ICS 11.220
CCS B41

NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB/T 17999.6-2008

Replacing GB/T 17999.5-1999

SPF chicken - Microbiological surveillance - Part 6: Enzyme-linked immunosorbent assay for SPF chicken

SPF鸡 微生物学监测 第6部分：SPF鸡 酶联免疫吸附试验

Issued on: December 31, 2008 **Implemented on: May 1, 2009**

Issued by: General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China;
Standardization Administration of China

Table of Contents

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

--- Section 3. SPF chicken serum neutralization test; Part

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

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

--- Part 6. SPF chickens ELISA; Part

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

--- Article 8. SPF chicken Salmonella testing;

--- Part 9. SPF chickens tube agglutination test; Part

--- Article 10. SPF chickens indirect immunofluorescence assay. This section GB/T 17999 Part 6. The partial revision of the reference to GB/T 18936-2003 "highly pathogenic avian influenza diagnostic techniques", GB/T 19167-2003 "infectious bursal Disease Diagnosis ", NY/T 538-2002" infectious coryza diagnostic techniques ", OIE" terrestrial animals (mammals, birds and bees) diagnosis Manual of Tests and Vaccines "the relevant provisions (fifth edition) of. This Part replaces GB/T 17999.5-1999 "SPF chickens ELISA." This section compared with the GB/T 17999.5-1999 The main changes are as follows:

--- Increased indirect enzyme-linked immunosorbent assay;

--- Added Appendix A "reagent preparation." This section of Appendix A normative appendix. 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, Jiang Qian, Han Lingxia, satellite Shao, Zhu fruit, single Zhong Fang, Liu Jiasen Secretary

Chand, Guodong Chun, Yu Haibo, Mengqing Wen. This part of the standard replaces the previous editions are.

--- GB/T 17999.5-1999. SPF chickens microbiological monitoring Part 6. SPF chickens ELISA

1 Scope

GB/T 17999.6-2008 is the Chinese national standard on spf chicken - microbiological surveillance - part 6: enzyme-linked immunosorbent 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 technical requirements ELISA test. Indirect ELISA This section applies to SPF chicken serum antibody detection of the following pathogens. Haemophilus paragallinarum Avian influenza virus (AvianInfluezaVirus), Newcastle disease virus (NewcastleDiseaseVirus), infectious bronchitis virus (InfectiousBronchitisVirus), infectious bursal disease virus (InfectiousBursalDiseaseVirus), reticuloendothelial hyperplasia Virus (ReticuloendotheliosisVirus), chicken infectious anemia virus (ChickenInfectiousAnaemiaVirus), avian reovirus Virus (viral arthritis) (AvianReovirus), avian (AvianEncephalomyelitisVirus), infectious laryngotracheitis gas Tube stomatitis virus (InfectiousLaryngotracheitisVirus), avian adenovirus III group (EDS) (AvianAdenovirusGroupIII), shower Ba-cell leukemia virus (LymphoidLeukosisVirus). This section double-antibody sandwich enzyme-linked immunosorbent assay suitable for SPF chickens lymphoblastic leukemia virus P27 antigen detection. Principle

2 Indirect enzyme-linked immunosorbent assay using known microbial antigens to detect unknown antibody, double antibody sandwich enzyme-linked immunosorbent assay has been adopted Known unknown antigen antibody detection.

3 indirect ELISA

3.1 Reagents

3.1.1 The envelope antigen negative and positive control sera, goat anti-chicken LgG HRP, saved and used according to instructions.

3.1.2 Appendix coating liquid diluent, substrate solution, stop solution preparation method A.

3.2 Equipment Microtiter plates, pipettes, 37 °C incubator, microplate reader.

3.3 Procedure

3.3.1 antigen-coated. the bird flu virus, infectious bronchitis virus, infectious bursal disease virus, infectious laryngotracheitis virus, new City disease virus, Haemophilus paragallinarum, avian adenovirus III group (EDS), Mycoplasma gallisepticum, Mycoplasma synoviae, Avian encephalomyelitis virus, lymphocytic Leukemia virus, reticuloendothelial hyperplasia virus, avian reovirus (viral arthritis), chicken infectious anemia virus antigen coating buffer dilute Diluted to working concentration, was added to each well microtiter plates, each well 100μL, at 4 °C refrigerator overnight.

3.3.2 washing. rejection net hole antigen solution, with the washing liquid to fill the holes, place 5min, then dumped a net, so to repeat three times.

3.3.3 was tested serum plus. The dilution will be subject to 100 dilution of serum added to each well 100μL, each operation are set to negative control Two holes, the positive control wells, blank comparison wells each, were added to the same negative serum dilution of positive serum and dilutions of each 100μL, Plus different serum samples should change the tip, 37 °C incubator role 30min.

3.3.4 Washing. with 3.3.2.

3.3.5 plus goat anti-chicken IgG-HRP. The dilution of the enzyme-labeled antibody diluted to working concentration added to each well 100μL, 37 °C incubator The role of 30min.

3.3.6 Washing. with 3.3.2.

3.3.7 color. the addition of solution 100μL, dark at room temperature for about 5min reaction (negative control wells start to produce a color when).