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

SN/T 1162-2020

Replacing SN/T 1162-2002

**Technical specifications for quarantine of infectious pancreatic
necrosis**

传染性胰脏坏死病检疫技术规范
(English Translation)

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Foreword

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

This document replaces SN/T 1162-2002 "Infectious Pancreatic Necrosis Virus (IPNV) Enzyme Chain Immunosorbent Test (ELISA) Diagnosis method".

Compared with SN/T 1162-2002, the main technical changes of this document are as follows.

- Added the description of clinical symptoms;
- Added SYBR Green fluorescent RT-PCR method;
- Added RT-PCR method;
- Modified the comprehensive judgment.

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

Drafting organizations of this document. Shenzhen Customs of the People's Republic of China, Shenzhen Institute of Inspection and Quarantine.

1 Scope

This document specifies the isolation of infectious pancreatic necrosis virus, SYBR Green real-time fluorescent reverse transcription polymerase chain reaction, reverse Record the detection methods of polymerase chain reaction and enzyme-linked immunosorbent assay.

This document is applicable to the epidemiological surveillance, detection and quarantine of infectious pancreatic necrosis.

2 Normative references

The following documents are indispensable for the application of this document. For dated reference documents, only the dated version applies to this article Pieces. For undated reference documents, the latest version (including all amendments) is applicable to this document.

GB/T 6682 Analytical laboratory water specifications and test methods GB/T 18088 Entry and Exit Animal Quarantine Sampling

3 Terms, definitions and abbreviations

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

BSA. bovine serum albumin (bovine serum albumin);

BF-2.blue fin fish cell line;

CHSE-214.Chinook salmon embryo cell line (chinook salmon embryo cell line);

Ct. cycle threshold (cycle threshold);

DEPC. Diethyl pyrocarbonate (diethy pyrocarbonate);

dNTP. deoxynucleoside triphosphate;

ELISA. enzyme-linked immunosorbent assay;

HRP. horseradish peroxidase (horseradish peroxidase);

OD value. optical density value (optic density value);

RNA. deoxyribonucleic acid (ribonucleic acid);

RTG-2.rainbow trout gonadal cell line;

RT-PCR. reverse transcription polymerase chain reaction (reverse transcription polymerase chain reaction).

4 Overview

Infectious pancreatic necrosis (IPN) is caused by infectious pancreatic necrosis virus (infectious pancreatic necrosis, IPN).

Pancreatic necrosis virus (IPNV) caused a very serious infectious and viral fish disease, mainly in salmon Fry and juvenile fish of the family fish (see Appendix A).

5 Reagents and materials

5.1

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

5.2

DEPC water. For the preparation method, see B.1 in Appendix B or purchase commercial reagents.

5.3

IPNV reference strain.

5.4

Cell line. CHSE-214, RTG-2 or BF-2 cell line.

5.5

HEPES. 4-hydroxyethylpiperazine ethanesulfonic acid.

5.6

Rabbit anti-IPNV reference antiserum.

5.7

Goat anti-IPNV antibody.

5.8

Enzyme-labeled goat anti-rabbit IgG. use according to the instructions.

5.9

Primer. Use DEPC water to prepare each primer into a 100 $\mu\text{mol/L}$ storage solution, and store it below $-20\text{ }^{\circ}\text{C}$; when using, take an appropriate amount.

Prepare 20 $\mu\text{mol/L}$ working solution to avoid multiple freezing and thawing. The specific sequence is as follows.

IPNV F. 5'-ACTACGAACCCCCAGGACAAA-3';

IPNV R. 5'-GTCTCGTCCTCTAGGCGGACGTA-3'.

Used for SYBR Green real-time fluorescent RT-PCR to amplify the 122 bp fragment of the IPNV VP2 gene.

IPNV PrD1.5'-AAAGCCATAGCCGCCCCATGAAC-3';

IPNV PrD2.5'-TCTCATCAGCTGGCCCCAGGTAC-3'.

Used for RT-PCR to amplify the 174 bp fragment between the IPNV NS and VP3 genes.

6 Equipment and equipment

6.1

Microplate reader.

6.2

Inverted microscope.

6.3

Constant temperature incubator.

6.4

PCR thermal cycler.

6.5

Gel imager.

7.1 Sampling

The sampling quantity shall be implemented in accordance with GB/T 18088. For fish with clinical symptoms, take the whole fish fry less than or equal to 4 cm in length, if the yolk sac should be removed for fish with yolk sac; fish fry with a body length of 4 cm to 6 cm should be taken from internal organs (including kidneys); fish with a body length of more than 6 cm should be taken from liver, kidney and spleen. Liver, kidney and spleen are taken from fish without clinical symptoms; ovarian fluid is also required for mature female fish. The egg shell is taken from fish eggs.

7.2 Sample processing

Should be kept below 10 °C for processing. First homogenize the sample with a tissue grinder, and then suspend it in the fine particles at a final dilution of 1:10.

Cell culture medium (see B.2 in Appendix B). If the sample has not been treated with antibiotics before homogenization, it must be added to the cell culture medium 1 000 IU/mL penicillin and 1 000 µg/mL streptomycin. Incubate at 15 °C for 2 h ~ 4 h or at 4 °C for 6 h ~ 24 h. 7 000 r/min Centrifuge for 15 min and collect the supernatant. The ovarian fluid does not need to be homogenized, it should be diluted more than 2 times. Centrifuge samples of