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

SN/T 5194-2020

**Technical specifications for quarantine of sheep infectious
pustular dermatitis**

羊传染性脓疱皮炎检疫技术规范

(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. Changchun Customs of the People's Republic of China, Yixing Customs of the People's Republic of China, Huizhou of the People's Republic of China Customs, Jilin Provincial Academy of Animal Husbandry and Veterinary Sciences.

1 Scope

This document specifies the clinical diagnosis, virus isolation, polymerase chain reaction and real-time fluorescent polymerase chain reaction of sheep infectious pustular dermatitis.

Reaction detection technology.

This document is applicable to the quarantine and inspection of sheep contagious pustular dermatitis.

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 regulations and test methods GB 19489 Laboratory Biosafety General Requirements

3 Terms and definitions

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

4 Biosecurity measures

In order to protect the safety of laboratory personnel, testing should be carried out by qualified personnel who have passed biosafety training.

Feeds and wastes should be implemented in accordance with the relevant regulations in GB 19489.

5 Reagents and materials

5.1

The water used should meet the requirements of Grade 1 water in GB/T 6682.

5.2

Preparation of primary cells from calf testis (see A.1 in Appendix A).

5.3 0.01 mol/L (pH7.2) PBS (see A.2 in Appendix A).

5.4 10% Sodium Lauryl Sulfate (see A.3 in Appendix A).

5.5 20 mg/mL proteinase K (see A.4 in Appendix A).

5.6 Primers and probes

A set of specific primers were synthesized based on the specific genes of sheep infectious pustular dermatitis. The size of the amplified product is 488 bp.

5.7 Positive control. a standard strain that can be traced to the source, or DNA containing the target fragment can also be used.

5.8 Molecular biology reagents. 10xPCR buffer, dNTP (2.5 mmol/L), Taq DNA polymerase (5U/ μ L), ExTaq Hs (5U/ μ L).

5.9 0.2 mL and 1.5 mL plastic centrifuge tubes.

5.10 Tips, used with pipettes.

5.11 Sterilized Erlenmeyer Flask. 250 mL, 500 mL.

6 Apparatus and equipment

6.1

Thermal cycler.

6.2

Fluorescent PCR machine.

6.3

Electrophoresis instrument.

6.4

Gel imaging system.

6.5

Biochemical incubator. $-5\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ ~ $50\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$.

6.6

Biological safety cabinet.

6.7

Electronic balance. with a sense of 0.1 g.

6.8

Homogenizer.

6.9

Biological microscope.

6.10

Pipette. measuring range 0.5 μL ~10 μL , measuring range 10 μL ~100 μL , measuring range 100 μL ~1000 μL .

6.11

High-speed refrigerated desktop centrifuge (up to 13 000 r/min).

7.1 Clinical symptoms

The clinical symptoms are mainly divided into lip type, hoof type and vulva type, see Appendix B in B.1.

7.2 Necropsy changes

The main lesions are shown in Appendix B in B.2.

7.3 Epidemiology

The main epidemiology is shown in Appendix B, B.3.

7.4 Initial diagnosis

Make a preliminary diagnosis based on clinical symptoms, changes in necropsy, and epidemiology.

8.1 Sample processing

Collect diseased materials such as pustules on the skin of suspected sheep scabs, add 0.01 mol/L (pH7.2) PBS and grind to make 1.10 (mass.volume) the suspension was centrifuged at 5 000 r/min for 30 min, the supernatant was taken, and the double antibody (penicillin, streptomycin) at a concentration of 10 000 IU/mL was added at a ratio of 20%.

After feeling overnight at 4 °C, store it for later use.

8.2 Sample inoculation and culture

After the primary cells of calf testis have grown up to a 25 cm² cell culture flask monolayer, discard the supernatant nutrient solution, and inoculate 1 mL of diseased material to treat the supernatant.