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

SN/T 1556-2020

Replacing SN/T 1556-2005

**Technical Specifications for Quarantine of Chicken Infectious
Rhinitis**

鸡传染性鼻炎检疫技术规范

(English Translation)

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Foreword

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

This document replaces SN/T 1556-2005 "Infectious Rhinitis Agar Immune Diffusion Test Operating Procedures".

Compared with SN/T 1556-2005, the main technical changes of this document are as follows.

- Added clinical diagnosis methods;
- Added pathogen isolation and identification methods;
- Added PCR detection method;
- Added hemagglutination inhibition test (HI);
- Added indirect enzyme-linked immunosorbent test (I-ELISA).

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 stipulates the isolation and identification method of infectious rhinitis in chickens, polymerase chain reaction technology, serum plate agglutination test, blood Operation method of coagulation inhibition test, indirect enzyme-linked immunosorbent test,

chicken infectious rhinitis agar immunodiffusion test.

This document is applicable to the diagnosis and quarantine of infectious rhinitis in chickens.

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 SN/T 2123 Regulations on the collection, transportation and storage of test samples for entry and exit animal quarantine

3 Terms and definitions

There are no terms and definitions that need to be defined in this document.

4.1 Comprehensive clinical diagnosis

Refer to Appendix A for the clinical symptoms and pathological changes of infectious rhinitis in chickens.

4.2.1 Reagents and materials

4.2.1.1

Sterilized cotton swabs, blood agar plates, sterilized vials, catalase.

4.2.1.2

Staphylococcus epidermidis producing nicotinamide adenine dinucleotide (or coenzyme 1, NAD).

4.2.1.3 5% carbon dioxide (CO₂) incubator, 4 °C or -20 °C ~ -30 °C refrigerator.

4.2.2 Sample collection and processing

The collection, storage and transportation of samples shall comply with the relevant sampling requirements of GB/T 18088 and SN/T 2123.

Collect the blood of diseased chickens aseptically and separate the serum. The serum should be fresh, transparent, non-hemolytic, and non-polluting, and packed in a sterile vial at 4 °C or -20 °C ~ -30 °C refrigerator storage or refrigerated conditions immediately send for inspection.

Aseptically collect the mucus, serous, or secretion of trachea and air sacs in the infraorbital sinus of sick chickens in the acute stage (incubation period 1 d~7 d) it is used for pathogenic diagnosis, and the bacteria in the infraorbital sinus cavity are the purest.

4.2.3 Isolation and identification of bacteria

The infraorbital sinus of the suspected chicken was opened aseptically, and the mucus or serum was dipped in a sterile cotton swab. Use a cotton swab to cross the blood agar plate. Scribe 5 to 7 lines, and then take NAD-producing *Staphylococcus epidermidis* and draw a vertical line from the middle of the horizontal line. Place the seeded plate in 5% dioxides. Incubate in a carbon incubator at 37 °C for 18 h~24 h, and observe the colony characteristics. Take the purified substance of the suspicious colony for catalase test, if there is a large amount Bubbles are positive for catalase. PCR can also be used to identify suspicious colonies, and at the same time, disease materials or suspicious colonies can be taken for animal testing.

See Appendix B for the method.

4.2.4 Judgment of results

If "satellite-like" dew-like, needle-sized small colonies appear on the blood agar medium, they are close to the NAD-producing epidermal grapes the colony at the coccus line is larger, with a diameter of up to 0.3 mm. The farther away from the NAD-producing *Staphylococcus epidermidis* line, the smaller the colony will be. Catalase is negative.

The result is judged to be positive; if there is no satellite phenomenon, but there are dewdrop-shaped, needle-sized small colonies and catalase is negative, it still needs to be used PCR or animal test identification to avoid missed detection of *Avian Bacillus paragallinarum* that does not require NAD. If there is no such phenomenon, it is judged as negative.

4.3.1 Reagents and materials

4.3.1.1

Unless otherwise specified, the reagents used in the test are of analytical grade, and the laboratory water should meet the requirements of GB/T 6682.

4.3.1.2 0.5% Np-40 (ethyl phenyl polyethylene glycol), 0.5% Tween-20, 100 mmol/L pH 8.3 Tris-HCl, 0.2 mg/mL egg White enzyme K, negative and positive controls.

4.3.1.3

Physiological saline, distilled water, 1.5 mL and 0.5 mL small centrifuge tubes, and pipette tips are all sterilized.

4.3.1.4

Thermal cycler, water bath, small centrifuge, micropipette, ice cubes.

4.3.1.5

Agarose, electrophoresis apparatus, horizontal electrophoresis tank, ultraviolet lamp or gel imager.

4.3.1.6 10 mg/mL ethidium bromide, loading buffer, TAE running buffer.

4.3.2.1.1 Nucleic acid extraction from tissue samples

Open the infraorbital sinus of the suspected chicken aseptically, and dip the mucus or serum into it with a sterile cotton swab and immerse it in 0.8 mL~1.0 mL normal saline.

Put the cotton swab on the tube wall as much as possible after placing it in the 1.5 mL centrifuge tube at room temperature for 0.5 h, and then discard it in the waste tank.

Centrifuge the centrifuge tube soaked with cotton swabs at 8 000 r/min~10 000 r/min for 10 min, carefully aspirate most of the supernatant, and save 20 μ L Around the liquid.

Use a pipette to mix the sediment in the centrifuge tube with the remaining liquid evenly, and add 15 μ L of 1x PCR buffer (containing 0.5% Np-40, 0.5% Tween-20, 0.2 mg/mL proteinase K and 100 mmol/L pH 8.3 Tris-HCl), press the centrifuge tube cap tightly, and water bath at about 50 °C for 1 h, Then 95 °C for 10 min, then ice bath for 10 min, PCR amplification or storage at -20 °C.

4.3.2.1.2 Nucleic acid extraction of colonies

Pick the colonies cultured in a 5% carbon dioxide incubator at 37 °C for 18 h~24 h, and add them to 0.5 mL small cells containing 10 μ L distilled water.

In the centrifuge tube, press the tube cap tightly, heat at 95 °C for 10 min, then perform PCR amplification after 10 min in ice bath or store at -20 °C.

Use 10 μ L of sterile water as a negative control sample, and use 9 μ L of sterile water plus 1 μ L of a known positive control as a positive control sample. approximately 50 °C water bath for 1 h, then 95 °C for 10 min, then ice bath for 10 min, PCR amplification or storage at -20 °C.

4.3.2.1.3 Other nucleic acid extraction methods

Equivalent commercial kits can be used for bacterial pathogen nucleic acid extraction.

4.3.2.2 PCR amplification

Design PCR detection primers for the hagA gene of Avian paragallinarum.