

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

SN/T 5108-2019

**Method for detecting HRMA of rotavirus, astrovirus, norovirus
and Zaru virus in border port**

国境口岸轮状病毒、星状病毒、诺如病毒、札如病毒四联HRMA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, People's Republic of China, Shenzhen Institute of Metrology and Quality Inspection.

1 Scope

This standard specifies the inspection objects, methods, and conclusions of four types of viruses. rotavirus, astrovirus, norovirus, and zaravirus.

Results reporting, biosafety requirements and determination of positive results.

This standard is applicable to laboratory tests for rotavirus, astrovirus, norovirus, and zaravirus carried by entry and exit personnel.

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 Application of polymerase chain reaction (PCR) in clinical diagnosis of WS/T 230 Technical requirements of SN/T 1193 genetic testing laboratory SN/T 1720 Surveillance regulations for rotavirus infection at entry-exit ports Directory of human-borne infectious pathogenic microorganisms (developed by the Ministry of Health of the People's Republic of China in 2006)

3 Terms and definitions

The following terms and definitions apply to this document.

Due to the composition of the fragments, they encode six structural proteins (VP1-VP4, VP6, VP7) and six non-structural eggs (NSP1-NSP6). Rota There are seven serogroups (A to G), among which groups A, B, and C can cause diarrhea in humans and animals.

The group length is about 6.8kb, and from the 5 ' end to the 3 ' end, a 5 ' non-coding region (5 ' non-coding data, NCR) of about 85 nucleotides is included.

Open reading frames OFLF1a, OR1b, and ORF2, a 3 'non-coding region of about 80 nucleotides and an approximately 30 nucleotides Polyadenylation tail. Astrovirus is an important pathogen that causes acute diarrhea in infants and young children, the elderly, people with low or lack of immune function.

7.6KB, including 3 open reading frames (ORFs) of ORFS1, ORFS2, and ORFS3. Norovirus can be divided into Five types. GI, GI, GIII, GI, and GV. Among them, GI, GI, and GI infect people and cause acute non-bacterial infection in adults and infants SN/T 5108-201919 ????? ???? , ???? ,?

The important pathogen of gastroenteritis is epidemic worldwide and infection can occur all year round.

Code box, ORF1 and ORF2. SV can be divided into 5 genotypes, namely GI, GII, GIII, GIV, and GV. Except for GIII-infected pigs, other all four types are infected.

In terms of PCR technology or real-time fluorescence PCR technology, the operation steps are greatly simplified, and the use of expensive probes is not required, and the time and cost are also reduced.

It is much lower, and the sample is directly analyzed by HRMA after PCR amplification. No transfer is required, and the closed-tube operation is truly realized, which reduces the pollution.

Risk of infection.

4 Abbreviations

The following abbreviations apply to this document.

- bp. basepair, base pair;
- DEPC. diehydrocarbonate, ethylene pyrocarbonate;
- HRMA. highsolutioning moments, analysis of high-resolution melting curves;
- PAGE. polyacrylamide electrotropisis, polyacrylamide gel electrophoresis.

5 Detection object

5.1

Patients with acute gastroenteritis suspected of diarrhea virus infection with symptoms of vomiting, diarrhea and fever, found at the port of entry and exit.

5.2

Other personnel applying for inspection.

6 Biosafety and PCR anti-pollution requirements

Laboratory biosafety should comply with the provisions of GB 1849. According to the Directory of Human Pathogens, the harm levels are all in the third category, and relevant operations can be carried out in the biosafety secondary laboratory. The anti-pollution measures of PCR are in accordance with WS/T 230 and Implementation of the provisions of Sn/T 1193.

7 Main instruments

The main instruments used in this method are as follows.

- Secondary biological safety cabinet;
- Desktop high-speed refrigerated centrifuge (maximum centrifugal force above 12,000rpm);
- Vortex oscillator;
- Refrigerator. 4 ° C, -20 ° C and -70 ° C;
- Ultra-clean workbench;
- Mini centrifuge;
- Rotor-GenenQ PCR thermal cycler (with high-resolution melting curve analysis module);

Note: This information is provided for the convenience of users of this standard and does not indicate endorsement and recommendation of the product. If other equivalent products have the same SN/T 5108-2019 ?????, ?????, ?????,?

Effect, you can use these equivalent products.

- microwave oven;
- autoclave;
- Ultra-pure water or double steamer;
- A set of micro-adjustable pipettes (including the following 5 specifications. 2.5 μ L, 20 μ L, 100 μ L,.200 μ L, 1 mL).

8 Main reagents

8.1

DEPC water without RNase.

8.2

RT-PCR primers should be purified above PAGE level. For the primer sequence, see 9.2.3.1.

8.3

RT-PCR basic reagent. Prime Biological Engineering (Dalian) Co., Ltd.

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

8.4

EvaGreen fluorescent dye.

9.1 Sample Collection

Collection of fecal specimens and vomit specimens was performed in accordance with Sn/T 1720.

9.2.1 Sample pretreatment

Take a stool sample or vomit with a size of about 100 mg of soybeans, or a 100 μ L water sample, add 900 μ L of physiological saline, shake vigorously and mix to make a 10% suspension; then centrifuge at 8000 r/min at 4 ° C for 5 min; To a sterilized clean centrifuge tube. Not immediately During the test, the samples were stored frozen in a -70 ° C ultra-low temperature refrigerator for future use.

9.2.2 Viral nucleic acid extraction

Take 9.2.1 100 μ L of the pretreated sample, add 1 mL of Trizol solution, shake and mix and leave it at room temperature for 5 minutes, then add.200 μ L of chloroform, cover the centrifuge tube, shake vigorously by hand and mix for 15 seconds, and leave it at room