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

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Technical specifications for rabbit coccidiosis quarantine

兔球虫病检疫技术规范

(English Translation)

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Foreword

This document is drafted in accordance with GB/T 1.1-2010.

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

Drafting organizations of this document. Chinese Academy of Inspection and Quarantine, Guangzhou Customs of the People's Republic of China, Taiyuan of the People's Republic of China customs.

1 Scope

This document specifies the technical specifications for sample collection, pathogen morphology observation and PCR detection during rabbit coccidiosis quarantine.

This document is suitable for the detection of coccidiosis in live rabbits and rabbit carcasses with liver, as well as the epidemic of coccidiosis in rabbit farms.

Scientific investigation and monitoring.

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 scale and test method

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 Overview of the disease

See Appendix A.

5 Materials

5.1

Unless otherwise specified, the chemical reagents used are of analytical grade. The test water requirement meets the requirements of first-grade water in GB/T 6682.

5.2

Genomic DNA extraction kit, PCR buffer, dNTP, DNA polymerase, DNA Marker, proteinase K, sodium chloride, Potassium dichromate, chloroform, SDS, isopropanol, agarose, absolute ethanol, etc., are all commercial reagents.

5.3

Saturated sodium chloride solution, 2.5% potassium dichromate solution, 0.5% SDS digestion solution, lysate, 20 mg/L proteinase K solution, Formula of phenol/chloroform/isoamyl alcohol solution, 70% ethanol, 3 mol/L sodium acetate solution, electrophoresis buffer, physiological saline See Appendix B for the method.

5.5

PCR detection negative and positive control. the positive control is one of 11 kinds of rabbit coccidia DNA genome, the negative control is healthy rabbit liver (Intestine) tissue sample.

6 Equipment and consumables

Balance, tweezers, glass slide, float tube, ziplock bag, 60 mesh small copper sieve, microscope, ice maker, refrigerator (4 °C, -20 °C, -80 °C), autoclave, refrigerated centrifuge, PCR machine, electrophoresis machine, gel imager or UV transilluminator, micro adjustable

pipette and Tips, 1.5 mL centrifuge tubes, etc.

7.1.1 Collection of samples from liver and intestinal lesions

Cut liver nodules and intestinal mucosal nodules, each of about 20 g, put them into a clean ziplock bag, and mark the number.

Record the rabbit's age, breed, breeding and sanitary conditions, etc., and store at 4°C for microscopic examination.

7.1.2 Collection of live rabbit feces samples

Live rabbits were quarantined for 30 days, and rabbit feces were collected twice on the first day of isolation and the 12th day of isolation.

Before sampling, put a layer of plastic wrap under the rabbit cage. Take samples from the fresh-keeping film after 3 hours. Regardless of the age of the rabbits, take the individual rabbits as the unit collects about 20 g of fresh feces. Put it into a clean ziplock bag, mark the number, and record the age, breed, and health of the rabbit the conditions, medications, breeding and sanitary conditions, etc., should be stored at 4°C for microscopic examination.

7.2.1 Microscopic examination of liver and intestinal lesions

Microscopic examination of the liver tissue showed atrophy of liver cells, proliferation of bile duct epithelium, showing papillary protrusions, and degeneration and necrosis of epithelial cells. Liver nodules A large number of coccidian oocysts can be seen on the tablet.

Intestinal mucosa necrosis and shedding, and coccidian oocysts can be seen on the intestinal mucosa smear.

7.2.2 Treatment and microscopic examination of live rabbit feces

7.2.2.1

Weigh 3 g of each stool sample, mash it with a glass rod and stir it into a suspension with 50 ml of normal saline.

7.2.2.2

Filter the fecal suspension through a 60-mesh small copper sieve into the second container, and pour the filtrate in the second container into a centrifuge tube.

7.2.2.3

Centrifuge at 3 000 r/min for 3 min, and discard the supernatant.

7.2.2.4

Add 10 ml of saturated sodium chloride solution to the precipitate and mix well.

7.2.2.5

Centrifuge at 3 000 r/min for 3 min.

7.2.2.6

Use a fishing net to get the surface floating liquid and shake it off in a container with water.

7.2.2.7

Transfer the oocysts into a petri dish that has been added with 2.5% potassium dichromate solution, mix well, the height of the liquid level is less than 1 cm, and mark it.

Place it in a shaking table at 28 °C for sporulation culture for 48 h~72 h, observe the sporulation situation every 12 h, and the sporulation rate reaches 80% or more.

The oocysts are stored in a refrigerator at 4 °C.

7.2.3 Morphological characteristics

Place the oocysts on a glass slide and observe under a 100x optical microscope. The oocysts are oval or approximately round, and a few are oval or pears.

Shape, morphology depends on the species of insects, the membrane wall is thicker, generally there are two layers, the eggshell-like structure can be clearly seen, colorless, light yellow, golden yellow Wait. After positioning the oocysts under a 100x optical microscope, further observe the shape and structure of the oocysts under a 400x optical microscope.

See Appendix D for the model diagram of Eimeria rabbit oocysts and the morphological characteristics of 11 species of rabbit coccidia oocysts.

7.3.1 PCR primers

The primer sequences used in this method are as follows.

7.3.2 Sample preparation

Take 25 mg of rabbit coccidia oocysts (or lesion tissue) and place them in a centrifuge tube to extract according to the method of extracting tissue DNA in Appendix E for DNA, commercial tissue DNA extraction kits can also be used.

7.3.3 PCR reaction system

Take 2 µL of the extracted sample DNA as a template and add it to the PCR reaction solution prepared in Table 1. At the same time set up a negative and positive control, Set up ddH₂O as a blank control.