

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

SN/T 5105-2019

**Real-time fluorescent RT-PCR detection method for border port
rotavirus (group B)**

国境口岸轮状病毒（B组）实时荧光RT-PCR检测方法
(English Translation)

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Foreword

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

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

This standard was drafted. Gongbei Customs of the People 's Republic of China, Xiamen Customs of the People 's Republic of China, Shenzhen Customs of the People 's Republic of China, Shenzhen Institute of Metrology and Quality Inspection.

1 Scope

This standard specifies the targets, detection methods, and results of real-time fluorescent RT-PCR for adult diarrhea rotavirus (Group B) at border crossings.

report.

This standard applies to laboratory testing of adult diarrhea rotavirus (Group B) at border crossings.

2 Normative references

The following documents are essential for the application of this document. For dated references, only the dated version applies to this article Pieces. For undated references, the

latest version (including all amendments) applies to this document.

GB 1849 General requirements for laboratory biosafety SN/T 1720 Surveillance regulations for rotavirus infection at entry-exit ports Application of polymerase chain reaction (PCR) in clinical diagnosis of WS/T 230 Directory of human-borne infectious pathogenic microorganisms (formerly Ministry of Health of the People's Republic of China)

3 Terms and definitions

The following terms and definitions apply to this document.

It is the main pathogen that causes diarrhea in humans, mammals and birds. Rotavirus has seven serogroups (A to G), of which A, B, and C groups can Causes diarrhea in humans and animals. Group A rotavirus is the most common and it is the leading cause of diarrhea in infants and young children. Subject of group B rotavirus infection it has an age limit and mainly infects young adults aged 15-45 years, but it can also infect the elderly and infants, so it is also called adult diarrhea rotavirus (Adultarearotavirus, ADRV).

A technology that combines reverse transcription (RT) of RNA and polymerase chain amplification (PCR) of cDNA. This standard uses a one-step inversion Transcription polymerase chain reaction (RT-PCR). In one-step RT-PCR, first using RNA as a template and using downstream specific primers, Transcription and synthesis of complementary DNA (cDNA) under the action of RNA-dependent DNA polymerase (reverse transcriptase). This step is called "reverse transcription".

Then, using cDNA as a template, PCR amplification was performed by DNA polymerase using upstream and downstream specific primers.

Eventually, a specific region (target fragment) of the RNA molecule is amplified several million times.

Using fluorescent dyes or fluorescently-labeled specific fluorescent probes, the products of PCR amplification after reverse transcription are labeled and tracked.

The accumulation of optical signals enables real-time online monitoring of the entire PCR reaction process, and the corresponding software can be used for qualitative and quantitative analysis of the results.

SN/T 5105-201919 ?????, ???? , ???? , ??

The fluorescent probe used in this standard is a molecular beacon probe. Molecular beacon probe is a fluorescently labeled oligo the nucleotide chain usually contains 25 to 35 nucleotides. Structurally, the molecular beacon probe can be divided into three parts, namely the circular region, the stem region, and the fluorescence and quenching groups. Loop region. generally composed of 15 to 30 nucleotides, which can specifically bind to the target molecule; stem region. oligonucleotide chain A 5 to 8 base pair DNA double-stranded

structure formed by complementary pairing of nucleic acid sequences at both ends, which can be used in the process of binding molecular beacons to target molecules.

Reversible dissociation occurs; fluorophores and quenching groups. fluorescent reporter groups (such as FAM) are generally attached at the 5' end, and fluorescent quenching groups (such as Dabcyl) is usually connected at the 3' end. In the free state, the molecular beacon has a hairpin structure. At this time, because the fluorescent group and the quenching group rely on each other, Nearly, the fluorescence is quenched; when combined with the target sequence, the spatial configuration of the molecular beacon changes, which makes the distance between the fluorescent group and the quenching group larger at both ends of the extended probe, fluorescence was restored. Molecular beacon technology has high specificity, and is easy to operate and sensitive High degree, especially it can be used for real-time quantitative detection, and can even be used for in vivo analysis.

In fluorescent PCR, the number of cycles that the fluorescence signal in each reaction tube goes through when it reaches a set threshold.

4 Abbreviations

The following abbreviations apply to this document.

DEPC. Diethylpyrocarbonate (diethylpyrocarbonate) PAGE. polyacrylamide gel electrophoresis (polyacrylamide intermediates) HPLC. High Performance Liquid Chromatography (HIGHPERFORMCELLIQIDROCHROMOTOGRAPHY) bp. basepair FAM. 6-carboxy-fluorescein DABCYL. 4,4-dimethylamino-azobenzene-4'-carboxylic acid (4,4-dimethylamino-azobenzenene-4'-carboxylic

5 Detection object

5.1

Patients with acute gastroenteritis who have symptoms of vomiting, diarrhea, and fever, and suspected diarrhea virus infection, were found at border crossings.

5.2

Other personnel applying for inspection.

6 Biosafety and PCR anti-pollution requirements

Laboratory biosafety shall comply with the provisions of GB 1849 and "List of Pathogenic Microorganisms Transmitted by Humans". PCR pollution prevention measures the provisions of WS/T 230 are implemented.

7 Main instruments

The main equipment is as follows.

- Class B biological safety cabinet;
 - Desktop high-speed refrigerated centrifuge (maximum centrifugal force above 12,000 x g);
 - Vortex oscillator;
 - Refrigerator. 4 ° C, -20 ° C and -70 ° C;
 - Ultra-clean workbench;
- SN/T 5105-201919 ?????, ?????, ??
- Mini centrifuge;
 - Fluorescence quantitative PCR instrument;
 - Electronic balance (d = 1mg);
 - microwave oven;
 - Electrophoresis;
 - autoclave;
 - Ultra-pure water or double steamer;
 - A set of micro-adjustable pipettes (including the following 5 specifications. 10μL, 20μL, 100μL, 200μL, 1mL).

8 Main reagents

8.1

DEPC water without RNase.

8.2

Primers and probes. RT-PCR primers should be purified above PAGE, probes should be purified by HPLC, primers and probe sequences See 9.2.3.1.

8.3

One-step fluorescence RT-PCR basic reagents. Bao Bioengineering (Dalian) Co., Ltd. One Step Primer Scrit TMT-PCR (PerfreetTime) 1).

1) This information is provided for the convenience of users of this standard and does not indicate endorsement or recommendation of the product. If other equivalent products have the same Effect, you can use these equivalent products.