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

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Piroplasmosis pathogen identification method

梨形虫病病原鉴定方法

(English Translation)

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Foreword

This standard refers to chapters 2.3.8, 2.3.9 and 2.5.6 of the 2004 edition of the OIE Terrestrial Animal Diagnostic Test and Vaccine Standards Manual.

Appendix A of this standard is a normative appendix, Appendix B, Appendix C, Appendix D, and Appendix E are informative appendices.

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

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

1 Scope

This standard specifies the identification method for the pathogen of pear-shaped diseases in cattle, sheep, horses and dogs.

This standard applies to the detection and identification of the pathogen of pear-shaped diseases in cattle, sheep, horses and dogs.

2.1 Reagents

---EDTA solution (1mg/mL) (see Appendix A);

---5% and 10% of Giemsa dye solution (see Appendix A);

---Methanol (analytical grade).

2.2 Instruments

- Optical microscope (8 times or more eyepieces and 60 times more objective lenses);
- Eyepiece micrometer;
- Mirror micrometer;
- 5mL syringe, 20 gauge needle;
- Slides.

3 Sample collection

3.1

Live animals. blood is taken from the tip of the tail or the tip of the ear to make a blood smear; or jugular blood can be aseptically collected in the anticoagulant EDTA solution. (1 mg/mL).

3.2

Dead animals. The death time during sampling is no more than 24h. The collected samples include peripheral blood from the hoof, ear and tail tip and the brain.

Organs such as cortex, kidney, liver and bone marrow (sorted by importance). The collected samples were quickly delivered to the laboratory under low temperature conditions (around 5 °C).

3.3

Tyler's disease sampling. In addition to blood collection, lymph nodes need to be punctured for smear.

4.1.1 Production of thin blood film

Drop 1 drop of blood on the left end of the clean glass slide, take a small amount of blood with a pusher, and apply a thin blood film from the near right end of the slide. After air drying, Fix with methanol for 1min, then dye with 10% Giemsa dye solution for 20min~30min, just apply the blood film as soon as possible to ensure good the quality of the dyeing.

4.1.2 Production of thick blood film

Drop 1 drop of blood (about 50 μ L) on a clean glass slide, heat it at 80 ° C for 5 min, then use 5% Giemsa dye solution.

Dyeing for 20min to 30min.

4.1.3 Production of organ smear

Lightly touch the fresh cut surface of the organ with a clean glass slide, or gently clamp a small piece of tissue with 2 clean slides, and push the glass vertically the piece makes the two slides leave a layer of tissue. After the smear is air-dried, it is fixed with methanol for 5 min, and then dyed with 10% Giemsa dye solution for 20 min~ 30min.

4.1.4 Preparation of lymph smears

The lymph fluid extracted by the syringe is pushed onto the slide glass, and then smeared with a needle to prepare a smear. After the smear is air-dried, it is fixed with methanol.