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

SN/T 2985-2020

Replacing SN/T 2985-2011

Technical Specifications for Equine Influenza Quarantine

马流行性感冒检疫技术规范

(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 replaces SN/T 2985-2011 "Technical Specification for Equine Influenza Quarantine".

Compared with SN/T 2985-2011, the main technical differences of this document are as follows.

--Refer to the "Handbook of Terrestrial Animal Diagnostic Tests and Vaccines" (2016 Edition) to optimize the test method;

- Added one-way radiation hemolysis test;

--- Added the H3N8 equine influenza virus nucleic acid detection fluorescent RT-PCR method.

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

Drafting organizations of this document. Beijing Customs of the People's Republic of China, Changchun Customs of the People's Republic of China, Tianjin of the People's Republic of China customs.

1 Scope

This document specifies the clinical diagnosis and laboratory diagnosis of equine influenza.

This document is applicable to the quarantine of entry and exit equine influenza.

2 Normative references

The contents of the following documents constitute an indispensable clause 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 19489 General requirements for laboratory biosafety SN/T 1687 Equine Influenza Hemagglutination Inhibition Test Operating Procedure

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.

EI. equine influenza (equine influenza) EIV. equine influenza virus HA. Haemagglutination (haemagglutination) HI. Haemagglutination inhibition test (haemagglutination inhibition) MDCK. canine kidney cells (madin-darby canine kidney) PBS. phosphate buffered saline RT-PCR. reverse-transcription polymerase chain reaction (reverse-transcription polymerase chain reaction) SRHT. single radial haemolysis test (single radial haemolysis test)

4.1 Clinical symptoms

Equine influenza is composed of two influenza A subtypes with different antigenicity in the Orthomyxoviridae influenza A virus genus.

(H7N7, called horse-1; H3N8, called horse-2) caused by an acute respiratory disease.

When susceptible horses get sick, the clinical symptoms are fever, harsh dry cough, nasal flow and viscous purulent secretions; when a horse has a certain degree of immunity, the above symptoms the condition may not be fully manifested.

4.2.1 Pathological anatomical changes

The visual lesions are conjunctival flushing, edema, ectropion, brick red or light yellow, and corneal turbidity often occurs. Upper respiratory tract mucosal filling Blood, edema and exudation, epithelial cell shedding and focal erosion. Swollen lymph nodes in the head and neck. Lung congestion, edema, no expansion all. The liver generally has no obvious eye disease.

4.2.2 Histopathological changes

It manifests as acute bronchitis and bronchiolitis, which are mainly characterized by the exudation of a large number of neutrophils in the lumen. Inflammation one Transient, usually only seen in the first 4 days after the onset of the disease, after which the inflammation subsides, and there are mild lymphocytes and plasma cell infiltration in the mucosa.

Severe cases are acute bronchial interstitial pneumonia, which is characterized by general thickening of the alveolar mediastinum, and the proliferating cells are histiocytes and lymph cell. In the liver, diffuse or nodular lymphocytes and tissue cell infiltration can be found in the sinusoidal spaces.

4.3 Epidemiology

Under natural conditions, only equine animals are susceptible, and there is no difference in age, sex and breed. A sick horse coughs and spews a virus-containing fly Mo, through the respiratory tract infection is the main mode of transmission of this disease. Experiments have shown that the disease can be spread through the air, or through contaminated feed.

Oral transmission of food and drinking water. The virus exists in the semen of recovered horses for a long time, so sexual intercourse is also an important way of spreading the disease.

The disease spreads extremely rapidly. The introduction of susceptible herds shows an explosive epidemic. After 1 week or a little longer, all susceptible horses are infected.

disease. The disease can occur throughout the year, and it occurs more frequently in the spring and autumn in northern my country. In some areas, it mostly occurs in late winter and early spring, while another in some areas, it is popular in summer.

5.1.1 Laboratory biosafety requirements

Isolation and identification of equine influenza virus pathogens should be carried out in a biosafety level 2 laboratory or a biosafety cabinet of the corresponding level.

The conditions should meet the requirements of GB 19489.

5.1.2.1 Equipment

High-speed bench-top refrigerated centrifuge, bench-top centrifuge, vortex shaker, refrigerator (4°C and -20°C), autoclave, microplate reader, Fluorescence PCR machine, PCR reaction tube or reaction plate, inverted microscope, micro adjustable pipette (10 µL, 200 µL, 1000 µL).

5.1.2.2 Materials and reagents

5.1.2.1 Sampling tools. cotton swabs, scissors, tweezers, syringes, 1.5 mL centrifuge tubes, mortars, sampling tools should be heated to $121^{\circ}\text{C}\pm 2^{\circ}\text{C}$, Autoclave for 15 minutes and dry, pH 7.2, 0.01 mol/L PBS, see Appendix A, antibiotics (penicillin and streptomycin) for the preparation method.

5.1.2.2

For chicken embryo inoculation. egg photoper, hole punch, alcohol lamp, 5% iodine tincture, 70% alcohol, 1.0 mL disposable sterile injection Ware, paraffin, 9~11 day old SPF chicken embryos.

5.1.2.3 Allantoic fluid activity determination. 96-well V-type micro-reaction plate, shaker, micropipette, Aldrich solution, chicken red blood cell suspension for the preparation method, see Appendix B.

5.1.2.4 For cell inoculation. canine kidney cells, newborn calf serum, cell culture flask, MEM culture medium. See Appendix C for the preparation method.

5.1.2.5 Virus antigen ELISA test. ELISA test kit.

5.1.2.6 For fluorescent RT-PCR. TriZol or other viral RNA extraction kits, reverse transcription magnetic beads (RT-PCR Ready-To-Go™ beads), Taq DNA polymerase, chloroform, isopropanol, absolute ethanol (analytical grade).

5.1.3 Sampling, sample delivery and processing

5.1.3.1

Take samples immediately after the appearance of clinical symptoms, including nasopharyngeal swabs or nasal and tracheal washes.

5.1.3.2

The swab is made of absorbent cotton or gauze wrapped around a metal wire. During sampling, the nasopharyngeal swab is sent into the nasopharynx through the anterior nasal passage and stay for about 1 minute, stick to the respiratory tract mucus, and immediately put it into the vial containing the transport culture medium. Nose and tracheal irrigation the objects are taken through nasal endoscopy.

5.1.3.3

The transport culture medium is PBS containing 40% glycerol, or phosphate broth containing 2% trypsin (plus 2% antibiotic) Solution (penicillin and streptomycin 10 000 IU/100 mL each) and amphotericin B (250 mg/mL mother liquor).