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

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**The detection of antibodies against ovine progressive
pneumonia virus. Protocol of agar gel immuno-diffusion test**

绵羊进行性肺炎抗体检测方法 琼脂免疫扩散试验

(English Translation)

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Foreword

Appendix A of this standard is a normative appendix.

This standard is proposed and managed by the National Certification and Accreditation Administration.

This standard was drafted. Shenzhen Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

Introduction

Sheep progressive pneumonia (Ovine Progressive Pneumonia, OPP) or sheep Medi-Wisner disease (Maedi-ViGB aDis-Ease, MVD) is a persistent viral infection in sheep caused by the MV/OPP virus, characterized by slow course, progressive wasting and Difficulty breathing, more manifestations of interstitial pneumonia and symptoms of cerebrospinal inflammation. Infected animals are the main source of infection, and only sheep and goats are known to the disease is susceptible and there is cross-infection between the sheep and the goat. The disease is mainly transmitted to the newborn lamb through colostrum and spreads through the respiratory tract.

Or spread through contaminated drinking water, feed and pasture. The incubation period of this disease is particularly long, after the animal is exposed to the virus for 1 to 3 years or more.

Clinical symptoms appear, followed by a progressive clinical course. Infected animals can be poisoned for life, and most sheep can have clinical symptoms and lesions. in in MV/OPP virus infection, neutralizing antibodies do not prevent the virus from spreading in the blood. The disease is currently in most countries where sheep are raised.

It has been determined to exist. The clinical and subclinical symptoms of MV/OPP are progressive monocytic inflammatory lesions in the lung, breast, and central nervous

system.

And indurated mastitis. MV/OPP has a serious impact on the breeding and improvement of sheep and the trade of sheep, resulting in economic losses it cannot be underestimated that the disease has been included in the Class B disease by the International Organization for Animal Diseases (OIE) and the second type of infectious diseases announced by the Ministry of Agriculture of China. Due to most infections Viral sheep and goats are asymptomatic but continue to be toxic and transmit the virus through colostrum or breastfeeding, or even through respiratory secretions.

Therefore, quarantine the disease by establishing a fast and reliable diagnostic method is an important means to control the disease.

Sheep progressive pneumonia antibody detection method agar Immunodiffusion test

1 Scope

This standard specifies the methods and procedures for detecting antibodies to progressive pneumonia (Meddy-Wisner disease) in sheep.

This standard is used to detect sheep progressive pneumonia (Meddy-Wisner disease) virus antibodies, suitable for quarantine, diagnosis, epidemic monitoring and Epidemiological Investigation.

2 Principle

Agar is a polysaccharide containing a sulfate group, which has a porous structure after gel formation, and the size of the pore diameter depends on the agar concentration. Antigen and antibody the molecular weight of the body is generally below 200,000, and the antigen and its specific antibody are blocked when diffusing from the high concentration region to the low concentration region in the gel.

The force is small and basically in the form of free diffusion. Due to the different molecular weight, structure, shape and charge of different antigen molecules, their diffusion system the number of diffusion varies in the gel. When the antigen and the corresponding antibody are diffused, they meet in the gel to form an antigen-antibody complex.

Object. If the ratio of the two is appropriate at the meeting, the largest composite is formed. As the molecular weight of the complex increases, the particles increase and are no longer Continue to spread to produce a precipitate, showing a "specific barrier" in the form of a line or band, called an immunoprecipitation line or an immunoprecipitation zone, so Whether the precipitation line or precipitation band between the antigen and the standard positive serum and the precipitation line or precipitation band between the antigen and the test serum to determine whether antibodies are present in the serum to be tested.

There are two most important antigens for MV/OPP virus, one is the envelope glycoprotein

of the virus, usually called gP135; the other is the core egg.

White P28, an antigen obtained by collecting the culture medium of infected cells when preparing these two proteins, and concentrating about 50 times by dialysis on polyethylene glycol preparation. The MV/OPP virus antibody was detected by this antigen, and the specificity was strong and the repeatability was good. In theory, these two antigenic proteins are related to each other.

The positive serum should be divided into two sedimentation lines, but the test results show that almost all the positive serums tested only form a distinct sedimentation.

Precipitation line.

3 Material preparation

3.1

Reagents. agarose, sodium chloride, sodium azide, Tris base, hydrochloric acid.

3.2

MV/OPP agar diffusion test antigen, standard positive serum. designated by the OIE reference laboratory or the relevant national authorities or provided by the designated laboratory, use according to the instructions.

3.3

Tested sheep serum. Blood was collected according to the conventional method, serum was separated, and the water was extinguished at 56 ° C for 30 min. The serum should be free of hemolysis and no bacterial contamination.

Store at 4 °C.

3.4

Equipment. balance, straw, measuring cylinder, plate (specification. 90.0mm x 15.0mm), triangular bottle, seven-hole plum blossom with outer diameter of 5.3mm Sample metal punchers, micropipettes and micro tips, observation lamps, water baths, autoclaves, etc.

4 Operation methods and steps

4.1

Preparation of 1% agar plates.

4.1.1

Preparation of Tris-HCl buffer. See Appendix A.

4.1.2

Preparation of 1% agar. Take 100 mL of Tris-HCl buffer solution, add 1.00 g of agarose, 8.50 g of sodium chloride, and dissolve well by heating.

Solution, add 0.01% sodium azide, add into the test tube, 16mL per tube for use.

4.1.3

Plate. The test tube agar is heated and melted. When the agar solution is naturally cooled to about 56 ° C, the diameter is 90 mm clean and sterilized.

Plates were placed on the platform, and each plate was filled with 16 mL of agar solution to a gel thickness of 2.8 mm. Covered to be naturally cooled and solidified, pour the plate