

# RESEARCH CENTER FOR ANIMAL HEALTH AND SAFETY (CISA-INIA)

**SOP/CISA/ASF/ELISA/2/2008**

**(STANDAR PROCEDURE OPERATION  
BLOCKING ELISA FOR SEROLOGICAL  
DIAGNOSIS FOR AFRICAN SWINE FEVER)**

**Rev. 1**

**Date: December 2008**

REV.	DATE	EPIGRAPH	CAUSE OF CHANGE
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## 1. PURPOSE

The main goal of this procedure is to describe the indirect ELISA technique to perform the African swine fever antibody detection

## 2. SCOPE

This procedure is applicable to porcine serum sample.

## 3. REFERENCES

### 3.1. DOCUMENTS USED IN THE PROCEDURE REDACTION

As a basic reference for the elaboration of this procedure it has been taken the criteria established in the next documents:

1. Protocol INGEZIM PPA COMPAC (1.1.PPA.K3), registered by MAPA nº 335 RD July, 2002/.

### 3.2. DOCUMENTS TO BE USED TOGETHER WITH THIS PROCEDURE

## 4. BACKGROUND INFORMATION

### 4.1. ABBREVIATION

ASF: African swine fever  
 ASFV: African swine fever virus  
 PC: Reference positive control  
 NC: Reference negative control  
 CO: Cut off  
 Mab: monoclonal antibody  
 IB: Immunoblotting  
 H<sub>2</sub>O d: destiled water

### 4.2. BACKGROUND

This kit is based on a blocking enzymatic immunoassay (Blocking Elisa). The antigen is fixed in a solid support (polystyrene plate). When a sample serum contains specific antibodies against the virus, they will bind to the antigen absorbed on plate while if the serum sample does not contain specific antibodies they will not bind the antigen. If we add a specific monoclonal antibody (Mab) against the viral antigen coated to the plate (conjugated with peroxidase), it will compete with the antibodies of the serum. If the serum samples contain specific antibodies, they will not permit the binding of the labeled Mab to the antigen whereas if it does not contain specific antibodies the Mab will bind to the antigen on the plate. After washing the plate to eliminate all non-

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fixed material from the plate, we can detect the presence or absence of labeled Mab by adding the specific substrate that in presence of the peroxidase will develop a colorimetric reaction. The antigen coated to the plate in our kit consists of a purified protein extract from the virus (the VP73), which is the major structural protein from the ASFV and the most antigenic one.

## **5. DESCRIPTION**

### **5.1. EQUIPMENT AND MATERIALS**

#### **KIT COMPOSITION:**

- Microtitration plates 8 x 12 wells
- Positive control
- Negative control
- Conjugate (100x)
- Washing solution concentrate 25x
- Diluent (DE01-1) (ready to use)
- Substrate TMB (ready to use)
- Stop solution

Store all plates and reagents at 4°C. Store the control sera at -20°C, otherwise they must be rejected within one month.

#### **OTHER MATERIALS AND REAGENTS NOT SUPPLIED WITH KIT**

- Shaker plate
- Incubator capable of 37°C
- Table centrifuge
- Reagent reservoir Polystyrene 50 ml (COSTAR Ref. 4870).
- Spectrophotometer UV/VIS with filter 450 nm annexed to a computer program to register and print results.
- Nitrile gloves
- Single channel pipette 1-10µl
- Single channel pipette 10-100µl.
- Single channel pipette 10-200µl.
- Single channel pipette 200-1000µl
- Multichannel pipette 5-50µl
- Multichannel pipette 50-300 µl
- Aluminium foie
- Adsorbent paper
- Pipetboy acu or equivalent
- Glass or plastic pipettes for volume of 1-10 ml
- Tips (1-10 µl., 1-200µ, 100-1000 µl).
- Chronometer
- Eppendorff tubes 0.5, 1.5 and 2 ml)
- Steril plastic tubes (10ml, 50 ml).
- Seal plates sealing film.
- Vortex

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## 5.2. PREPARATION

- **Washing solution** dilute one part of the 25 x concentrate washing solution provided in the kit with 24 parts of distilled or deionizer water (eg. 40 ml concentrate washing solution + 960 ml deionizer water). When ready this solution remains stable at 4°C.
- **Controls:** make aliquots and store at -20°C till use. The control sera need to be diluted ½ in diluent prior to be used, in the same way than the sera samples. This dilution can be done directly into the plate by adding 50 µl of diluent plus 50 µl of control sera.
- **Conjugate:** to make immediately before use. Dilute the needed quantity of conjugate provided in the kit 1/100 into the supplied diluent. Shake very well the solution before the use. Prepare only the quantity need for each time because the remainder volume has to be rejected.

### SAMPLE PREPARATION:

- Sample porcine serum. Samples need to be diluted ½ in diluent prior to be used; this dilution can be done directly into the plate by adding 50 µl of diluent plus 50 µl of sample sera.

Each sample must be identifying with a register number at CISA and with an identification number (sample ID) in case there are more than one sample from the same sender.

## 5.3. METHODS

1. **All reagents** (except conjugate) must be allowed to come to room temperature before use.
2. **Addition of sera:** Add 50 µl of supplied diluent to each well. Add 50 µl control sera (PC and NC) and add 50 µl of each sample sera to test on each remainder wells. Incubate for 1 hour at 37°C or overnight (18 hours) at 18-25°C.
3. **Empty** the wells into a container with NaOH solution .
4. **Wash the plates:** Wash the plates at least four times with washing solution.
5. **Add 100 µl of specific conjugate** prepared previous instructions to each well. Seal the plate and incubate 30 minutes at 37°C
6. **Wash the plates:** Wash the plates five times as previously described. Then blot them onto paper towels.
7. **Add 100 µl of substrate solution per well and** incubate for approximately 15 minutes at room temperature.
8. **Stop** the reaction by addition of 100 µl stopping solution per well
9. **Reading plates:** The results can be obtained using a spectrophotometer to read microtitre plates at 450 nm wavelengths.

## 5.4. RESULTS

**NOTE:** At the moment of reading results, each plate is analyzed as individual plate: controls values of each plate are used to calculate CO and PC/NC value of each one. In this way, sera will be analyzed respect to the controls of its plate.

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### VALIDATION OF THE TEST:

The test could be considered valid when the OD of the NC is, at least, 4 times higher than the OD of the PC:

DO NC	= ≥ 4
DO PC	

### Cut Off Calculation:

To achieve a correct interpretation of the results it is necessary to calculate the CUT OFF which will permit to define negative, ambiguous and positive sera.

The CUT OFF is calculated by a simple equation:

$$\begin{aligned} \text{Positive Cut Off: } & \text{NC} - [(\text{NC} - \text{PC}) \times 0.5] \\ \text{Negative Cut Off: } & \text{NC} - [(\text{NC} - \text{PC}) \times 0.4] \end{aligned}$$

For calculating the % of competition (x %) of a sample:

$$X\% = \frac{\text{NC} - \text{SAMPLE OD}}{\text{NC} - \text{PC}}$$

### Results Interpretation:

- Sera with optical density **lower** than Positive CUT OFF can be considered as **positive sera to ASFV antibodies**.
- Sera with optical density **higher** than Negative CUT OFF can be considered as **negative sera**.
- Sera with optical density **between** both CUT OFF can be considered as **doubtful sera**, and they have to be confirmed by another technique

## 5.5. CRITICAL POINTS

1. **The washing steps** could be done using an automatic washing machine or a multichannel pipetting device suitable for dispensing 300 µl on each well. After the incubation periods, the washing steps must be done following the next instructions:
  - Throw out the content of the plate by a brusque turn over of the plate to avoid the possible mixture of the content from one well to another.
  - Dispense a volume of 300 µl of washing solution on each well.
  - Shake delicately the plate, avoiding the contamination between wells.
  - Turn over the plate brusquely to empty the wells.
  - Repeat the process as much times as is indicated on the instructions of the Kit.
  - Prior to empty the content of the last washing step, verify that the next reagent to be added to the plate is ready to use.
  - Do not maintain the plate on dry more time than strictly needed.
  - After the last step of washing shake the plate turned over an absorbent filter paper.
2. **Do not use highly haemolysed or contaminated samples).**

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## 5.6. SAFETY CAUTIONS

- Read the protocol previously.
- Bring all reagents to room temperature prior to use.
- Do not mix instructions or reagents from different kits.
- Avoid any reagent contamination
- Do not use the components after expiration dates and do not mix components from different lots.
- Do not eat, smoke or drink while the manipulation of reagents.
- Do not pipette by mouth.
- Use a new tip for each serum sample.
- Always include PC, LC and NC.
- Substrate must be handle with care, it is very sensible to light and contamination.
- Stop solution is a strong acid. Handle with care.

**6. APPENDIX**

*Appendix 1. FORM CISA/PPA/ELISA/2/2008*

**ENTRY REGISTER CISA:**

**DATE:**

**TECHNICIAN:**

**BATCH NUMBER:**

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

**RESULTS:**

**OD PC**

**OD NC**

**POSITIVE Cut Off:**

**NEGATIVE Cut Off:**

**OBSERVATIONS:**