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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB/T 17999.10-2008

Replacing GB/T 17999.9-1999

SPF chicken - Microbiological surveillance - Part 10: Indirect immunofluorescent assay for SPF chicken

SPF鸡 微生物学监测 第10部分：SPF鸡 间接免疫荧光试验

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Foreword

GB/T 17999 "SPF chickens microbiological monitoring" is divided into 10 parts.

--- The first part 1. SPF chicken microbiological monitoring General; Part

--- Article 2. SPF chicken hemagglutination inhibition test; Part

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--- Chapter 4. SPF chicken serum plate agglutination test; Part

--- Article 5. SPF chicken agar diffusion test;

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--- Article 7. SPF chicken embryo susceptibility testing; Part

--- Article 8. SPF chicken Salmonella testing;

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--- Article 10. SPF chickens indirect immunofluorescence assay. This section GB/T 17999 Part 10. This Part replaces GB/T 17999.9-1999 "SPF chickens indirect immunofluorescence assay." This section compared with the GB/T 17999.9-1999 The main changes are as follows:

--- Increasing the normative Appendix A "Preparation of Reagents" and informative Appendix B "cell counting method";

--- A detailed definition of the experimental conditions and the establishment of sample to be tested criteria. Appendix A of this section is normative appendix, Appendix B is an informative annex. This section proposed by the People's Republic of China Ministry of Agriculture. This part of the National Animal Epidemic Prevention Standardization Technical Committee (SAC/TC181) centralized. This section is drafted. Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, China Animal Health and Epidemiology Center, Jinan Spa law Adams Poultry Limited. The main drafters of this

section. song even East Liujia Sen, Han Lingxia, satellite Shao, Zhu fruit, single Zhong Fang, Jiang Qian, Secretary Chand, Yu Haibo, Mengqing Wen. This part of the standard replaces the previous editions are.

--- GB/T 17999.9-1999. SPF chickens microbiological monitoring Part 10. SPF chickens indirect immunofluorescence assay

1 Scope

GB/T 17999.10-2008 is the Chinese national standard on spf chicken - microbiological surveillance - part 10: indirect immunofluorescent assay for spf chicken, in the field of health care technology. The /T suffix marks it as a recommended standard: it is not compulsory by itself, but it becomes binding as soon as a contract, a tender or a customer specification calls it up - which in practice is how most foreign buyers meet it. It was issued on 31 December 2008 by the General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China; Standardization Administration of China, and has been in force since 1 May 2009. Classification: ICS 11.220, CCS B41. This page is published from the official record of the standard held by the Chinese standards administration: the identification, the dates, the classification and the issuing body are taken from there. The clause text, the tables and the numeric limits are in the document itself, which is delivered complete in English translation.

This section GB/T 17999 specifies the indirect immunofluorescence test technical requirements. This section applies to SPF chickens chicken infectious anemia virus (ChickenInfectiousAnaemiaVirus, CIAV) antibody Testing. Principle

2 Indirect immunofluorescence test for virus-infected cells as antigen and serum to be tested in the specific antibody binding, and then labeled with a fluorescent dye Anti-antibody binding, the formation of the antigen - antibody - anti-antibody complexes. Fluorescent dye under the action of ultraviolet light or violet light, a visible fluorescence excitation Light, and therefore appear to explain the presence of fluorescent markers, but also reflects the presence of specific antibodies. Often using a known antigen and a fluorescent dye Labeled anti-antibody to detect the corresponding antibodies.

3.1 Reagents

3.1.1 CIAV DELROSE virus strains.

3.1.2 negative and positive sera.

3.1.3 was tested serum.

3.1.4 phosphate buffered saline (PBS) (see Appendix A).

3.1.5 fluorescein isothiocyanate (FITC) labeled anti-chicken IgG (diluted according to the instructions).

3.1.6 buffer or glycerol mounting medium (see Appendix A).

3.2 Equipment

3.2.1 antigen sheet.

3.2.2 humidified chamber. 3.2.3 37 °C incubator.

3.2.4 fluorescence microscopy. Procedure 4

4.1 Preparation of infected cells With DMEM medium containing 15% fetal bovine serum MDCC-MSB1 cells were cultured at 39 °C and 5% carbon dioxide environment. Cells grown to 5 x 106 per milliliter when Ge (cell count see Appendix B), inoculated CIAV, and replaced with a bovine serum-containing DMEM 5% newborn Medium, cultured 36h ~ 48h.

4.2 Preparation of antigen sheet

4.2.1 cytopathic (cell volume increased 2 to 3 times) from about 50% to 75%, the cell cultures were collected to 1500r/min centrifugal 10min, supernatant was discarded, the cell pellet was washed with PBS three times, and the cells were counted, resuspended in PBS cell concentration.

4.2.2 Take 10μL cell suspension (containing about 10 cells 5), was added dropwise in each well of the antigen in the sheet, while normal cells prepared pictures.

4.2.3 drip dry good antigen slices with ice-cold acetone fixed 4 °C 10min, stored at -20 °C, use within a week.