foreword
1 Scope
2 Normative references
3 Terms and definitions
3.1 Multiplex Polymerase Chain Reaction (MPCR) Multiplex Polymerase Chain Reaction
3.2 Diarrheogenic Escherichia coli
4 Abbreviations
5 Method summary
6 Equipment and materials
7 Medium and reagents
8 Inspection procedures
9 Operation steps
9.1 Sample preparation, enrichment culture and separation

Foreword

This standard was drafted in accordance with the rules given in GB/T 1.1-2009.

This standard was proposed and managed by the General Administration of Customs of the People's Republic of China.

Drafting organizations of this standard. Zhengzhou Customs of the People's Republic of China, PLA Center for Disease Control and Prevention, China Animal Health and Epidemiology Medical Center, Gongbei Customs of the People's Republic of China, Fuzhou Customs of the People's Republic of China.

1 Scope

This standard specifies enteropathogenic Escherichia coli (EPEC), enterohemorrhagic Escherichia coli (EHEC, Also known as Shiga toxin-producing Escherichia coli), enterotoxigenic Escherichia coli (ETEC), enteroinvasive Escherichia coli (EIEC), Intestinal-aggregating Escherichia coli (EAEC) five rapid detection methods for diarrhea-causing Escherichia coli.

This standard applies to all kinds of import and export foods and their raw materials, except for those with special inspection methods. Other products can refer to the use of this standard.

2 Normative references

The following documents are indispensable for the application of this document. For dated reference documents, only the dated version is applicable to this file. For undated reference documents, the latest version (including all amendments) is applicable to this standard.

GB/T 6682 Analytical laboratory water specifications and test methods GB 19489
Laboratory Biosafety General Requirements GB/T 27403 Laboratory Quality Control
Specification Food Molecular Biology Testing

3 Terms and definitions

The following terms and definitions apply to this document.

3.1 Multiplex Polymerase Chain Reaction (MPCR) Multiplex Polymerase Chain Reaction

Also known as multiple primer PCR or multiple PCR, it is to add more than two pairs of primers in the same PCR reaction system to amplify multiple primers at the same time.

PCR reaction of a nucleic acid fragment, the reaction principle is that the template gene sequence is first denatured by high temperature to become single-stranded.

Under suitable reaction conditions, the two primers designed according to the template sequence are respectively sent to the corresponding complementary sequence on the two strands of the template DNA.

Are annealed and combined with each other, and then the four kinds of deoxyribonucleic acid (dNTP) are used as substrates under the action of DNA polymerase to make the primers Extension, and then continue to repeat the cycle of denaturation, annealing and extension, so that the gene fragments to be amplified will be amplified geometrically.

3.2 Diarrheogenic Escherichia coli

Diarrhea-causing Escherichia coli is a type of Escherichia coli that can cause diarrhea in the human body. It is also a kind of Pathogens that contaminate food and cause human disease. According to virulence factors, pathogenic mechanism, epidemiological characteristics, it will mainly cause diarrhea in the large intestine There are 5 types of serobacteria. enteropathogenic E.coli (EPEC), enterohemorrhagic Escherichia coli Enterohemorrhagic E.coli (EHEC, also known as Shiga toxin-producing Escherichia coli), enterotoxigenic Escherichia coli (Enteroinvasive E.coli, ETEC), enteroinvasive E.coli (EIEC), intestinal aggregation adhesion Sexual Escherichia coli (enteroaggregative E.coli, EAEC).

4 Abbreviations

The following abbreviations apply to this document.

DEC Diarrheogenic Escherichia coli EPEC Enteropathogenic Escherichia coli EIEC
 Enteroinvasive Escherichia coli ETEC Enteroinvasive Escherichia coli EHEC
 Enterohemorrhagic Escherichia coli EAEC Enteroaggregative Escherichia coli escV protein
 secretion regulation gene gene encoding LEE-encoded type III secretion system factor
 bfpB bundle-forming pilus B gene bundle-forming pilus B stx1A Shiga toxin I gene Shiga
 toxin one stx2A Shiga toxin two gene elt heat-labile enterotoxin gene heat-labile enterotoxin
 invE invasive plasmid regulator astA Aggregative heat-stable enterotoxin A gene
 enteroaggregative heat-stable enterotoxin A estla heat-stable enterotoxins initially
 discovered in the isolates from pigs estlb heat-stable enterotoxins initially discovered in the
 isolates from human aggR Aggregative adhesive fimbriae regulator pic intestinal
 colonization factor gene protein involved in intestinal colonization eae gene encoding
 intimin for Escherichia coli attaching and effacing ipaH nvasive plasmid antigen H-gene
 Polymerase chain reaction dNTP deoxyribonucleoside triphosphate

5 Method summary

According to the pathogenic mechanism of diarrheal Escherichia coli, this standard detects pathogenic EPEC (bfpB, escV), EIEC (invE), ETEC (elt, estla, estlb), EHEC (escV, stx1A, stx2A), EAEC (astA, aggR, pic), using multiple PCR Technology, according to the size of the band of the PCR amplified product to determine the pathogenic type of diarrheal Escherichia coli.

6 Equipment and materials

6.1

Constant temperature incubator, 36°C ±1°C, 42°C ±1°C.

6.2

Homogenizer.

6.3

Oscillator.

6.4

Sterile homogenization cup or sterile homogenization bag; capacity 500mL.

6.5

Sterile petri dish; diameter 90 mm.

6.6

One-time inoculation loop.

6.7

Pipettes. the ranges are 1 mL, 5 mL and 10 mL respectively, and the scale is 0.1 mL.

6.8

Micropipette. 0.5 μ L ~ 2 μ L, 2 μ L ~ 20 μ L, 20 μ L ~.200 μ L.

6.9

Sterilize PCR reaction tube.

6.10

Electronic balance. Sensitivity 0.1g, 0.01g.

6.11

Constant temperature water bath. 100°C.

6.12

Refrigerated centrifuge. temperature control 4°C~8°C, maximum speed not less than 13 000 r/min.

6.13

Nucleic acid protein analyzer or ultraviolet spectrophotometer.

6.14

PCR thermal cycler.

6.15

Nucleic acid electrophoresis instrument, including power supply, electrophoresis tank, gel preparation tank (length >10 cm) and comb.

6.16

Gel imaging system.

7 Medium and reagents

Except for special instructions, all experimental reagents are of analytical grade. The experimental water meets the requirements of first-grade water in GB/T 6682.all the reagents are all aliquoted in containers that are not contaminated by DNase.

7.1

Nutrient broth. see A.1.1 in Appendix A.