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

SN/T 5281-2020

**Technical Specifications for Quarantine of Avian Tembusu Virus
Disease**

禽坦布苏病毒病检疫技术规范
(English Translation)

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Foreword

This document is 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. Fuzhou Customs of the People's Republic of China, Institute of Animal Husbandry and Veterinary Medicine, Fujian Academy of Agricultural Sciences, People's Republic of China Nanjing Customs, Dongguan Zhongding Testing Technology Co., Ltd., Xiamen Hanjunke Testing Technology Co., Ltd., Qingdao, the People's Republic of China customs.

1 Scope

This document specifies the clinical diagnosis and reverse transcription-polymerase chain reaction identification technology for avian tambusu virus disease.

This document is applicable to the clinical diagnosis and laboratory quarantine of avian tambusu 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

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.

4.1 Symptoms

When some or all of the following conditions occur in poultry (especially breeding poultry and laying poultry), it can be used as one of the basis for preliminary diagnosis. rapid body temperature Increased, feed intake plummeted; weak feet, ataxia or (and) drooping wings; plummeted egg production rate; breeder and egg poultry will start to lay the laying rate of breeding (egg) poultry rises slowly, the laying rate continues to be low, there is no peak laying or (and) the laying rate is sometimes high and sometimes low, the reserve breed (egg) poultry opens Laying is delayed or (and) uneven laying.

4.2 Pathological changes

When poultry (especially laying poultry) have the following macroscopic lesions, it can be used as one of the basis for preliminary diagnosis. breeding poultry, laying poultry ovary (egg Blisters) bleeding; liquefaction of the yolk in the follicles of breeding birds and laying birds, deformed follicles; light red blood or light yellow turbidity in the abdominal cavity of breeding birds and laying birds Liquid; the ovaries (follicles) of breeders and egg poultry are atrophy, and the number of follicles decreases. Focal hemorrhage in the liver of poultry or (and) mild hemorrhage in the brain tissue.

4.3 Clinical diagnosis

When poultry (including reserve breeders and egg poultry) have the above symptoms, combined with necropsy lesions, it can be preliminarily diagnosed as suspected avian tambusu virus disease.

Further laboratory confirmation is required.

5 Sample collection

According to clinical symptoms and pathological changes of necropsy, aseptically collect poultry, dying poultry or dead poultry (especially breeding poultry, egg Poultry) ovaries and liver samples, the collected samples should be sent to the laboratory for testing within 24 hours under refrigerated conditions.

6.1 Equipment

6.1.1

Tissue homogenizer.

6.1.2

High-speed desktop refrigerated centrifuge.

6.1.3

Micropipette.

6.1.4

Thermal cycler.

6.1.5

Electrophoresis instrument.

6.1.6

Electrophoresis tank.

6.1.7

UV gel imaging system.

6.2 Reagents

6.2.1

Water should meet the requirements of first-class water specified in GB/T 6682.

6.2.2

Phosphate buffered saline (PBS), see Appendix A.1 for the preparation method.

6.2.3

RNA extraction reagent Trizol.

6.2.4

Trichloromethane.

6.2.5

Isopropanol.

6.2.6

DEPC water, the preparation method is shown in Appendix A.2.

6.2.7 75% ethanol, the preparation method is shown in Appendix A.3.

6.2.8

Reverse transcriptase.

6.2.9

Taq DNA polymerase.

6.2.10

RNase inhibitor.

6.2.11

Deoxynucleotide triphosphate dNTPs (including dCTP, dGTP, dATP, dTTP).

6.4.1 Use inactivated avian tambusu virus as a positive control; use other inactivated avian RNA viruses (such as duck hepatitis I virus) as negative Control; Take DEPC water as a blank control. The control sample and the sample to be tested are operated synchronously to extract RNA.

6.4.2 In a 1.5 mL sterile centrifuge tube treated with DEPC water, add 250 µL of tissue homogenate supernatant or control sample respectively, and then Add 750 µL RNA extraction reagent Trizol and mix well for 15 s. Add 200 µL of pre-cooled chloroform, mix well, and leave at room temperature Leave it for 10 min.

6.4.3 After centrifugation at 12 000 r/min for 15 min at 4 °C, carefully aspirate 600 µL of the supernatant, and add 1.5 mL of DEPC water-treated quencher.

Add 600 µL of isopropanol pre-cooled at -20°C into the microbial centrifuge tube, and mix by inversion.

6.4.4 After centrifugation at 12 000 r/min for 15 minutes at 4 °C, gently pour off the supernatant, add 800 µL of 75% ethanol to wash twice, Place it upside down on absorbent paper for 5 minutes and dry at room temperature.

6.4.5 Add 20 µL of DEPC water, mix gently to dissolve the RNA on the wall of the centrifuge tube, and centrifuge at 2 000 r/min for 30 s. RNA extracted RT-PCR amplification should be carried out within 2 h or stored in a refrigerator at -20°C for later use.

6.4.6 RNA extraction can also use a commercially available equivalent commercial nucleic acid RNA extraction kit, and operate in accordance with the requirements of the instructions.