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**Protocol of indirect immunofluorescent assay for African swine  
fever**

非洲猪瘟间接免疫荧光试验操作规程

**(English Translation)**

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### Foreword

This standard refers to the Diagnostic Test and Vaccine Standards Manual (2004 edition) published by the International Organization of Animal Health (OIE) on Africa.

Indirect immunofluorescent antibody test method for diagnosis of swine fever.

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. Guangdong Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China is responsible for drafting.

### 1 Scope

This standard specifies the method for detecting antibodies to African swine fever by indirect immunofluorescence assay.

This standard applies to the detection of antibodies to African swine fever.

### 2 Instruments and equipment

#### 2.1

Fluorescence microscope.

## **2.2**

Invert the microscope.

## **2.3**

Carbon dioxide incubator and ordinary constant temperature incubator.

## **2.4**

Refrigerator.

# **3 Test materials**

## **3.1**

African swine fever standard strain.

## **3.2**

African swine fever standard negative serum and positive serum.

## **3.3**

Anti-porcine fluorescent antibodies.

## **3.4**

PK-15 cells (porcine kidney cells) or Vero cells (African green monkey kidney cells).

## **3.5**

fetal bovine serum.

## **3.6**

Tested serum. inactivated at 56 ° C for 30 min.

## **3.7**

Distilled water (pH 7.4), see Appendix A for preparation.

## **3.8**

Cell base medium, cell growth medium and cell maintenance solution. For the preparation method, see Appendix A.

3.9 0.01mol/L pH7.4PBS (phosphate buffer), see Appendix A for the preparation method.

## **3.10**

Buffered glycerol. Prepared using neutral, non-autofluorescent glycerol (analytical grade). See Appendix A for preparation.

## **3.11**

Acetone. Pre-cooled using a front-loading refrigerator.

#### **4.1 Virus culture**

PK-15 cells or Vero cells are cultured with cell growth medium. After the cells are grown into a single layer, the culture solution is discarded and the standard for African piglets is added.

The strain makes the virus liquid completely cover the cells. Adsorbed in a 37 ° C 5% carbon dioxide incubator for 0.5 h ~ 2 h, discard the virus solution, and exchange Fresh cell maintenance solution, placed in a 37 ° C 5% carbon dioxide incubator, observed daily, more than 50% of cells appear CPE (cytopathic Change) when harvesting. Cells that were not infected with the virus were cultured at the same time and used for comparison.

#### **4.2 Preparation of antigen tablets**

Harvest the harvested African swine fever virus infected cells into a cell suspension with a concentration of  $5 \times 10^5/\text{mL}$ , drop 2~3 drops on the glass slide, and apply A uniform sheet was formed, and it was naturally air-dried at room temperature, and fixed with pre-cooled acetone for 10 minutes to prepare an antigen sheet. Store at -20 °C.

#### **4.3 Preparing cell pairs for photos**

Cell-pair photographs were prepared as described in Section 4.2 of PK-15 cells or Vero cells grown into cell monolayers.

#### **4.4 Sample loading**

After the test serum was diluted 1:10 with PBS, an appropriate amount was added to the antigen sheet and the cell pair photograph to prepare the specimen to be inspected and finely Cell control specimens. The specimen was placed in a wet box and incubated in a 37 ° C incubator for 1 h.