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

SN/T 5181-2020

**Technical specification for quarantine of hematopoietic organ
necrosis of goldfish**

金鱼造血器官坏死病检疫技术规范

(English Translation)

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Foreword

This document was drafted in accordance with the rules given in GB/T 1.1-2020.

1 Scope

This document specifies the clinical symptoms of carp herpes virus type 2, testing equipment and equipment, reagents and materials, sampling, and diagnostic methods.

Law and comprehensive judgment.

This document is applicable to the diagnosis, monitoring and detection of necrosis of goldfish hematopoietic organs.

2 Normative references

The contents of the following documents constitute an indispensable clause 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 experimental methods GB/T 18088 Entry and Exit Animal Quarantine Sampling GB 19489 Laboratory Biosafety General Requirements

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.

4 Overview

Gold Fish Haematopoietic Necrosis (GFHN) is caused by carp herpes virus type 2 (Cyprinid Herpesvirus 2, CyHV 2) is a viral infectious disease that infects goldfish, crucian carp and other carps and causes the disease (see Appendix A).

5 Clinical symptoms

After goldfish and others are infected with GFHNV, they will show lethargy, loss of appetite or abolishment, and their gills will turn white. Dying fish can see a lot of blood from the lower edge of the operculum overflows naturally, the body surface is congested, the abdomen is swollen, and the base of the orbit is congested. After anatomy, muscle hemorrhage can be seen, and the spleen and kidney have different course Degree of bleeding, hepatosplenomegaly, and spotting bleeding in the swim bladder.

6 Equipment and equipment

6.1

Ordinary refrigerators and ultra-low temperature refrigerators.

6.2

Ice maker.

6.3

Sterile tissue grinder.

6.4

Secondary biological safety cabinet.

6.5

Refrigerated high-speed centrifuge.

6.6

Thermal cycler.

6.7

Horizontal electrophoresis apparatus.

6.8

Gel imager.

6.9

Real-time fluorescent PCR instrument.

6.10

Micropipettes of different specifications, with ranges including 0.5 μL ~10 μL , 10 μL ~100 μL , 100 μL ~1000 μL .

6.11

Autoclave.

6.12

Consumables such as scissors, tweezers, alcohol cotton balls, centrifuge tubes and PCR tubes.

7 Reagents and materials

7.1

Water. Comply with the first grade water specification in GB/T 6682.

7.2

Phosphate buffer (see B.1).

7.3

CTAB solution (see B.2).

7.4

Extract 1 (see B.3).

7.5

Extract 2 (see B.4).

7.6

Anhydrous ethanol, analytically pure.

7.7

TE buffer (see B.5).

7.8 10xPCR amplification buffer (see B.6).

7.9

Taq enzyme. Store at -20°C, avoid repeated freezing and thawing or drastic temperature changes.

7.10

dNTPs. containing 10 mmol/L each of dCTP, dGTP, dATP, and dTTP.

7.11

TBE running buffer (see B.7).

7.12

Ethidium bromide (see B.8).

7.13 6x loading buffer (see B.9).

7.14 2x real-time fluorescent PCR master mix (see B.10).

7.15

Primer for PCR. the concentration is 20 μ mol/L.

7.16

Primers and probes for real-time fluorescent PCR. the concentration is 10 μ mol/L.

8 Sampling

Sampling objects include healthy, diseased and dying goldfish and crucian carp, etc. The sampling quantity shall be implemented in accordance with GB/T 18088.

The laboratory should meet the requirements of GB 19489.

Under ice bath conditions, every 5 to 10 fish are used as a sample, and at least 0.1 g of tissue is collected for each sample. Body length less than 4 cm Take the whole fish from the fish fry of 4 cm~6 cm; take all the internal organs (including kidney) from the fish fry of 4 cm~6 cm;

(Use alcohol cotton ball to remove the mucus on the gill surface), brain.

9.1.1 Establish a comparison

From the extraction of sample DNA, a positive control, a negative control, and a blank control need to be set up. Take the positives that are known to contain GFHNV Sex tissue was used as a positive control; healthy fish tissue was used as a negative control; an equal volume of sterile deionized water was used as a blank control.

9.1.2 Nucleic acid extraction (CTAB method)

Under aseptic conditions, homogenize the obtained tissue in a sterile mortar, and use pre-cooled phosphate buffer at a ratio of 1.10 (mass. volume) the tissue was resuspended, centrifuged at 8 000 r/min at 4°C for 5 min, and the supernatant was taken for inspection.

Take 450 μ L of the tissue suspension into a 1.5 mL centrifuge tube, add 450 μ L of CTAB