

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

SN/T 1165.1-2002

Protocol of competitive ELISA for bluetongue

蓝舌病竞争酶联免疫吸附试验操作规程

(English Translation)

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Foreword

BLuetongue competition enzyme-linked immunosorbent assay (C-ELISA) is based on the International Organization for Animal Disease (OIE) "Mammal, poultry Developed on the basis of the Diagnostic Test and Vaccine Standards Manual for Bees A and B Diseases and Practical Experience.

Appendix A of this standard is an informative annex.

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

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

1 Scope

This standard specifies the competitive enzyme-linked immunosorbent assay for blue tongue disease.

This standard applies to ruminant bluetongue antibody detection.

2 Normative references

The terms in the following documents become the terms of this standard by reference to this standard. All dated references, followed by all Modifications (not including errata content) or revisions do not apply to this standard, however, parties to agreements based on this standard are encouraged to study is it possible to use the latest version of these files? For undated references, the latest edition applies to this standard.

GB/T 6682-1992 Analytical laboratory water specifications and test methods (eqv ISO

3696:1987)

3 Abbreviations

The following abbreviations apply to this standard.

3.1

BTV. Bluetongue virus.

3.2

C-ELISA. Competitive enzyme-linked immunosorbent assay.

3.3

McAb. monoclonal antibody.

3.4

PI. percent inhibition.

3.5

goat anti-mouse IgG-HRP. horseradish peroxidase (HRP)-labeled goat anti-mouse IgG.

3.6

OD value. optical density value.

4 Principle

The test serum and BTV group-specific monoclonal antibody (McAb) were added to the wells of the ELISA plate coated with BTV-VP7 antigen at 37 ° C.

Under the reaction, the two compete for binding to the corresponding specific coating antigen in the well, and the enzyme-labeled goat anti-mouse IgG-HRP is added to wash away the unbound anti-antigen.

Body, then add substrate to develop color. The OD value of the reactant was measured using a microplate reader, and the percentage of inhibition (PI) was calculated to determine the test result.

5 Reagents and materials

5.1

coating antigen. Bluetongue virus VP7 antigen, provided by the designated unit.

5.2

Serum. Blue-tongue standard strong positive, weak positive serum, negative serum, provided by the designated unit.

5.3

Serum to be tested. bovine serum was inactivated at 56 ° C ~ 57 ° C for 30 min, and goat serum was inactivated at 58 ° C ~ 59 ° C for 30 min.

5.4

Bluetongue virus group-specific monoclonal antibodies. provided by the designated unit.

5.5

Sheep anti-mouse IgG-HRP. diluted according to the working concentration specified in the instructions.

5.6

Coating solution, washing solution, diluent, substrate solution, stop solution. See informative Appendix A.

5.7

Water. It meets the secondary water specification of the analytical laboratory of GB/T 6682-1992.

6 Instruments and equipment

6.1

Microplate reader.

6.2

Removable 96-well microtiter plate.