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

**GB/T 18643-2021**

Replacing GB/T 18643-2002

**Diagnostic techniques for Marek's disease**

鸡马立克氏病诊断技术

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## Foreword

This standard was drafted in accordance with the rules given in GB/T 1.1-2009. This standard replaces GB/T 18643-2002 "Diagnostic Techniques for Marek's Disease in Chickens". Compared with GB/T 18643-2002, this standard The main technical changes are as follows:

- Modified the description of the differential diagnosis section (see 3.4, 3.4 in the.2002 edition);
- Added virus isolation and detection methods (see Chapter 4, Appendix A);
- Added PCR detection method (see Chapter 6, Appendix B);
- Increased fluorescence quantitative PCR detection method (see Chapter 7, Appendix C). The revision of this standard refers to the OIE "Terrestrial Animal Diagnostic Experiment and Vaccine Standard Manual" (2017 Edition), and combines domestic and foreign technical research The new achievements are consistent with the international advanced technology. Please note that some of the contents of this document may involve patents. The issuing agency of this document is not responsible for identifying these patents. This standard was proposed by the Ministry of Agriculture and Rural Affairs of the People's Republic of China. This standard is under the jurisdiction of the National Animal Health Standardization Technical Committee (SAC/TC181). This standard was drafted. Harbin Institute of Veterinary Medicine, Chinese Academy of Agricultural Sciences, China Center for Animal Health and Epidemiology. The main drafters of this standard. Liu Changjun, Gong Zhenhua, Zhang Yanping, Lu Ping, Wang Xiaomei, Li Yang, Gao Yulong, Qi Xiaole, Li Kai, Wang Zhiliang. The previous editions of the standard replaced by this standard are as follows:

---GB/T 18643-2002.

Chicken Marek's disease (Marek's disease, MD) is a highly infectious tumor disease in chickens characterized by lymphocyte proliferation. The disease is caused by Gallidherpesvirus 2 (GaHV-2), that is, Marek's disease virus (Marek's disease virus, MDV) caused. MD generally occurs in birds over 3 weeks of age, and mostly occurs between 12 weeks of age and 30 weeks of age. MD can cause a variety of serious diseases, among which lymphoid tissue hyperplasia is the most common and most important one. The typical MD mainly invades the nervous tissues, and the mortality rate rarely exceeds. After 10%~15%, it can last for several weeks to several months. Acute MD can cause lymphoma in the internal organs of affected chickens, and the incidence is generally 10%~30%, the incidence rate can be as high as 70% during an outbreak. The mortality rate may increase rapidly within a few weeks, and then the death will stop, or it may be within a few months. Maintain a stable mortality rate and gradually decline. At present, the most common is the acute type of MD that produces extensive internal organ lymphoma. MD is easily related to other tumor diseases in chickens, avian leukosis (AL) and avian reticuloendothelial tissue in clinical diagnosis. Reticuloendotheliosis (Reticuloendotheliosis, RE), confusion occurs. It is generally necessary to conduct differential diagnosis through epidemiology and histopathology. MD can be prevented by immunization with live vaccines. Immunization is a non-clearing immunity, which cannot eliminate pathogens and cannot prevent MDV wild virus in the environment. Infection and detoxification of infected chickens. Due to the widespread application of MD vaccines and the characteristics of the vaccines, the clinical inoculation and wildness of MD vaccine strains. The infection of the virus strain is complicated and requires laboratory diagnostic techniques to confirm the diagnosis. The isolation of MDV from infected chicken tissues can be used for the detection of the disease. Usually use lymphocytes and kidney cells isolated from anticoagulant blood samples. Or the spleen cell suspension can be used as the material for virus separation, and the feather pulp tissue slurry can also be used as the MDV separation material. Cell suspension or feather pulp. The tissue slurry can be inoculated with chicken kidney cells (Chicken kidney cells, CKC), duck embryo fibroblasts (Duck embryo fibroblast, DEF) or chicken embryo fibroblast (Chicken embryo fibroblast, CEF) for virus isolation. Applied agar immunodiffusion test (Agar gel immunodiffusion, AGID). Serological detection of MDV antigen in feather pulp can confirm MDV infection. AGID. It is the most commonly used method to detect antibodies. However, because MD vaccine strains can also stimulate the body to produce corresponding antibodies, antibody detection is. The clinical diagnosis of MD only has reference value. PCR (Polymerase chain reaction, PCR) method can be used to detect MDV, The PCR method can also identify MDV serotype I wild strains and vaccine strains.

Chicken Marek's Disease Diagnosis Technology

## 1 Scope

GB/T 18643-2021 is the Chinese national standard on diagnostic techniques for Marek's

disease, 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 30 April 2021 by the State Administration for Market Regulation; Standardization Administration of China, and has been in force since 1 November 2021. 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 standard specifies the clinical diagnosis of Marek's disease in chickens, as well as virus isolation, agar immunodiffusion test, PCR detection and fluorescence quantification. Technical requirements and comprehensive judgments for laboratory testing such as PCR testing. This standard applies to the detection, diagnosis, quarantine and epidemiological investigation of Marek's disease in chickens.

## 2 Abbreviations

The following abbreviations apply to this document. AGID. Agar immunodiffusion test (Agarimmunodiffusion) AL. Avian leukosis (Avianleukosis) CEF. Chicken Embryo Fibroblast (Chickenembryofibroblast) CKC. Chicken kidney cells (Chickenkidneycells) DEF. Duck Embryo Fibroblast (Duckembryofibroblast) CPE. Cytopathic effect (Cytopathiceffect) DEF. Duck Embryo Fibroblast (Duckembryofibroblast) DNA. Deoxyribonucleic acid (Deoxyribonucleicacid) EB. Ethidium bromide (Ethidium bromide) FBS. Fetal Bovine Serum (Fetalbovineserum) LL. Avian lymphoid leukemia (Lymphoidleukosis) M199. M199 Medium (M199 Medium) MD. Chicken Marek's disease (Marek's disease) MDV. Chicken Marek's disease virus (Marek's disease virus) PCR. Polymerase chain reaction (Polymerasechain reaction) PBS. Phosphate buffer (Phosphate-bufferedsalinebuffer) RE. Avian Reticularendotheliosis (Reticularendotheliosis)

## 3 Clinical diagnosis

3.1 Clinical symptoms In chickens, the clinical symptoms of sick chickens are severe weight loss, paralysis, and the legs are stretched back and forth in a "split" position, skin and hair follicle nodules, and Blindness, etc., generally can be used as symptoms of MD, and it can be preliminarily speculated to be MD.

### 3.2 Necropsy changes

3.2.1 Visceral type The pathological changes of visceral MD include extensive diffuse lymphoma in the liver, gonads, spleen, kidney, lung, front stomach and heart. Liver of young chicken The viscera is generally moderately enlarged, but the liver of adult chickens is severely enlarged.