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

SN/T 5096-2019

**Real-time fluorescent RT-PCR detection method for rumored
lone star virus in border ports**

**国境口岸蜚传孤星病毒实时荧光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., Huh Hehot Customs.

1 Scope

This standard specifies the detection targets, biosafety, and PCR of real-time fluorescent RT-PCR detection methods for tick-borne solitary virus at border crossings.

Anti-pollution requirements, instruments, reagents, and inspection procedures.

This standard applies to the detection of tick-borne lone virus.

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 19489 General requirements for laboratory biosafety
SN/T 1293 Tick monitoring regulations at border crossings
Application of polymerase chain reaction (PCR) technology in WS/T 230 clinical diagnosis
List of pathogenic microorganisms transmitted from human to human (Ministry of Health of the People's Republic of China Health Science and Education [2006] No. 15)

3 Terms and definitions

The following terms and definitions apply to this document.

Lone Star virus LSV

A member of the Bunyaviridae family of white cricket virus, a single-stranded negative-segment RNA virus with S, M, and L fragments. Among them, L segment encodes RNA-dependent RNA polymerases, M fragments encode glycoproteins Gn and Gc, and S fragments encode nucleocapsid and non-structural proteins.

Lone star virus is mainly transmitted by ticks. After tick bites, people may develop symptoms such as tick-borne spot fever.

4 Acronyms

The following abbreviations apply to this document.

bp. base pair

Ct value. cycle threshold
DEPC. diethyl pyrocarbonate
FAM. 6-carboxy-fluorescein
RNA. ribonucleic acid
ROX. 6-carboxy-x-rhodamine
RT-PCR. reverse transcription polymerase chain reaction
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5 Detection objects

5.1

Tick samples collected on-site at border crossings.

5.2

Persons suspected of being bitten by ticks and unable to exclude lone star virus infection.

6.1 Biosafety requirements

According to the requirements of GB 19489 and the "List of Pathogens of Human Infections", the biological safety requirements of solitary virus related materials are as follows.

- Handling of uncultured solitary virus infectious materials should be performed in a Biosafety Level 2 (BSL-2) laboratory;
- Lone star virus-related inactivated and non-infectious materials can be handled in a Biosafety Level 1 (BSL-1) laboratory-Lone star virus infectious material is transported and packaged in Category B, UN number UN 3373.

6.2 PCR anti-contamination requirements

PCR anti-contamination measures are implemented in accordance with WS/T 230.

7 Instruments

7.1

Real-time PCR instrument.

7.2

Secondary biological safety cabinet.

7.3

Autoclave.

7.4 -70 °C ultra-low temperature refrigerator.

7.5

Refrigerated centrifuge.

7.6

Vortex oscillator.

8.1 DEPC water

DEPC-treated RNase-free water.

8.2 RNA extraction kit

QIAamp Viral RNA kit, see the kit manual for details, including AVL, AW1, AW2, and AVE, etc. 1). a

8.3 One-Step Fluorescent RT-PCR Kit

Ag-Path-IDTM One step RT-PCR Kit2). b

1) This information is provided for the convenience of users of this standard and does not indicate endorsement of the product. If other equivalent products have the same effect, these equivalent products can be used.

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8.4 Primers and probes

The sequence is shown in Table 1.

Table 1 Primer and probe sequences

Primer/Probe Name Sequence (5'-3' ') Monitoring Period F CTCCTTAACCCTCAGATA 134 bpR CCTCTTTATGGTCCCTTA Probe FAM- TCATCAACATCTCGTCTCATTCGCT -BHQ1 Note. The 5' end of the probe is labeled with the FAM fluorescent reporter and the 3' end is labeled with the BHQ1 fluorescent quenching group. Other equivalent fluorescent markers and Quenching groups or equivalent primers and probes or other equivalent commercially available detection kits can also be used.

9.1.1 Collection of tick samples

According to SN/T 1293.

9.1.2 Collection of human serum samples

3 mL ~ 5 mL of internal venous blood was collected aseptically within 3 days of the onset of the suspected case, and allowed to stand at room temperature for 30 minutes to coagulate, 1 500 r/min ~ 3 000 r/min Centrifuge for 10 minutes, collect the serum in a 2 mL sterile screw-top plastic tube, register it with a low temperature resistant oily marker, and send it to the laboratory at low temperature.

Detection. If it cannot be delivered to the laboratory within 24 hours, the serum should be stored in a refrigerator at -70 ° C.

9.2.1.1 Tick samples

On the sterile operation platform, take out the ticks and place them in sterile dishes, soak them in 75% alcohol for 20 minutes, and blot them with sterile filter paper.