ICS 11.220
CCS B41

NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB/T 18089-2008

Replacing GB/T 18089-2000

Isolation, identification and serum neutralization antibody test in bluetongue virus

蓝舌病病毒分离、鉴定及血清中和抗体检测技术

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

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Standardization Administration of China

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Foreword

This standard replaces GB/T 18089-2000 "Bluetongue micro serum neutralization test and the isolation and identification of the virus." This standard and GB/T 18089-2000 compared to the main changes are as follows:

--- Standard name has been modified;

--- Remove the bluetongue virus identified in a virus neutralization test portion;

--- Increase in the identification of viral identification RT-PCR method. Appendix A of this standard is a normative appendix. The standard proposed by the People's Republic of China Ministry of Agriculture. This standard by the National Standardization Technical Committee on Animal Epidemic Prevention. This standard was drafted. People's Republic of China Yunnan Exit Inspection and Quarantine, Shenzhen People's Republic of China Entry-Exit Inspection and Quarantine Bureau. The main drafters of this standard. Zhou Xiaoli, Yang Jianming, Ai Jun, flower Qun Yi, Yang flower, Zhangqi Yan, Yan tree hair, Dong Jun, Ye Lingling Xu Vega. This standard replaces the standards previously issued as follows:

--- GB/T 18089-2000. Bluetongue virus isolation, identification and serum and Antibody detection technology

1 Scope

GB/T 18089-2008 is the Chinese national standard on isolation, identification and serum neutralization antibody test in bluetongue virus, 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 standard specifies the bluetongue virus isolation, identification and serum neutralizing antibody detection techniques. This standard applies to the bluetongue virus isolation, identification and animal infected with bluetongue virus serum neutralizing antibody test.

2 Symbols and Abbreviations

The following symbols and abbreviations apply to this standard. BHK21

--- baby hamster kidney cell line; BLP

--- buffered lactose peptone broth; BT

--- bluetongue; bp

--- base pairs; CPE

--- induced cytopathic effect; C6/36

--- mosquito cell lines;

--- Of DEPC diethylpyrocarbonate; SPF

--- specific pathogen; TCID50

--- median tissue culture infective dose; Vero

--- African green monkey kidney cell line.

3 Bluetongue virus isolation

3.1 Materials 10 days to 11-day-old SPF chicken embryos.

3.2 Equipment and Apparatus Chick opening, Egg or Egg lamp box, a mortar or tissue masher, inverted microscope.

3.3 Test Procedure

3.3.1 Sample Anticoagulant heparin (10IU per 5mL blood with heparin), organs (spleen, liver, kidney, lymph nodes), semen, Culicoides. For virus isolation Samples should be stored in the refrigerated container home, sent to the laboratory within 24h, and processed immediately.

3.3.2 Blood Sample Preparation Take anticoagulant heparin 5mL, with phosphate buffered saline (PBS, recipe see Appendix A) Blood samples were washed 3 to 4 times, each time 2000r/min Centrifugal 10min, the supernatant was added after the times of BLP (recipe see Appendix A), set in an ice bath with sonication (40μA, 1min). By the Department

Reasonable sample that day, saved using the pre-4 °C, -70 °C to save the remaining sample set aside.

3.3.3 Preparation of tissue samples Collected aseptically animal spleen, lymph nodes, liver, kidney each 5g, cut into pieces added BLP10mL (including penicillin 2000IU/mL, streptomycin 2000μg/mL), set Mortar grinding, 4 °C refrigerator extraction 4h, the supernatant 5mL treated with sonication (100μA, 1min ~ 2min) or freezing and thawing three times, 3000r/min centrifugal 20min, supernatant using, use the pre-stored at 4 °C, -70 °C remaining sample set Paul Deposit reserve.

3.3.4 semen sample preparation After taking a sperm sample 0.5mL, with BLP as

1.5 dilution, 3000r/min centrifugal 20min, supernatant was discarded, and then make BLP