

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

SN/T 5099-2019

**Real-time fluorescent RT-PCR detection method for Hendra
virus at border port**

**国境口岸亨德拉病毒实时荧光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. China Academy of Inspection and Quarantine, Xinjiang International Travel Health Care Center, Shenzhen Inspection and Quarantine Scientific Research Institute Institute, Guangzhou Customs, People's Republic of China.

1 Scope

This standard specifies the biosafety requirements for Hendra virus testing at border crossings, the collection, transportation and preservation of specimens, and the handling of specimens.

Real-time fluorescence RT-PCR detection method for Della virus.

This standard applies to the detection of nucleic acids of Hendra virus carried by entry-exit personnel 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. 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 WS233 General Guidelines for Biosafety in Pathogenic Microbiology Laboratories Regulations on the Management of Transportation of Highly Pathogenic Pathogenic Microorganism Bacteria (Virus) or Samples that Infect Humans Number 45) Measures for the Administration of Depository Institutions of Human-Transmitted Pathogenic Microorganisms (Poisons) (formerly the Ministry of Health of the People's Republic of China) 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 3.1 do not. Hendra virus is a zoonotic virus that was first reported in 1994 by Hendra in the suburb of Brisbane, Queensland, Australia Found to cause severe respiratory diseases. So far, human cases of HeV infection have been those in close contact with infected horses.

Therefore close contact is one of the routes of transmission of HeV. Diseases caused by HeV virus are typically characterized by severe dyspnea and high mortality the rate is also manifested in human contact infections. Humans have significant respiratory symptoms in the early onset of infection, with fever and myalgia; some appear nervous Symptoms, often with moderate encephalitis.

The real-time fluorescence RT-PCR method is based on the conventional RT-PCR, and a specific real-time fluorescence probe is added. The probe is A segment of oligonucleotide with a reporter group and a quenched real-time fluorescent group at each end. When the probe is complete, the actual emission from the reporting group the fluorescence signal is absorbed by the quenching group at the time. during PCR amplification, the 5'-3' exonuclease activity of the Ta enzyme is used to digest the probe to degrade the when the fluorescent group and the quenching group are separated, the real-time fluorescence monitoring system can receive the real-time fluorescence signal. Real-time inside each reaction tube the number of cycles that the fluorescence signal goes through when it reaches the set threshold is expressed by the value of Ct.

SN/T 5099-2019 ?????, ?????, ??????

The Ct value is 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) FAM. 6-carboxy-fluorescein (6-carboxy-fluorescein) BHZL. black hole quenching group

5 Detection object

Suspicious cases found at the port of entry and exit.

6 Laboratory biosafety and PCR anti-pollution requirements

According to GB 1849, "List of Human-Transmitted Pathogenic Microorganisms" and "Highly Pathogenic Pathogenic Microorganisms (Poison) Species that Infect Humans" the requirements of the "Regulations for the Management of Sample Transportation", the biosafety requirements of the relevant materials are as follows.

- Hendra virus damage is classified into the first category;
- Personal protection complies with GB 1849;
- Laboratories should follow GB 1849 and WS233. The operation of uncultured infectious materials should be at Biosafety III (BSL-3).
Or ABSL-3) in the laboratory, and inactivated materials in the biosafety class II (BSL-2) laboratory;
- Hendra virus infectious material transport packaging is classified as Class A, and the UNI number is UNI 2814;
- The used experimental supplies should be treated in accordance with GB 1849 for the treatment of waste;
- The packaging and transfer of suspected Hendra virus materials should comply with the former Order No. 45 of the Ministry of Health of the People's Republic of China
- PCR pollution prevention measures are implemented in accordance with the provisions of WS/T 230.

7 Instruments

The main instruments used in this standard are as follows.

- Real-time fluorescence PCR instrument;
- Ultra-clean workbench;
- Secondary biological safety cabinet;

- autoclave;
 - Low temperature high-speed centrifuge. maximum centrifugal force.2000g;
 - Vortex oscillator;
 - Refrigerator. 4 ° C, -20 ° C and -70 ° C;
 - Pipette. 10 µL, 20 µL, 100 µL, 200 µL and 1 mL; --- constant temperature water bath;
 - Sterile syringe.
- SN/T 5099-2019 ?????, ?????, ?????,?

8 Reagent

The main reagents used in this method are as follows.

- Nucleic acid extraction reagent. ZIAMPVIRALRNA Kit from Germany Qiagen Company extracted virus nucleic acid 1);
- Real-time fluorescent RT-PCR reagent. Ag-Path-IDTM OnsiteRT-PCR Kit, product of American company Amion 2);

1) and 2) are provided by the designated unit. This information is provided for the convenience of users of this standard and does not indicate approval of the product. If other equivalent products Have the same effect, you can use these equivalent products.

- DEPC water without RNase;
- Primer and probe sequences are shown in Table 1.

Table 1 Primers and probes for real-time fluorescence RT-PCR detection of Hendra virus

Primer/Probe Name	Sequence (5'-3 ')
HeV-F	CTTCGACACAGAGACGACGACACAA
HeV-R	CCCACGCTCGGTCTGAGCACAATAAT
HeV-P	FM-TGCGCATCTCTCTCTCTCGCT-CGRAM-TAMRA

9.1 Collection of specimens

3 to 5 mL of venous blood was collected aseptically within 5 days after onset, centrifuged at 500 g for 10 min, and the serum was collected in a 2 mL sterile screw plastic tube.

The collected samples were numbered and registered with a low temperature oil-resistant marker.

9.2 Preservation of specimens

The collected specimens should be stored at 4 ° C. The biosafety requirements for sample storage should be in accordance with