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

SN/T 5279-2020

**Technical specification for quarantine of eel herpes virus
infection**

鳎疱疹病毒感染检疫技术规范

(English Translation)

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Foreword

This document is drafted in accordance with GB/T 1.1-2020.

This document was proposed and managed by the General Administration of Customs of the People's Republic of China.

Drafting organizations of this document. Xiamen Customs of the People's Republic of China, Haikou Customs of the People's Republic of China, Shenzhen of the People's Republic of China customs

1 Scope

This document specifies the PCR and fluorescent probe PCR detection methods for eel herpes virus.

This document is applicable to the quarantine and identification of eel herpes virus infection and its epidemiological investigation, diagnosis and monitoring.

2 Normative references

The contents of the following documents constitute the indispensable clauses of this document through normative references in the text. Among them, dated quotations Only the version corresponding to that date is applicable to this document; for undated references, the latest version (including all amendments) is applicable Used in this document.

GB/T 6682 Analytical laboratory water specifications and test methods GB/T 18088 Entry and Exit Animal Quarantine Sampling SC/T 7103 Technical Specification for Quarantine and Sampling of Aquatic Animal Origin

3 Terms, definitions and abbreviations

There are no terms and definitions that need to be defined in this document. The following abbreviations apply to this document.

HVA. Herpesvirus anguillae PCR. Polymerase chain reaction (Polymerase chain reaction)
Taq enzyme. Thermus Aquaticus DNA Polymerase

4 Overview of HVA infection

The eel herpes virus infection is caused by the eel herpes virus. The English name of the virus is Herpesvirus anguillae (HVA).

As Anguillid herpesvirus 1 (AngHV1). Infected eels may not show clinical symptoms, and the clinical symptoms produced do not have typical characteristics.

Sex. See Appendix A for the epidemiology and pathogenic characteristics of HVA.

5 Reagents and materials

If the water is not specified, it should meet the first-grade water specifications in GB/T 6682. Unless otherwise specified, all reagents used are of analytical grade.

6 Apparatus and equipment

6.1

Constant temperature water bath.

6.2

Desktop refrigerated high-speed centrifuge.

6.3

Thermal cycler.

6.4

Electrophoresis instrument.

6.5

Gel imager.

6.6

Fluorescent PCR machine.

7.1 Diagnosis of clinical symptoms

When HVA is infected, the clinical symptoms of eel are very different between groups and individuals. The common clinical symptoms are pectoral fins, the operculum and gill filaments are bleeding. Clinical symptoms can also be manifested as loosening and hyperplasia of mucus on the body surface, followed by shedding, and mucus defect plaques on the body surface. Follow as the disease progresses, the lesion reaches the dermis, ulcers occur, perforation and internal organs are exposed, the head is congested and red (red head), and the abdominal skin is bleeding.

Congestion of the gills, part of the fin rays and anus, rotten gills, protruding eyes and abdominal distension, swelling of the liver, discoloration or redness, swelling of the kidneys, swelling of the gallbladder, and intestine Inflammation, continued death, etc.

7.2 Sampling

The sampling quantity refers to the regulations of GB/T 18088 and SC/T 7103. According to the freshness of the sample, it can be oxygenated and refrigerated at 4 °C.

Or frozen and transported to the laboratory.

7.3 Sampling site

7.3.1

For fish with body length > 4 cm, gills, liver, spleen, kidneys and other parts can be taken for testing.

7.3.2

For fish with body length ≤ 4 cm, the whole fish can be rounded up for testing.

7.4 Tissue DNA extraction

7.4.1

Grind the tissue block into a homogenate shape with a grinding tool in an ice box, take about 25 mg of the tissue homogenate in a 1.5 mL centrifuge tube, and place it in a 1.5 mL centrifuge tube.

Add 500 μL of lysis buffer (see B.1) and proteinase K (see B.2) at a final concentration of 100 $\mu\text{g}/\text{mL}$. Place the above centrifuge tube Incubate in a 55 °C water bath for 1 h~2 h to fully lyse the tissue. Add 350 μL extract 1 (see B.3) and mix well 12 000 g Centrifuge for 5 min. Take the upper aqueous phase, add 350 μL extract 2 (see B.4), mix well and centrifuge at 12,000 g for 5 min. Take the upper water phase, Add 1.5 times the volume of absolute ethanol pre-cooled at -20 °C, mix well, place at room temperature for 10 minutes, centrifuge at 15,000 g for 10 minutes, and discard the supernatant. instant Centrifuge at time and carefully aspirate the remaining supernatant with a pipette. Leave it at room temperature for 5 minutes to dry the precipitate. Add 10 μL of sterilized double distilled water and flick the tube wall Dissolve the precipitate and use it as a PCR template.

7.4.2

Tissue DNA extraction can also use equivalent commercial DNA extraction kits and use them according to the instructions.

7.5.1 PCR amplification system

10xPCR buffer (see B.5) 5 μL , 20 $\mu\text{mol}/\text{L}$ PCR primer (5.2) 1 μL each, 25 mmol/L MgCl_2 5 μL , 10 mmol/L dNTPs 1 μL , 5 U/ μL Taq enzyme (5.1) 1 μL , DNA template 5 μL , add double distilled water to a total volume of 50 μL .

7.5.2 PCR amplification conditions

The reaction conditions are preheating at 94 °C for 5 min, then denaturation at 94 °C for 40 s, annealing at 65 °C for 45 s, and extension at 72 °C for 60 s, a total of 40 cycles.

Finally, extend at 72 °C for 10 min and store at 4 °C. The PCR reaction conditions can also be set according to the instructions of the commercial Taq enzyme.

7.5.3 Establish a comparison

Take the nucleic acid extract of the eel tissue sample that has been diagnosed as positive by PCR as a positive control template. Fetch has been diagnosed by PCR as Negative eel tissue sample nucleic acid extract was used as a negative control template. The blank control is sterile deionized water.

7.5.4 Agarose gel electrophoresis

Prepare a 1.5% agarose gel plate (containing 0.5 $\mu\text{g}/\text{mL}$ EB) with TBE running buffer (see B.6), about 0.5 cm thick. will 6 μL of sample and 2 μL of loading buffer (see B.7) are mixed and added to the sample well, and a DNA standard Marker is set up as a control. 30 mA electricity Swim for about 0.5 h, and stop when the bromophenol blue indicator reaches the bottom of the gel plate. Observe the nucleic acid band under the gel imager.