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

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Technical specifications for quarantine of avian nephritis

禽肾炎检疫技术规范

(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. Tianjin Customs of the People's Republic of China, Jinzhou Medical University, Qinghai Island Customs of the People's Republic of China.

1 Scope

This document specifies virus isolation and identification, reverse transcription-polymerase chain reaction, and real-time fluorescent reverse transcription-polymerase for avian nephritis Technical requirements for chain reaction and indirect enzyme-linked immunosorbent assay.

This document is applicable to the quarantine of avian nephritis.

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 experimental methods 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.

AN. avian nephritis (avian nephritis);

ANV. avian nephritis virus.

4.1 Introduction to Avian Nephritis

Refer to Appendix A for an introduction to avian nephritis.

4.2 Clinical symptoms

This disease can significantly inhibit the growth and weight gain of chicks within 1 month of age, and other symptoms are not obvious. Generally presents a transient recessive infection, this disease After artificial inoculation of chicks without special pathogenic microorganisms, the concentration of urate in plasma was 2-10 times higher than the normal concentration, and returned to normal 21 days later.

4.3 Pathological changes

The pathological changes of this disease are mainly manifested in the kidneys, and other organs are generally unchanged. As the age increases, the degree of disease is lessened, 4 days old the chicks inside are the most severely diseased and may even die. Necropsy of dead chicks, kidney, spleen, peritoneal surface and legs are seen A large amount of urate deposits around the tendons, and only mild nephritis lesions occurred at 2 months of age.

A preliminary diagnosis of avian nephritis can be made based on epidemiology, clinical symptoms and pathological changes, and further diagnosis depends on laboratory diagnosis.

5.1 Biosecurity measures

See GB 19489 for test environment and protection requirements.

5.2.1.1 Equipment

Biological safety cabinets, centrifuges, carbon dioxide incubators, refrigerated high-speed centrifuges, grinders, inverted microscopes, etc.

5.2.1.2 Water

It meets the first-level water requirements of GB/T 6682.

5.2.1.3 Reagents

Trypsin digestion solution, cell growth solution, cell maintenance solution, double antibody solution, please refer to Appendix B.1~B.4 for specific preparation.

5.2.1.4 Cell

Chicken kidney cells (CKC).

5.2.2.1 Sample collection and preparation

5.2.2.1.1

When there are no clinical symptoms, live poultry shall aseptically collect cloacal swabs of sick chickens (with visible feces), and young poultry shall collect fresh feces;

After the animal died, the contents of the kidney and rectum were aseptically taken as disease feed.

5.2.2.1.2

The sample is placed in isotonic phosphate buffered saline (PBS) at pH 7.0 containing antibiotics.

5.2.2.1.3

Stool and crushed tissues are made into 10%-20% (mass concentration) suspension with PBS containing antibiotics, and the swab is immersed in 3 mL Shake well in PBS containing antibiotics. Incubate at 4 °C for 4 h or overnight, or at room temperature for 2 h. The swab was discarded after repeated squeezing.

5.2.2.1.4

Centrifuge at 4 °C 3 000 r/min for 10 min, take the supernatant and filter and sterilize it through a 0.22 µm filter membrane.

5.2.2.2 Sample transportation and storage conditions

Samples for immediate laboratory testing should be stored at 4 °C or placed in a refrigerator for fast delivery, samples that cannot be tested immediately Store frozen at -70°C.

5.2.2.3 Sample inoculation

Inoculate the prepared samples in a monolayer of CKC cells cultured in a 24-well cell culture plate, inoculate at least 4 wells for each sample, set the correct for habitual cell control, at least 4 wells, 0.1 mL~0.2 mL per well, adsorbed at 37°C for 1 h, decanted excess liquid, added 1 mL of maintenance solution, and placed Incubate in a 5% carbon dioxide constant temperature incubator at 37°C for 3 to 5 days.

5.2.2.4 Observation of results

24 hours after the sample is inoculated, observe the cell status daily under an inverted microscope and make corresponding records. If the cell control well is normal, and when cells become rounded, clustered or plaques are formed due to cell shedding in the seeding sample wells, transfer to the sample after freezing and thawing three times. If the culture plate is newly overgrown with a single layer of cells, if the observation still shows similar cytopathic changes and is becoming more obvious, the cell culture can be harvested and placed Put it into the refrigerator at -70 °C for identification. If there is no change between the control cells and the inoculated sample cells, pass them blindly for two more passages. If there is a fine According to the above principles, harvest the cell culture and put it in the refrigerator at -70 °C; if there is no cytopathic disease, it is avian nephritis virus.

The result is negative.

5.2.2.5 Culture identification

Since avian enteroviruses can cause the same CPE, the harvested culture should be identified. Reverse transcription-polymerization can be used Enzyme chain reaction (RT-PCR), real-time fluorescent RT-PCR and enzyme-linked immunosorbent assay (ELISA) methods to identify whether it is avian kidney Inflammatory virus infection.

5.3.1 Instruments and equipment

5.3.1.1

PCR thermal cycler.

5.3.1.2

Electrophoresis instrument.

5.3.1.3

UV gel imager.

5.3.1.4

High-speed desktop refrigerated centrifuge.