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

SN/T 5282-2020

**Technical Specification for Quarantine of Virus Diseases in
Notian Village**

虾偷死野田村病毒病检疫技术规范
(English Translation)

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Foreword

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

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, Yellow Sea Fisheries Research Institute of Chinese Academy of Fishery Sciences.

1 Scope

This document specifies the clinical symptoms, histopathology, nested polymerase chain reaction, and real-time fluorescent reverse transcription of Nodamura virus disease.

Detection methods of polymerase chain reaction and in situ hybridization.

This document is applicable to the epidemiological investigation, diagnosis, quarantine and monitoring of Noda Village virus disease.

2 Normative references

The contents of the following documents constitute indispensable clauses of the documents 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 SN/T 1193 Technical requirements for genetic testing laboratories

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 of the disease

Shrimp stealing Noda village virus disease is also called shrimp stealing virus disease, its pathogen is the stealing Noda village virus (CMNV), which belongs to Noda village disease Nodaviridae Alphanodavirus. For details, see Appendix A.

5 Reagents and materials

5.6

Xylene.

5.7

Paraffin wax.

5.8

Hematoxylin dye solution.

5.9 0.5% Eosin ethanol solution.

5.10

Neutral resin.

5.11

Reverse transcriptase. 20 U/ μ L, stored at -20°C, do not freeze-thaw repeatedly or temperature changes drastically.

5.12

RNase inhibitor. 20 U/ μ L, stored at -20°C, do not freeze-thaw repeatedly or temperature changes drastically.

5.13

Trizol reagent. RNA extraction reagent.

5.14

Taq enzyme. Store at -20°C, do not freeze-thaw repeatedly or change the temperature drastically.

5.15

dNTPs, including dCTP, dGTP, dATP, dTTP each 10 mmol/L.

5.16

The sequences of primers and probes for real-time fluorescent RT-PCR are as follows.

6 Equipment and equipment

6.1

Slicer.

6.2

Microscope.

6.3

Tissue grinder.

6.4

High-speed refrigerated centrifuge.

6.5

Real-time fluorescent PCR thermal cycler.

6.6

PCR thermal cycler.

6.7

Horizontal electrophoresis apparatus.

6.8

Micro pipette.

6.9

Gel imager.

7 Clinical symptoms

Sick shrimp mostly sink to the bottom of the water, rarely move on the surface, soft shell, slow growth, in many cases, white abdominal muscles can be seen. Anatomy visible Hepatopancreas atrophy, jejunum and stomach.

8.1.2 Dehydration, embedding and sectioning

Treat the fixed tissue with 70% ethanol for 1 h, 80% ethanol for 1 h, 95% ethanol for 1 h, and absolute ethanol for 30 min (repeat twice), treat with xylene absolute ethanol (volume ratio 1.1) for 30 min, and treat with xylene for 30 min (repeat twice), Xylene paraffin (volume ratio 1.1) treatment for 15 min, paraffin treatment for 15 min (repeated twice), paraffin embedding, sectioning, section thickness the degree is about 3 μm . The cut slices are used for dyeing or in situ hybridization. The time of each step can be adjusted appropriately according to the sample situation.

8.1.3 Dyeing

Treat the sections with xylene for 5 min (repeated twice), and treat with xylene absolute ethanol (volume ratio 1.1) for 5 min.

Alcohol treatment for 5 min, 95% ethanol treatment for 5 min, 80% ethanol treatment for 5 min, 70% ethanol treatment for 5 min, double distilled water washing, hematoxylin Fine dyeing, double-distilled water washing, 1% hydrochloric acid ethanol treatment, double-distilled water washing, 0.5% eosin staining, double-distilled water washing, 70% ethanol treatment 5 min, 80% ethanol treatment for 5 min, 95% ethanol treatment for 5 min, absolute ethanol treatment for 5 min (repeated twice), xylene anhydrous Ethanol (volume ratio 1.1) is treated for 5 minutes, xylene is treated for 5 minutes (repeated twice), and finally sealed with neutral resin and observed under a microscope.

The time of each step can be adjusted appropriately according to the sample situation.

8.1.4 Results judgment

8.1.4.1

Typical histopathological lesions are. eosinophilic inclusion bodies can be seen in the epithelial cells of hepatopancreas tubules of hepatopancreas tissue; muscle tissue Visible coagulation necrosis, blood cell infiltration in the necrotic muscle fibers, and nuclear pyknosis of the nucleus infiltrating the blood cells (see Appendix D);

8.1.4.2

Histopathological methods are only used for preliminary judgment. If a typical lesion is observed in the test sample, it is judged as a suspicious case and you need to use Diagnosis by other methods of this standard.

8.2.1 Sample collection

Sampling according to the standard of GB/T 18088, and preferentially selecting individuals with clinical symptoms. Take hepatopancreas, gills and appendages.