

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

SN/T 5100-2019

**Detection method for Kyasanur Forest disease virus by
real-time fluorescence RT-PCR at frontier ports**

**国境口岸卡萨诺尔森林病病毒实时荧光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, Hangzhou Fuji Biotechnology Co., Ltd., Xinjiang International Travel Health Care center.

1 Scope

This standard specifies the biosafety requirements for the detection of Casanor forest disease virus at border crossings, the collection, transportation and preservation of specimens, and specimens.

Processing, real-time fluorescent RT-PCR detection method for Casanor forest disease virus.

This standard applies to the detection of nucleic acids carried by persons entering or leaving the country at the 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 file.

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 General Guidelines for Biosafety in Pathogenic Microbiology Laboratories Regulations on the Management of the Transport of Species or Species of Highly Pathogenic Pathogenic Microorganisms Infecting Humans no. 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 the virus belongs to the mammalian tick-borne virus group of the Flaviviridae and was discovered in 1957. It originated from the forest of Kassanol in Nataka, India.

Sick monkey. Since then, 400 to 500 human cases have been reported each year. Symptoms of the disease include high fever and frontal headache, accompanied by He has bleeding symptoms, such as nasal bleeding, throat bleeding, gums, and gastrointestinal bleeding. Ticks are the main vector of transmission and can cause fever, Blood, myalgia, and encephalitis, with a mortality rate of 3% to 5%, are mainly distributed in western India. A researcher collected by Chinese researchers from 1989 Nanzianyinivirus was isolated from serum samples of fever patients and identified as a KFDV variant by molecular biology and other methods, suggesting that in my KFD occurred in the Hengduan Mountains in Yunnan, China.

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; when the PCR is amplified, the 5'-3' exonuclease activity of the Ta enzyme is used to degrade the probe to make 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.

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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 BHZ1. Black hole quenching group (blakehozuencher1)

5 Detection object

Suspicious cases found at the port of entry and exit.

6 Laboratory biosafety and PCR anti-pollution requirements

According to the requirements of GB 1849, "List of Pathogens Infecting Humans" and "Regulations on Management of Transportation of Species or Species of Highly Pathogenic Pathogens Infecting Humans", the biosafety requirements of relevant materials are as follows.

- The degree of damage of the forest disease virus of Casanor No. 1 is classified into the first category;
- Laboratories should follow GB 1849 and WS233. Operation of uncultured infectious materials should be performed in a Biosafety III (BSL-3 or ABSL-3) laboratory, and inactivated materials should be in Biosafety II (BSL-2) performed in the laboratory;
- Cassanoor forest disease virus infectious material transportation 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.
- Packing and transfer of suspected Casanor forest disease 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 method are as follows.

- Real-time fluorescence PCR instrument;

- Ultra-clean workbench;
- Secondary biological safety cabinet;
- autoclave;
- Cryogenic high-speed centrifuge. Maximum centrifugal force.2000s.
- 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.

8 Reagent

The main reagents used in this method are as follows.

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- Nucleic Acid Extraction Reagent. Virus nucleic acid is extracted with ZIAgenVIRalRNA Kit from Germany Ziagen Company 1);
- Real-time fluorescence RT-PCR reagent. Ag-Path-IDTM OnsiteRT-PCR2);
- DEPC water without RNase.
- Primer and probe sequences are shown in Table 1.

Table 1 Primers and probes for real-time fluorescent RT-PCR detection of Casanor forest disease virus

Primer/Probe Name Sequence (5'-3 ') KFDV-F TGGAAGGCCCTGCGCTGAAAGAG
KFDV-R TCCATCCCCCTCGACCGCAT KFDV-P
FAM-ATTGAGAGAGAGCGCGCGCGTCCGCG-BHZ1

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 Administrative Measures for Depository Institutions.