CENTRO DE
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OIE
Reference Laboratory

SEROLOGICAL DIAGNOSIS OF AFRICAN SWINE FEVER BY ELISA COMMERCIAL KIT

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ELISA COMMERCIAL KIT for SEROLOGICAL DIAGNOSIS of AFRICAN SWINE FEVER

*Equipment required

- Chamber 37ºC
- ELISA reader with 450 nm wavelength filter.

*General laboratory material

- Latex or nitrile gloves
- Permanent marker
- Racks for microcentrifuge tubes

1. MATERIALS AND REAGENTS

- 10 ml tube
- Multichannel pipette
- Pipetboy
- Micropipettes of 200µl
- Reagents of the kit: ASF COMPACT ELISA (INGENASA®)

2. METHODOLOGY:

2.1 Label the sensitised plate provided in the kit. Identify the control wells.

2.2 Dilute test and control sera directly in the kit plates:

- *Add 50 µl of sample diluent*
- *Add 50 µl of sera (test and control sera)*
- *Cautiously mix the content, avoiding the contamination from one well to another.*

2.3 Cover the plate and incubate for 1 h at 37 ºC.

2.4 Wash the plates four times with the washing buffer*. Then blot them onto paper towels.

- ***Washing buffer***: The washing buffer provided in the kit is x25 concentrated. Dilute the washing solution provided in the kit in 24 parts of distilled water (e.g.: 40 ml of concentrate + 960 ml of distilled water)

2.5 Add 100 *µl* of conjugate per well.

** Dilute the conjugate of the kit with the supplied diluent (dilution 1/100) (e.g. for one plate: 110 µl of conjugate + 11 ml de diluent)

2.6 Cover the plates and incubate 30min. at 37 ºC.

2.7 Wash the plates four times with washing buffer. Then blot them onto paper towels.

2.8 Add 100 µl of substrate (is ready to use in the kit) per well.

2.9 Incubate 15 min. at room temperature without light.

2.10 Add 100 µl of stop solution.

2.11 Read the plates using a spectrophotometer UV/VIS at 450 nm wavelengths.

3. INTERPRETATION OF THE RESULTS

- VALIDATION OF THE TEST:

The test could be considered valid when the OD of the NC (Negative Control) is, at least, 4 times higher than the OD of the PC (Positive Control).

$$\frac{OD\ NC}{OD\ PC} > 4$$

If the test is not valid, the following steps are not necessary because no conclusions could be obtained from the OD results.

Once the test is valid, the cut off values must be calculated based on the following formulas. Finally, the OD values of each sample must be compared with both

cut offs, being classified on greater, lower or between both cut offs, and consequently on positive, negative or doubtful sera.

- CUT OFF CALCULATION:

  Positive cut off: CN – ((CN-CP) x 0.5)
  Negative cut off: CN – ((CN-CP) x 0.4)

- RESULTS INTERPRETATION:

  Positive sera: OD < Positive cut off
  Negative sera: OD > Negative cut off
  Ambiguous sera: OD *between* both cut offs

- CP: Positive Control
- NC: Negative Control
- OD: Optic Density

*__Recommendation__: positive and doubtful results should be confirmed by confirmatory techniques: immunoblotting, indirect immunofluorescence or immnuoperoxidase test.

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