

ICS 11.020
CCS C 62

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

SN/T 5097-2019

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

**国境口岸蜚传巴贾病毒实时荧光RT-PCR检测方法
(English Translation)**

Issued on: September 3, 2019

Implemented on: March 1, 2020

Issued by: General Administration of Customs

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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, Guangzhou Customs of the People's Republic of China, Shenzhen Inspection and Quarantine Science Research Institute, Hohhot Customs, People's Republic of China.

1 Scope

This standard specifies the detection targets, biological safety, and PCR antifouling of tick-borne Borna disease virus real-time fluorescence RT-PCR detection at border crossings.

Dyeing requirements, instruments, reagents and inspection procedures.

This standard applies to the detection of tick-borne Baja 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 Pieces. 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.

Bajaja virus; BHAV

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

BHAV is mainly transmitted by tick bites, causing illness in humans and livestock. Under normal circumstances, a person infected with BHAV may experience lethargy, vomiting, Nervous system symptoms such as meningitis and paralysis; abortion and death may occur after infection in livestock.

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 reation Tick-borne Baja virus.indd 1.2019/9/12 14.01.05 ?????? ?????, ?????, ??

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 rule out Baja virus infection.

6.1 Biosafety requirements

Biosafety requirements for Baja virus-related materials in accordance with the requirements of GB 19489 and the Directory of Pathogens in Human Infections as follows.

- The degree of harm of Baja virus is classified as the third category;
- Handling of uncultured Baja virus infectious material should be performed in a Biosafety Level 2 (BSL-2) laboratory;
- Baja virus-related inactivated and non-infectious materials can be handled in a Biosafety Level 1 (BSL-1) laboratory;
- Baja virus infectious materials are 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

Benchtop 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 AVE1).
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	GATGGTTGCATACACTGA	
Amplified fragment is 166 bpR	CTTGGCATCATCTTTCCA	
Probe FAM-	ATCCTTAAGGAGTTCGGTGAGGA	-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

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, and centrifuged at 1 500 r/min ~ 3 000 r/min After 10 minutes, the serum was collected in a 2 mL sterile screw-top plastic tube, registered with a low-temperature oil-resistant marker, and sent to the laboratory for testing at low temperatures.

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